Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2019 Volume 1 (A to M)

Contents

Project	**1. A New Framwork for Computational Biomechanical Models and 3Rs in Musculoskeletal Research**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 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 goal of this research study is to demonstrate that computer models are capable of accurately predicting the forces generated and sustained by the jaw/skull muscles and bones of rabbits while they feed. This will be achieved by comparing the predictions from computer models to a new set of direct experimental measurements on rabbit muscles and bone. This will be pursued through three specific methodological objectives, namely to:	

	1. collect data on bone motion and muscle physiology on rabbit mastication. 2. collect anatomical and image data on bone and muscle in rabbits. 3. combine data from (1) and (2) to build and validate computer models of rabbit feeding biomechanics.
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 goal of this project is to demonstrate that computer simulation approaches can contribute significantly to reduction, replacement and refining of the use of animals in biomechanical research and medical device design. Many experimental studies in animal biomechanics are highly invasive, causing pain and distress to the animals before they are euthanized. In theory, once of digital model has been created, computer simulation has the potential to completely replace (or maximally reduce) the use of animals in that area of biomechanical research and/or medical device design. The anatomy (e.g. muscle size, bone properties) and/or behaviour (e.g. chewing motions, bite forces) of a digital model can manipulated or altered continuously without any harm or distress to a real animal. This can allow, for example: a model analysis to be extended to a different strain/breed of the same species (or a morphologically similar species) by digital modification of the anatomy/behaviour; elements of anatomy to be modified in multiple ways (e.g. removal of teeth/bone) to examine the consequences of different surgical approaches; and for implant devices to be digitally inserted into models to examine their mechanical impact and performance, all without the need for any experimentation on real animals.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to 18 male New Zealand White rabbits over the course of the next four years to generate the data required to build and thoroughly test the accuracy of our models. The maximum of 18 rabbits was decided on based on analysis of previously published data by an expert statistician to ensure we are using an appropriate number of animals
In the context of what you propose to do to the animals, what are the expected adverse effects and the	The surgical procedures and some of the associated experiment methods may cause moderate pain, harm, suffering or distress. Rabbits will undergo surgery under anaesthesia. During this surgery metal beads

likely/expected level of severity? What will happen to the animals at the end?	and one strain gauge will be attached to the skull and jaw bone, and sonomicrometry crystals and EMG electrodes will be implanted in muscles. After surgery, the rabbit will be given a minimum 48 hour recovery period before the feeding experiments take place. During this period the rabbits may experience moderate pain and suffering due to soreness from the surgery. During the feeding experiment, biplanar x-ray videography, EMG, sonomicrometry and strain gauge data will then be collected simultaneously while the rabbits feed normally on a variety of food types within a Perspex box. At the end of the experiments most of the animals will be euthanized immediately using a Schedule 1 method due to the need to carry out medical imaging (CT, MRI) and anatomical dissection to build the computer models. However, a small number of the rabbits will undergo a further experiment before euthanasia. These rabbits will undergo surgical in-situ experiments on their chewing muscles to quantify their various properties that determine how much force they generate. This will be carried out under non-recovery anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To demonstrate that a computer model is accurate or valid requires a large amount of data about the anatomy (e.g. muscle size, bone properties) and mechanics of feeding (e.g. jaw motions, bite forces) used by rabbits. This data does not exist for rabbits, or indeed any other experimental animal. Therefore a systematic anatomical and biomechanical investigation of rabbit feeding is required in which all the primary aspects of jaw anatomy and feeding mechanics are measured from a small cohort of rabbits. Constructing models from medical imaging (CT/MRI) data of those same rabbits can then directly and immediately validate computers simulation. Only in this way can models we truly validated and their potential for achieving 3Rs in future studies be demonstrated.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We consulted with a chartered statistician to calculate the sample size required for our analysis. We have based our sample size calculations on the relationship between bone strain and jaw muscle activity measured in rabbits by **REDACTED (published material)**. We have already collected high quality data from 3 rabbits in a pilot study and

	therefore 14 more rabbits are required. Given the success rates of measurements from our pilot experiments, and our general experience using these techniques, we anticipate an 80% success rate. Therefore, we request permission to work on up to 18 rabbits. It is worth emphasising that we will be collecting data from the majority of jaw/feeding muscles, along with data on their force production capabilities, and combining this data in our models. Hence, our predictive capabilities may exceed those possible from the data of **REDACTED (published material)**, and therefore we may find that a smaller number of rabbits is sufficient for our goal. We will therefore carry out an interim analysis after first 8 rabbits are tested to see if the minimum number of animals can be reduced.
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.	Rabbits are the first-choice experimental animal for dental implant design studies because of their size and easy handling. According to international standards regarding species suitable for testing implants in bone, rabbits represent an important species. Although the rat is also a frequently used model, it is not really regarded as a suitable model for testing dental implants and bone remodeling due to significant differences in bone composition, healing, and anatomy to humans. Therefore because rabbits represent by far the most widely used species in this context we proposed to use them to demonstrate the capacity of validated computer modeling as a means of achieving replacement, reduction and refinement of animal use in future studies of dental surgeries and implant design. We have the facilities and expertise for housing this species. In this project we will use male New Zealand White rabbits because their large body size (~3kg) is more amenable to the surgical procedures and x-ray imaging than smaller breeds of rabbits.

2. A study on treatment and penetrance of inherited cardiac conditions

Project duration

5 years 0 months

Project purpose

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

Key words

cardiac channelopathies,, genetic cardiomyopathies, diet and cardiovascular disease, , air pollution and cardiovascular disease

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

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?

Inherited cardiac conditions (ICC) are cardiac diseases caused by abnormalities in the DNA (mutations) and can be transmitted from parents to children. In this project we will investigate two different types of ICCs:

Inherited arrhythmia syndrome (channelopathies)
Genetic cardiomyopathies.

These conditions usually affect adolescents and young adults.

Channelopathies are caused by abnormalities in the electrical properties of the heart that lead to irregular heart rhythms (arrhythmias) which can sometimes lead to sudden death. Novel treatments have been proposed to prevent arrhythmias. However these have not been widely tested. Channelopathies vary in their severity: in some family members there is no evidence of any arrhythmias but in others there are severe arrhythmias which can cause sudden death. The factors that determine this variability are not known, though some studies suggest that diet and pollution are important.

Genetic cardiomyopathies are conditions characterized by alteration in the structure and

function of the heart. We intend studying 2 types of cardiomyopathies which cause heart failure and serious symptoms including death. We can identify family members who carry the genetic defects very early before they develop the cardiomyopathy. However currently we do not have any treatments to prevent or reverse them. Again, there is variability in the severity of the condition within a family. Diet and pollution have also been suggested as potential factors that influence the severity of the condition.

This project has 3 main objectives

To develop new treatments for arrhythmias and the 2 types of cardiomyopathies
To better understand whether dietary modifications affect the severity of these conditions
To better understand whether pollution modify the severity of these conditions

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?

This project could identify new treatment strategies for these patients that could significantly improve their symptoms and prolong their lives. Identifying environmental pollutants which influence the severity of these conditions would be a valuable asset to the public health community

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 10700 over 5 years

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

We will inject certain drugs to induce irregular heart rhythms (arrhythmias). The onset of arrhythmias can cause difficulty in breathing in the animals. We will perform the study under anaesthesia to reduce animal distress. Some of the genetic alterations we study can cause thickening of the heart muscle or enlargement of the pumping chambers of the heart. If these changes become severe they can cause heart failure. The typical signs of heart failure are decreased food intake, weight loss and difficulty in breathing. We will monitor the heart structure and function with heart scans. To prevent the onset of heart failure the in vivo studies will be terminated when the changes in heart structure are moderate. We will give drugs or substances that can cause adverse effects such as decreased food in intake and weight loss. The animals will be carefully monitored for these signs. The animals will have operations that can cause infection or bleeding which will be

prevented by appropriate care of the animals. The severity level of this study is going to be moderate All animals will be humanely killed at the end of the in vivo studies.
Replacement

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

Arrhythmias and enlargement or thickening of heart muscle cannot be studied in single cells or tissues slices. They must be studied in vivo using animal models.

Reduction

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

We have carefully designed the study to reduce animal use . We have carefully calculated the minimum number of animals needed for each experiment. Animals used for the in vivo studies will also be used for the in vitro studies after they have been sacrificed. We will also use tissue from each animal in more than one in vitro experiment, so that the numbers needed will be drastically reduced

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.

Mouse models reliably reproduce the clinical signs of these inherited cardiac conditions. Therefore, mice are the lowest sentient animal suitable for this work.

We will terminate the study well before the development of advanced cardiomyopathy, so it is very unlikely that the animals will develop any symptoms of heart failure which can happen in advanced cardiomyopathy.

We will run a pilot to determine whether we can minimize the stress to the animals when we induce arrhythmias under general anaesthesia.

Phenantrene, a common air pollutant, has been associated with weight loss at very high doses which are well above the range proposed in the study. It is therefore unlikely that the animals will experience weight loss. However we will monitor animal weight closely to ensure this does not happen.

3. A study on treatment and penetrance of inherited cardiac conditions

Project duration

5 years 0 months

Project purpose

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

Key words

cardiac channelopathies, genetic cardiomyopathies, diet and cardiovascular disease, air pollution and cardiovascular disease

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

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?

Inherited cardiac conditions (ICC) are cardiac diseases caused by abnormalities in the DNA (mutations) and can be transmitted from parents to children. In this project we will investigate two different types of ICCs:

Inherited arrhythmia syndrome (channelopathies)
Genetic cardiomyopathies.

These conditions usually affect adolescents and young adults.

Channelopathies are caused by abnormalities in the electrical properties of the heart that lead to irregular heart rhythms (arrhythmias) which can sometimes lead to sudden death. Novel treatments have been proposed to prevent arrhythmias. However these have not been widely tested. Channelopathies vary in their severity: in some family members there is no evidence of any arrhythmias but in others there are severe arrhythmias which can cause sudden death. The factors that determine this variability are not known, though some studies suggest that diet and pollution are important.

Genetic cardiomyopathies are conditions characterized by alteration in the structure and function of the heart. We intend studying 2 types of cardiomyopathies which cause heart failure and serious symptoms including death. We can identify family members who carry the genetic defects very early before they develop the cardiomyopathy. However currently we do not have any treatments to prevent or reverse them. Again, there is variability in the severity of the condition within a family. Diet and pollution have also been suggested as potential factors that influence the severity of the condition.

This project has 3 main objectives

To develop new treatments for arrhythmias and the 2 types of cardiomyopathies
To better understand whether dietary modifications affect the severity of these conditions
To better understand whether pollution modify the severity of these conditions

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?

This project could identify new treatment strategies for these patients that could significantly improve their symptoms and prolong their lives.

Identifying environmental pollutants which influence the severity of these conditions would be a valuable asset to the public health community

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 10700 over 5 years

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

We will inject certain drugs to induce irregular heart rhythms (arrhythmias). The onset of arrhythmias can cause difficulty in breathing in the animals. We will perform the study under anaesthesia to reduce animal distress.

Some of the genetic alterations we study can cause thickening of the heart muscle or enlargement of the pumping chambers of the heart. If these changes become severe they can cause heart failure. The typical signs of heart failure are decreased food intake, weight loss and difficulty in breathing. We will monitor the heart structure and function with heart

scans. To prevent the onset of heart failure the in vivo studies will be terminated when the changes in heart structure are moderate.

We will give drugs or substances that can cause adverse effects such as decreased food in intake and weight loss. The animals will be carefully monitored for these signs.

The animals will have operations that can cause infection or bleeding which will be prevented by appropriate care of the animals.

The severity level of this study is going to be moderate

All animals will be humanely killed at the end of the in vivo studies.

Replacement

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

Arrhythmias and enlargement or thickening of heart muscle cannot be studied in single cells or tissues slices. They must be studied in vivo using animal models.

Reduction

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

We have carefully designed the study to reduce animal use . We have carefully calculated the minimum number of animals needed for each experiment. Animals used for the in vivo studies will also be used for the in vitro studies after they have been sacrificed. We will also use tissue from each animal in more than one in vitro experiment, so that the numbers needed will be drastically reduced.

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.

Mouse models reliably reproduce the clinical signs of these inherited cardiac conditions. Therefore, mice are the lowest sentient animal suitable for this work.

We will terminate the study well before the development of advanced cardiomyopathy, so it is very unlikely that the animals will develop any symptoms of heart failure which can happen in advanced cardiomyopathy.

We will run a pilot to determine whether we can minimize the stress to the animals when we induce arrhythmias under general anaesthesia.

Phenantrene, a common air pollutant, has been associated with weight loss at very high doses which are well above the range proposed in the study. It is therefore unlikely that the animals will experience weight loss. However we will monitor animal weight closely to ensure this does not happen.

Project	**4. Adaptation in the musculoskeletal system**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project observes the changes in muscle, tendon and bone in response to increases and decreases in daily muscle activity. Many changes associated with voluntary exercise or electrically activated movement are beneficial, but the best programmes for that exercise are not well understood in term of the cellular responses within the musculoskeletal system. In some cases (such as the rare disease **REDACTED** which we study in this project), excessive exercise might even accelerate the disease process. We need to understand the signals that cells respond to in terms of the useful responses like increased strength, endurance and ability to reduce the	

	blood glucose level, but also why muscle is lost so quickly when it is not used, in conditions like bed rest during a period of illness, or after a leg fracture
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)?	If we understand the cellular responses underlying adaptation to exercise we may be able to advise individuals more effectively in terms of the most productive approach to gaining strength or endurance. In situations in which muscle function has been lost, such as laryngeal paralysis, we may be able to advise how best to activate the muscles to restore function. For sufferers of the rare disease **REDACTED**, we will continue to characterise the disease and to test potential therapies based on replacement of the missing enzyme function or therapies that modify the protein breakdown pathway or avoid the build-up of toxic substances that otherwise cause severe pain and damage to the joints.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice, 200-300 over 5 years Rabbits, 20 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 receive implanted muscle pacemakers to produce programmed activity in one hind limb, designed to interfere as little as possible with normal locomotion and behaviour. Animals will be killed humanely and the muscles taken for analysis after a period of a few weeks of training. Sometimes, the limbs will be scanned by X-ray techniques before the muscles are removed to investigate changes in bone and joint structure. Severity is considered moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no non-animal model of the interaction between muscle, tendon bone and joints. So work that involves the whole musculoskeletal system requires the use of mammalian species which have a very similar arrangement to human.
2. Reduction Explain how you will assure the	We make the very best use of every sample, measuring gene expression with sensitive techniques that require very little tissue, and

use of minimum numbers of animals	performing microscopic techniques on samples from the same muscles. Because we use implantable stimulators we can exercise one limb so that the other acts as the control (unexercised) limb. We thus do not need an additional group of animals as controls.
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 model of muscle activation we use is highly developed making use of miniaturised electronics to produce muscle pacemaker systems that can be programmed by an external controller, like the latest heart pacemakers. That means that the pattern of activity can be adjusted for each individual. We use a standard of technique that mirrors the best veterinary practice. Our model of the rare disease **REDACTED** is much milder than the severe human form, and represents only the early stages of this painful joint disease. Even so, the mechanisms seem to be the same so we can still test new therapies because we anticipate that prevention of the early stages will have a significant benefit in later life when the symptoms would otherwise progress to a severe stage.

Project	**5. ADME (Adsorption, distribution, metabolism and excretion) and Tissue Residue 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)	The aims of the project are: 1. the study of the adsorption, distribution, metabolism and excretion (ADME) of veterinary medicines and feed additives in food producing animals 2. residue depletion studies of veterinary medicines and feed additives in food producing animals 3. the study of the ADME of plant protection	

	products in food producing animals 4. to supply samples of animal tissues containing tissue-bound (incurred) residues of veterinary medicines for analytical method development and validation, quality control (QC) and performance assessment purposes.
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)?	ADME studies and reside depletion studies are part of the regulatory requirements which have to be met before a veterinary medicine or feed additive can be approved for use. Similarly ADME studies are part of the regulatory requirements for the approval of plant protection products. ADME studies and reside depletion studies are part of the information required to ensure the safety of consumers exposed to food products derived from animals which have been exposed to the chemical. Animals can be exposed through direct administration for veterinary medicines and feed additives, or through consumption of feed/fodder containing residues of plant protection products. In addition to issuing approvals for the use of veterinary medicines, regulatory authorities also conduct monitoring surveys of veterinary medicine in tissues from food-producing animals. This monitoring is designed to ensure that only approved veterinary medicines are being used and that approved medicines are being used in the correct manner. To conduct this monitoring, analytical methods to determine residues in tissues are required. The validity of analytical measurements is critical, since important decisions are made based on them. EU legislation (Commission Decision 2002/657/EC) requires that new analytical methods are developed and validated using tissues containing incurred residues. Better, more valid, more robust and better understood chemical data, produced with the use of incurred tissue, will allow regulatory bodies to make better judgements on the risks associated with residues and help to safeguard human health and protect the food chain. The use of incurred tissue also allows laboratories to assess and monitor their performance and to
	identify problem areas

What species and approximate numbers of animals do you expect to use over what period of time?	Total numbers of animals expected to be used over the duration of the project are listed below with expected numbers per year indicated in brackets Pig, juvenile & adult 410 (82 per year) Chicken, neonates, juvenile & adult 1500 (300) Turkey, juvenile & adult 300 (60) Cattle, juvenile & adult 150 (30) Sheep, juvenile & adult 225 (45) Goats, juvenile & adult 180 (36) Rabbits, juvenile & adult 300 (60).
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 animals to be used are all food producing animals, the tissues of which are eaten by consumers. The animals will, after a period of acclimatisation, be given one or more doses of veterinary medicine or feed additive or plant protection product. Veterinary medicine may be given in feed, or in solution (in some cases the solution will be contained within a capsule), or by injection if this is the normal route by which the medicine is administered. Feed additives are given in feed. Plant protection product are given in solution (possibly encapsulated). The likelihood of any adverse effects is very low since the veterinary medicine will be generally be used at normal therapeutic levels and higher levels will only be used when reliable information is available to show that the planned dosing level has been studied in the species of interest and has not caused adverse effects. Feed additives will be used at levels at which they are normally included in feed for the species being studied. Plant protection products will be given orally and will only be used at levels similar to levels shown to have had no effect on laboratory vertebrate animals. All animal care/welfare and procedures will only be performed by trained or supervised staff. Animals will be closely monitored and the veterinary surgeon consulted if there are any adverse effects. The administration of substances as liquids (either as encapsulated doses or oral dose) will only be carried out by appropriately trained staff. Sizes of capsules and needles/dose volumes/rates will be in line with good practice guidelines. If scientific requirements require any deviations from good practice guidelines then this will only be done

	with the agreement of a veterinary surgeon. At the end of each study, the animals will be killed humanely and organs and tissues collected for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The relevant regulations and guidelines for the conduct of ADME or residue depletion studies specify that animals must be used. For procedures designed to produce incurred residues for validation of analytical methods, options such as the use of tissue culture have been considered as replacements for animal procedures. These procedures are unsuitable as the biochemistry of vertebrates is complex, and substances pass through different organs and tissues before being deposited or excreted. Within each one, many chemical and biochemical processes may occur. These do not occur in isolation, and many substances undergo a variety of them. Using a single compartment model from tissue culture would not reproduce this complexity. Further, tissue culture and similar techniques cannot produce the quantities of tissues required for use in chemical processes. Analytical methods require large samples (2-10 g), so significant quantities of tissues are needed. Only animal studies can combine the requirements of the complexity of the system, the applicability of the product and the volume of material required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies will only be conducted when appropriate data of suitable quality is not already available for a particular chemical in the species of interest. A detailed study plan will be produced for each study which will contain the number of animals to be used. The study plan will be reviewed against the relevant guidelines to ensure that the study will not have to be repeated because minimum requirements for animal numbers were not met and to ensure that excessive numbers of animals are not being used. For procedures designed to produce incurred residues for validation of analytical methods, the

	minimum number of animals that will be required to generate a given mass of material will be used. This can be estimated accurately from the expected bodyweight and the proportion of a given organ / tissue within each animal or from the expected rate of production of materials such as milk or eggs.
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 listed in the protocols are all used in the human food chain. Hence, these are the most suitable to carry out ADME, residue depletion and incurred residue studies. As far as possible, all protocols are designed to reduce the pain, distress and harm suffered by the animals under test. This includes dosing and treatment via feed or water wherever possible, minimising invasive treatments and regular, in-depth veterinary involvement designed to mitigate any and all adverse effects to the greatest possible degree. Animals are, wherever possible housed with conspecifics, and are always within sight / sound of conspecifics. Inspection / checking regimes are thorough to ensure that in the event of any animal suffering adverse effects; these will be noted and hence treated as rapidly as possible. Species-specific enrichment will be provided to ensure welfare needs are met and to allow natural behaviours. This can be: Social; housing social animals with conspecifics when possible or interspecific for some animals, and regular human social interaction Structural; pen/cage design providing structural features to enhance the animals living space providing opportunities to exercise, play and explore. Cognitive to give a choice over resting or feeding area and bedding material. Enrichment will be rotated to give varied stimulation and will be agreed with the NVS, NACWO and study director. Sometimes enrichment is restricted to certain items for example when animals are housed in metabolism cages

Project	**6. Advances in Tropical Fish Aquaculture**	
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
	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 overall aim of the programme is to develop techniques for the culture of tropical fish. Success will ultimately increase the numbers, diversity and availability of farmed species within the aquarium trade. This will reduce the number of fish collected from the wild, protecting natural fish stocks from overexploitation, and natural habitats from destructive collection practices. In addition, farmed fish are better adapted to life in captivity compared to wild collected fish. Key objectives will include: I) An investigation of the reproductive behaviour and spawning requirements of tropical fish species in captivity,	

	including their environmental (temperature and light levels) and dietary requirements (food types and feeding frequency). II) Research into rearing techniques for eggs and the early life stages of tropical fish, examining a variety of aquarium designs and husbandry techniques in order to enhance development. III) Assessment of novel diets for the early life stages of tropical fish. The provision of suitable food items for early life stages has been identified as arguably the most important factor contributing to the success or failure of farming activities and is considered a 'bottleneck' in rearing efforts.
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)?	Farmed fish offer significant improvements for animal welfare over their wild counterparts. Wild collected animals are transported over long distances and for long periods of time. Fish often experience stressful conditions during transport, often leading to death. Fish require quarantining prior to sale in order to eliminate parasites. Farmed fish however can be produced and distributed within the destination market and subsequently need only be transported over comparatively short distances and time periods, thereby reducing their carbon footprint. It is recognised that farmed fish are considered to be healthier, being pre-adapted to life in captivity having been weaned onto commercial diets from the early stages of their development. The scale of the ornamental fish trade is too vast to be accounted for by the farming of tropical fish within the foreseeable future. However, establishment within destination markets could form part of a sustainable management strategy. It could off-set some of the environmental impacts of the traditional supply network, such as long-distance overseas air travel, multi-stage distribution network, extensive infrastructural demands, wild collection, handling and shipping mortality.
What species and approximate numbers of animals do you expect to use over what period of time?	Our primary aim is to study a selection of tropical fish species, all of which are popular within the global aquarium trade. We plan to use approximately 30 to 60 adults, 1,000 juveniles and 15,000 larvae per species.

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?	All procedures that are included are classified as mild in severity and in general all the methodologies are modifications to those that have been tried and tested. These will be performed by well-trained and highly experienced staff. Some observational work will be carried out on the development of embryos within the eggs and hatchlings younger than 3 days of development (prior to first-feeding hence too young to be under the Act). At the end of the project mature adults will be maintained as a source of eggs and larvae for future projects while juveniles will be reared to adulthood for use in future studies. Fish no longer required will be euthanased at the end of the project using a Schedule 1 method or re-homed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The basis for this study is the development of culture techniques for live animals and therefore there are no non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduced numbers of animals is achieved by using a small group size over an appropriate number of replicates and where possible a factorial design will be used.
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 aim to use in this study are some of the most popular and heavily traded groups of tropical fish. We aim to study a variety of tropical fish species, all of which are popular within the global aquarium trade. Most research to date on the farming of tropical fish has focused on a number of model groups of fishes. Therefore, there are solid foundations from which commercial culture techniques can be built. Fish showing signs of illness will be given the proper treatment or killed by a schedule 1 method.

Project	**7. Analysing mechanisms of cancer dissemination and therapy 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)		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 over-arching aim of this work is to achieve a better understanding of the mechanisms governing the spread of cancer and its response to therapy. This knowledge will help to inform the development of better strategies for cancer prognosis and treatment. It will also enhance our understanding of mammalian tissue organisation.	
Why is it important to undertake this work?	Cancer is a major cause of mortality in the UK, responsible for over a quarter of all deaths. Even modest improvements in treatment will benefit the lives of thousands.	
What outputs do you think you will see at the end of	The outputs from this work will include. 1. A better understanding of how the interplay	

this project?	between cancer cells and their microenvironment influences both how cancer spreads and its response to therapies 2. Improved treatment regimens for controlling or eliminating cancer in pre-clinical models 3. Improved understanding of how to mimic the complexities of the tumour microenvironment in reductionist systems 4. In practical terms, the new information generated will be shared via publications, research presentations at conferences (both national and international), and, if appropriate, the reporting of improved methods 5. Improved imaging methods and new prognostic strategies are also potential outputs, although not the immediate aim of this project
Who or what will benefit from these outputs, and how?	There are likely to be multiple beneficiaries from the outputs above. These include: 1. If any of the genes implicated are 'druggable' targets then we expect that biotechnology and pharmaceutical companies may utilise the information produced in their drug discovery programs. If possible we will try to develop some targets with the appropriate technology transfer teams. 2. Another possibility is that the identification of important regulators of cancer dissemination may lead to improved cancer prognosis. This would enable better clinical management of patients. Thus there is a small, but real, possibility of patient benefit arising from this work within 5-10 years. In support of this, work from the previous PPL has recently obtained funding to explore its importance in large human patient cohorts. This is evidence that the type of work here is being exploited to try to directly improve patient management. 3. This work will benefit the basic research community by increasing our knowledge of mammalian physiology and cell biology. 4. An improved understanding of the details of how the tumour microenvironment influences the metastatic process and how tumours respond to therapy should enable better in vitro models to be developed. Indeed, the development of improved 'organotypic' culture models is

	an active area of research in the REDACTED lab . This may ultimate reduce the number of animals used in research.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	A major mechanism to maximise the output of the work alongside the publication of primary research papers, will be presentation of the work at both big international meetings and smaller more methods oriented workshops. These latter formats have the advantage that details of approaches that were ultimately sub-optimal can be shared. Our group already collaborates widely and this provides another avenue to share details of approaches that were ultimately sub-optimal and how methods were improved. If sufficiently transformative changes are implemented to methods, then we will look to publish specific protocols papers and post on bioxriv.
Explain why you are using these types of animals and your choice of life stages.	This project aims to understand how cancer cells interact with other cells in the body, and how their 'communication' affects the spread of cancer and its response to therapy. To do this, it is necessary to work with animals that have similar organs to humans – for example, lungs and mammary glands. This leads to the choice of mice. The main cancer types that we study occur in adults, and occasionally, young adults. Therefore, we work with juvenile and adult mice.
Typically, what will be done to an animal used in your project?	Procedures will be performed that lead to the development of cancer in mice. In the majority of cases, this will be through injected cancer cells. Mice will then receive therapies similar to those being used or developed to treat patients. Advanced imaging methods will be used to monitor the spread of tumours and how they react to therapies. Surgical procedures, such as the implantation of devices to aid imaging or the removal of primary tumours, will also be performed on a subset of mice. Extensive post-mortem tissue analysis will be performed to maximise the information obtained from each animal.

What are the expected impacts and/or adverse effects for the animals during your project?	Mice will develop tumours. Initially, these have little effect on the mice, but as they become larger they might affect mobility. Further, as tumours begin to spread they can affect weight, breathing, and behaviour. Depending on how much the animal is affected, the duration of the adverse effect may range from a small number of days to a small number of weeks.
What are the expected severities and the proportion of animals in each category (per animal type)?	Moderate severity is expected for about a third of the animals.
What will happen to animals at the end of this project?	used-in-other-projects
Why do you need to use animals to achieve the aim of your project?	We are interested in understanding how cancer cells interact with other non-cancerous cells. Therefore, we need to work in systems where the non-cancerous cells are as similar to the human as possible, both in their intrinsic features and their tissue organisation. The latter point of accurate tissue organisation is particularly hard to recreate using reductionist in vitro systems. Large parts of our work involve either studying breast cancer, lung cancer, or metastasis to the lung and this requires the use of organisms that have lungs and mammary glands. The mouse is well suited to this as it is a small mammal with relatively simple husbandry requirements. Further, well-established methods exist for genetic alteration in mice, which facilitate analysis of how cancer cells interact with non-cancerous cells.
Which non-animal alternatives did you consider for use in this project?	Yes, we have considered and use alternatives for much of the work in our group. Our group has made extensive use of complex co-culture models. Zebrafish models of cancer can be informative, but fish lack lungs and mammary glands, which are key tissues for the cancers that we study.
Why were they not suitable?	It is currently not possible to replicate the complexity of mammalian tissue structures in culture models. For example, the grape-like architecture of alveoli and their associated blood vessels cannot be mimicked even in state of the art 3D cultures. A second issue, is that the immune system only functions effectively in an organismal

	context with appropriate white blood cell movement and function within lymph nodes. Finally, metastasis is the transit of cancer from one organ to another. To study this requires not just recreating the environment of a single tissue, but to have multiple tissues linked together via the blood and lymphatic circulation. This is not achievable in simplistic non-animal models.
Enter the estimated number of animals of each type used in this project.	mice: 6000
How have you estimated the numbers of animals you will use?	The estimate is based on several factors. First, we have taken into account the numbers of mice used in the previous five years' experience. Second, we have taken into account the current number of researchers within the group. Third, we continually re-evaluate the numbers of mice required for each experiment using power calculations. Indeed, our laboratory employs a highly trained statistician who assists with experimental planning. This will allow us to determine the number of animals required per experiment. By combining this with the group size and availability or resources for data analysis, we are able to estimate how many experiments we will run per year (roughly 20) and therefore the numbers of mice required. Numbers of mice used for breeding are based on best practice and we maintain between 5 - 10 strains.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Experiments will be designed to not falsely detect effects and not miss effects ($\alpha = 0.05$ and $\beta = 0.1$ in technical terms). Based on our previous experience of how variable our measurements are and how big the effect we are looking is, then most experiments involve 5 – 10 mice per group and 4 – 10 groups. If the necessary in vivo data does not exist, then experimental design will be informed by a combination of prior in vitro data generated in the laboratory, existing publications, and the cumulative experience of >15 years mouse tumour work.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in	<u>Production, Breeding, Maintenance and Phenotyping</u> Mouse lines are routinely maintained by keeping 2-3 breeding pairs, with around 3-4 litters/year total 75-100 animals per strain/year. For crosses to enable

your project?	characterisation specific phenotypes it is likely that 5-6 breeding pairs will be kept with 6-8 litters/year total 350-400 animals per strain. We anticipate maintaining up to a maximum of 10 lines at any one time. To minimise breeding, lines under sporadic use are maintained at lower levels, and frozen whenever practicable. Lines will be maintained in collaboration with other licences wherever possible to minimise redundant breeding. Defining mechanisms of cancer dissemination and therapy failure We always aim to maximise the amount of data we get from each mouse. Whenever possible we try to use the same mouse for both intravital imaging and analysis of spontaneous metastasis. We take care to divide tumours into multiple pieces so that we can perform histological, transcriptomic, and flow cytometry analysis on tissue for the same mouse. We always aim to maximise the amount of data we get from each mouse. Whenever possible we try to use the same mouse for both intravital imaging and analysis of spontaneous metastasis. We take care to divide tumours into multiple pieces so that we can perform histological, transcriptomic, and flow cytometry analysis on tissue for the same mouse. We also utilise a new designs of imaging windows to obtain longitudinal data about changes in tumours and their response to therapy in the same mouse. This enables more data to be obtained for each mouse. Further, we are now using repeated imaging in the ear, which can be done with no surgical intervention. These experiments with repeated tracking of tumours are more powerful than simple endpoint assays as they reveal the 'full history' of the tumour. Therefore, we need fewer mice to draw robust conclusions. We are committed to implement further longitudinal imaging modalities in the coming years.
Which animal models and methods will you use during this project?	We will use mice in this project. As the focus is cancer biology, this necessitates the generation of tumours. For the generation of primary skin and breast tumours, we will most often employ intra-dermal and sub-cutaneous

	injections. For the study of lung tumours and lung metastases, intra-venous injections will be the route of choice. On rarer occasion, we will study metastasis to other organs and therefore use other injection routes. When using genetic models, we will endeavour to use external agents to deliver a special gene editing enzyme that initiates tumorigenesis. This reduces the complexity of the mouse crosses and avoids generating tumour prone mice outside of experimental requirement. We take care to ensure that the extent of tumour burden is the minimum required to observe the cancer behaviour that is being studied in the specific experiment. In particular, the use of microscopic resolution imaging methods means that we are able to evaluate tumour spread with greater sensitivity and, therefore, a lower overall tumour burden in the mouse.
Why can't you use animals that are less sentient?	We have considered zebrafish and fruit fly cancer models, but these do not replicate key structures that we are interested in, such as the mammary gland and lungs. We do use terminal anaesthesia to obtain very detailed information about tumours. However, to understand properly responses to therapy it is necessary to analyse the same tumour before and after giving treatment, hence the use on non-invasive longitudinal imaging.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We will stay up to date via regularly communication with BRF staff, other sientists in the field and regular visits to the following website https://www.nc3rs.org.uk/3rs-resources .
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	We try to minimise any possible adverse effects. In particular, the use of fluorescently labelled cells coupled with microscopic analysis of tissues enables us to detect small metastases. This reduces the overall tumour burden needed to be able to detect metastasis from primary sites. Similar benefits are expected from the use of non-invasive fluorescent or bio-luminescent imaging. If the purpose of the experiment is simply to observe cellular behaviours in the primary tumour, then we would not grow the tumours to the larger size that some of the metastasis experiments require. Regular monitoring by BRF staff is in place and we strive to keep updated with the latest environment improvements, such as enhanced environmental stimulation.

	Surgical procedures will be performed with suitable anaesthesia and animals monitored post-surgery to ensure that they recover well. Where appropriate we will also use suitable analgesia. An example of our commitment to refinement is that we were the first group in the UK to implement an improved design of imaging window.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We are aware of NC3Rs, and ARRIVE guidelines. We also discuss with colleagues in other research groups new improvements that lead to refinement.

Project	**8. Analysis and Therapy of Neuromuscular 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
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the work carried out under this licence is to develop an effective therapy for Duchenne muscular dystrophy and to find the genes responsible for other neuromuscular diseases so that treatments can be found.	
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)?	DMD is the most common of all the neuromuscular disorders with a prevalence of 1 in 5000 male births. Current treatment is restricted to palliative care which has only modest benefits and is associated with a number of severe side effects. An effective treatment developed through this programme of work will improve the quality of life for DMD patients and their families.	

	Additionally, the clinical development of effective therapies for DMD is paving the way for the development of experimental therapies for other relevant muscle-related disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 17,950 mice over the course of the 5 year 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?	Animal models used in this programme of work are mostly mildly affected (~95%). The most likely adverse effect comes from the genetic modification found in a small percentage (~5%) of moderately affected animals. These animals show signs of muscle weakness, reduced mobility and disease progression similar to the human condition. Mice are monitored and scored to assess welfare and are treated with nutritional support where appropriate. Animals showing signs of deterioration are euthanized. Animals may undergo moderate procedures such as injection, imaging and repeated motor function tests which are minimally invasive and no significant adverse effects are expected. General anaesthetic may be used and any pain following procedures will be controlled with analgesics. Denervation and nerve crush should not result in lasting pain and reduced mobility, and the animals are closely monitored for any possible, rare adverse effects and treated with analgesics where necessary. If an adverse effect were to occur, animals would be euthanized.
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 mimic the action of a drug on muscle from cell line work alone because muscle in the body receives signals from the nerve in order to work properly and in general, the drugs for treatment have to be delivered by the blood stream. The regulatory authorities also demand that we have preclinical data in mice before going on to human clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of	Calculations are carried out to determine the necessary number of animals for each experiment, ensuring significance of results but also minimising the number of animals used in

animals	each study. Wide selections of tissue samples are taken from each animal used in a study to try to cover every eventuality and prevent having to use more mice in a repeat experiment. This ensures maximum use of every animal that we work with. Samples are put in long term storage enabling us to access them if and when required at any time in the future.
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 best model to use as mouse muscle is very similar to human muscle. The *mdx* mouse has a mutation in dystrophin and is a good model for DMD and for use in the development of therapies. It is only very mildly affected because mice can regenerate their muscles after damage much more easily than man can. The double knockout (dKO) mouse lacks dystrophin and utrophin and is a moderately affected model of DMD that better mimics the disease progression in man. However, due to this increased severity we minimise the use of this model, using the mdx mouse in preference where possible.

Project	**9. Analysis of genes involved in the Plasmodium life cycle**	
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)	Malaria is one of the most important infectious diseases affecting humans, with 219 million cases and nearly 0.5 million deaths reported in 2017. As we still lack an effective vaccine and with the recent emergence of resistance against the frontline antimalarial drug artemisinin, we urgently require new antimalarial drugs. For this we need a much better understanding of how infection leads to such a severe disease, and more systematic ways to identify and prioritise new drug targets. In addition, we aim to identify new drugs that kill the liver stage of the parasite, which could be used to as a prophylactic to prevent establishment of an infection after a mosquito bite.	

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 aims to gain a better understanding of parasite biology, in particular those aspects that are essential for parasite growth and transmission, in order to identify new drug targets. This work will therefore advance our knowledge of malaria and the knowledge gained may, in the long-term, lead to the discovery of new strategies for treating malaria, and hence contribute to reducing the burden of this devastating disease. Findings and materials will be made freely available to other scientists through REDACTED, publication in open access, peer-reviewed journals or on open access platforms, and presentations at scientific conferences and meetings. This will reduce duplication and increase the speed and impact on the whole malaria research community.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the next 2 years we anticipate to use an average of 1900 mice and 100 rats per year of which all will be wild type.
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 life cycle of the malaria parasite, both in the vertebrate host and in the insect vector is multifaceted and in order for the malaria parasite to complete its life cycle, we will require both the mammalian host (rodents) as well as the mosquito vector. In order to maintain our mosquito colony, these require regular blood feeds which are essential for female mosquitoes to produce eggs. The resulting mosquitoes will be used throughout the project as part of the different protocols. There are not adverse effects expected as mice do not show hypersensitivity (skin reactions) at the site of mosquito bites. All rats will be used in mild protocols only where large amounts of biological material is required and where parasite infection efficiency is critical. The majority of mice (~70%) will be used in mild protocols, where they will be used for the production, selection and analysis of wild type or mutant parasite lines with the aim to identify parasite genes that contribute to drug resistance, virulence, disease and transmission. Infection of rodents will either happen via intraperitoneal or intravenous injection or by exposure to mosquito bite under

	anaesthesia. In order to study the complete and very complex life cycle of the parasite, infected mice might be used to infect mosquitoes via mosquito bite under terminal anaesthesia. The remainder of mice will be used in moderate protocols where either the infection levels require to be higher in order to obtain sufficient material for the data analysis, when we require to image animals to monitor infections or where compounds are used in order to identify novel drug targets. Identification of drug targets using novel compounds will be initially performed in pilot studies on a small number of mice. Some mice can be at risk of potential adverse effects resulting from using novel compounds but this will be closely monitored and affected animals will be humanely killed if it exceeds the severity threshold. Pain relief will be given as needed according to a regime recommended by the vet. From our experience obtained over the last 5 years of research, we know we can monitor and predict infections by performing regular blood smears. Parasite numbers in the blood will be monitored routinely by obtaining small samples of blood using a heel pin prick to sample from the tail. Hence we know that the malaria infection itself does have a low risk of potential adverse effects, provided the correct strain of host and parasite are used. Parasites will be isolated for in depth analysis, genotyping, cryopreservation and cloning using a method that causes only momentary discomfort such as tail vein sampling. To select for edited parasites or to screen for active compounds against parasite infection at different stages, infected rodents will be treated with known antimalarials by the least severe route which may include administration in drinking water, injection (intravenous, intraperitoneal or subcutaneous) or oral gavage. Other agents administered might include haemostasis modifying agents or anemia inducing agents, for example to prevent blood clot formation when taking blood samples. In some cases we will need to image the parasite infections in vivo under anaesthesia using equipment available within the REDACTED which might require administration of light-emitting substances to enhance the contrast. All substances will be administered via the most suitable and least

	invasive route using a combination of volumes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. All animals will be humanely killed at the end of the procedures. The experimental protocols that we are using are well established and designed to cause the least pain, suffering and distress.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have access to in vitro cultured human malaria parasites in our laboratory which we seek to use as much as possible to replace the use of animals and ensure the relevance of our animal research for understanding human disease. However, malaria parasites of humans are not experimentally usable at many stages of their life cycle, including the liver stage, where there is a strong emphasis on the development of new antimalarial drugs. In addition, genetic manipulation of rodent malaria parasites is much more efficient than human parasites, which very significantly increases the speed of our work. Finally, mechanisms of infection and disease that involve complex interactions between pathogens and hosts cannot always be modelled in vitro, meaning whole organism studies are sometimes essential. For these reasons, we use rodents as they offer the best characterised and least severe whole-animal model for Plasmodium infections. We are, however, constantly working on developing new methods and are for example, currently in the process of testing a new protocol that would use commercially available blood sources for the maintenance of mosquito colonies. Until this protocol is established, we will use surplus, non-GM mice available from existing breeding programmes whenever possible to maintain our mosquito colony.
2. Reduction Explain how you will assure the use	The development of improved genetic systems for Plasmodium is a major aim for our research

of minimum numbers of animals	programme. Experimental groups, controls and sample sizes are based on data and results from previous experiments being performed over the last 5 years on a previous licence. As data generated on this new licence is an extension of the already existing data, experimental groups, controls and samples sizes stay unchanged. Our genetic screening approaches using pools instead of analysing single genes have already dramatically reduced the number of mice required to characterise the functions of a given parasite gene. For example, from our work so far on a previous licence we observed that, to assay 58 pools of mutant parasites (with 100 mutants selected at random and then pooled) each pool being screened in one mouse, including several types of biological and technical replicates to control for experimental variability, 174 mice were used. This generated 2578 mutant phenotypes which corresponds to over half of the REDACTED genome being covered. If we had used conventional techniques to study these genes, it would have used at the very minimum, 2578 mice, which in triplicate would require 7734 mice. Our approach has therefore saved, at a conservative estimate, >7500 mice. We constantly make further improvements that increase the number of parasite mutants we can study using a single mouse. Additionally, all mutant parasites and the data resulting from these screens are entered into the REDACTED database pre-publication and are made freely available to avoid duplication of research. Based on this data and from experience obtained over the last 5 years, all screening experiments will be performed using the same experimental setup wherever feasible (pools of 100 mutants selected at random including controls that have been the same across all previous experiments and are used as reference to control for variability) and using biological triplicates as well as technical duplicates with the reasoning that if we lose one data point (mouse) due to, for example a failed infection, we still have two remaining data points (mice) which is sufficient for our data analysis and hence means that we do not need to abort the entire experiment. If we would

	reduce to duplicates then the loss of one data point (mouse) would mean the experimental data generated is not reliable enough and hence it would result in wasteful usage of mice. As we have never lost 2 data points (mice), using 4 mice per experiment would again be a wasteful usage of mice. To control for variability, all experiments include either intrinsic controls of known genes (which if feasible are the same that were included in previous experiments for consistency) or mock infections. In experiments where large amounts of biological material is required and where available biomass and transfection efficiency are critical, rats will be used in preference to a large number of mice, thus reducing the overall number of animals used. Additionally, we are currently working on reducing the number of rats required by improving the transfection protocol which would mean less starting blood culture is required. Data generated from the different steps will be constantly analysed by an existing computational pipeline, uploaded to our **REDACTED** webpage and will inform the design and execution of any following experiments in order to reduce the amount of animals being used for each experiments. Pilot studies will be performed whenever required for example to determine the optimal time points for imaging or to test and validate novel antimalarial compounds
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 seek to minimise any adverse effect of infection by choosing the parasite-host system with the least severe adverse effects but which still provides a robust scientific answer to a particular important question. We will be using rodents because they are physiologically the most well defined animal models for malaria. For different scientific questions we use a range of protocols with different severity limits, monitoring regimes and different carefully defined endpoints to ensure the welfare costs

	are minimised. We are able to monitor infections closely and in the vast majority of animals, studies will be concluded before malaria symptoms occur. We have also refined our method to obtain blood samples for monitoring infections, and are now able to use a heel prick pin to puncture the tail and only draw one drop of blood instead of performing a full tail bleed which tended to cause more tissue damage. All procedures are reviewed and optimised regularly by the animal training team.

Project	**10. Animal Models of Respiratory 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
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To identify effective new drugs for the treatment of chronic respiratory diseases such as asthma and Chronic obstructive pulmonary disease (COPD). To achieve this objective some basic research is required to further our understanding of these diseases. Potential new drugs will be tested in rodents in which disease or aspects of disease have been generated. Existing treatments for asthma (e.g. corticosteroids and bronchodilators) only relieve the symptoms of disease, without affecting disease progression. Furthermore, corticosteroids are ineffective in approximately	

	10% of asthma patients. The use of corticosteroids is also limited by undesirable side effects. Current treatments for COPD are largely ineffective and incapable of halting disease progression. Therefore new, effective medicines are needed to treat patients with these severe respiratory diseases.
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 is expected to identify novel medicines and along with a better understanding of the fibrotic disease process in diseases such as asthma and COPD, which will lead to new medicines to treat patients and improve their quality of life.
What species and approximate numbers of animals do you expect to use over what period of time?	Only rodents (mice and rats) will be used in this project. It is anticipated that less than 1500 rodents will be used each year for the 5 year duration 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?	Reproducing aspects of human respiratory diseases may lead to those animals experiencing some signs of the diseases such as lethargy and breathlessness on exertion. In the majority of cases this is anticipated to give rise to no more than mild discomfort, with a small minority of animals experiencing moderate discomfort. Animals may undergo procedure involving injections. For all procedures anaesthesia will be used where appropriate and animals carefully monitored to ensure no animal experiences discomfort exceeding moderate. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon. At the end of each experiment all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present, there is no alternative in vitro technology that replaces the need to use animals since there is a requirement for all components of an immune response must be present within the systems used to investigate novel drugs to better predict the effects of that
	potential new treatment in man

2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure the fewest number of animals are used, only the most effective drugs that have been pre-screened for activity in vitro will be examined in animals. Our experience with the experimental protocols will be applied to ensure appropriate group sizes are used to identify statistically significant differences between groups, whilst minimising the numbers of animals undergoing the protocol. Group sizes are constantly reviewed and experts in statistics are consulted to ensure the minimum numbers of animals are used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are the mammalian species of lowest neurophysiological sensitivity in which these fibrosis models have been developed. Several of the animal models in this project are well established both in-house and within the literature and have been shown to model different aspects of human disease. As our understanding of human disease increases animal models will be continually reviewed to ensure that they are relevant to human disease. Selection of the most appropriate model to use to study a particular process in disease will be based on prior knowledge of that model. All procedures have been ethically reviewed and all animals undergoing procedures are monitored closely by trained staff that work closely with a veterinary surgeon. In addition, distress scoring sheets are used to monitor disease severity and these are under constant review to ensure the correct level of disease is achieved with minimum stress to animals. Humane endpoints are employed to limit suffering and disease burden. Refinements to disease models are continuously assessed and applied where appropriate
Project	**11. Anorexia and cachexia in ageing 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)	About 50% of cancer patients suffer with loss of appetite (anorexia) and wasting disease (cachexia), which can worsen the chances of a successful outcome. This proportion rises to 80% in terminal cancer patients and is considered as the immediate cause of death in a large proportion of these individuals. However, anorexia and cachexia are associated with a range of other types of disease related to inflammation (e.g. colitis and irritable bowel syndrome) or infection (e.g. influenza or parasitic worms). Furthermore, anorexia can also be aggravated by some of the treatments that are used to combat disease, notably cancer chemotherapy. This project aims to understand what the causes of	
	disease-related anorexia and cachexia are. We believe that parts of the brain which respond to natural toxins found in some foods may be responsible for reductions in appetite, while the causes of wasting may be similar to those experienced with natural ageing.	

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 laboratory is well placed to make a major impact on understanding the brain networks that may be behind anorexia and cachexia, as we have a long-term expertise in similar networks which control appetite under healthy conditions. We therefore have available the necessary models and tools to carry out the research effectively. By separating different mechanisms which modify food intake and body composition, we may be able to develop new interventions to bring benefit to those suffering with disease without affecting normal appetite and tissue metabolism.
What species and approximate numbers of animals do you expect to use over what period of time?	Although the brain's wiring is complex, it is very similar between humans and mice. This gives us the opportunity to use mice to understand normal and abnormal brain function. In fact, the breeding of genetically-modified mice, in which we can introduce transgenes (changes in the mouse genes or the introduction of the human genes) that control normal physiology, has massively accelerated our understanding and our ability to target diseases with drugs. Our techniques are minimally invasive and we need far fewer experimental animals than in the past in order to progress knowledge. We expect to use around 4400 experimental mice over a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The transgenic mice we use tend to grow and behave in the same ways as normal mice, both in health and disease. Normality is very important, because if a mouse is strikingly different or behaves abnormally, it is unlikely to be very helpful in understanding physiology. Very often we will need to induce illness in our mice, so that we can model cancer, inflammation or infection. However, we do not want our mice to become too ill, since this would mask the things we are trying to study.

	Therefore, we are collaborating with experts in each type of disease who are very experienced in studying mouse models. To reduce stress, we like to handle our mice (often daily) in order to get them used to being picked up. This means that, when the time comes for an experiment, we can give them an injection (either under the skin, into a vein or directly into the brain) without them hardly noticing. We can also do a range of physiological tests on the mice, sometimes in their home cages, but often after acclimatising them to other cages. Thus, we might put them in a scanner to see how much muscle and fat they have, or measure their metabolic rate. Occasionally, we even train our mice to poke their noses into holes to break an infrared beam or to press a little lever, which provides them with a sugar reward. This can tell us whether they have lost their motivation to eat or if they are suffering with anxiety-like symptoms. Invariably, the parameters we measure are much simpler: for example, how much food do they eat or how much do they weigh. Often, we need to do surgery on our mice in order to manipulate how the brain responds to diseases or drugs. In this case, we carry out the surgery with the mice under general anaesthetic, plus we give the mice pain killers and sometimes local anaesthetics, to make sure that they do not feel any pain. The mice recover very rapidly, so they can be returned to their home cages to carry on living as usual. At the end of our experiments, all the mice are killed humanely. We can then harvest tissues post mortem and continue gathering useful information.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although it is difficult to study healthy behaviour or disease in anything other than a living mouse, we still find out a lot about brain cells by studying them isolated from the rest of the body. We have to kill the mice humanely, but this allows us to take slices of brain and put them in a dish. We can then record the minute electrical activity of individual brain cells. To enable us to identify the right cells in

	the complex brain, we have bred transgenic mice in which specific cell types glow fluorescently under our microscopes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For an individual experiment, data provided from similar studies in the past or from pilot studies, allows us to make precise calculations of the minimum number of animals we will need to provide robust results.
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.	With between us, over 75 years of experience working with mice, our lab members have greatly improved the way in which we carry out experiments. One major advance has been the use of remote radiotelemetry. This is where, during surgery, we implant a small radiotransmitter under the skin or in the abdomen of the mouse. Later, these devices allow us to monitor things like body temperature, blood pressure and brain activity, which tells us how the mice are “feeling” without having to disturb them. We now use transgenic mice to identify, control or record the activity of individual cell types in the brain. This allows us to determine how different cells respond to diseases or drugs and how they communicate with each other, without using the very invasive old techniques. Since we can manipulate the mice while they are still in their home cage, we can record their behaviour, whether they are secreting hormones, or if their metabolism is changed, with minimal disturbance. To do this we breed mice that have so-called “designer receptors” expressed in just a single cell type. The designer receptors lay dormant and the mice behave as usual. But, by then giving the mice a “designer drug” or by shining a light through an optic fibre, we can activate or inhibit selective brain cells, while studying changes in behaviour or physiology. All the time, our techniques are improving and our equipment is miniaturising, so it is now even possible to see and record the activity of specific brain cells in freely moving mice, using tiny camera lenses attached to the mouse’s head.

Project	**12. Antibody production in rodents**	
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 purpose of this licence is to produce antibodies in the bloods and tissues of rodents, and supply these bloods and tissues to research establishments that lack the expertise, capacity or facilities in their own establishments to conduct the work. Requirements for the antibodies are specific to each research program, and may support a broad range of objectives, specific to the client. For example: the characterisation, location and expression patterns of new proteins identified during research, creation of targeted medicines or development of new diagnostic assays. Antibodies will only be produced if they do not	

	currently exist, or those that do exist are of poor quality.
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 provision of these services under this licence will allow important programs of scientific research to progress more rapidly, and thus increase the speed at which new potential therapies for human and animal disease can be identified and developed. The provision of this type of centralised service is beneficial because it facilitates the development of a high level of expertise in the staff performing the service.
What species and approximate numbers of animals do you expect to use over what period of time?	The numbers of animals used is not expected to exceed 4000 mice and 1000 rats over 5 years and will either be bred from or supplied for studies.
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 authorises the immunisation of rodents to produce antibodies for future research. Most of the rodents are expected to experience no more than mild clinical signs. A small number may develop clinical signs due to adverse side effects of the antigens, which may be controlled by special care, veterinary treatment or by killing the animal if it appears to be developing adverse effects which are worse than predicted. Anaesthetics will be used as advised by a veterinary surgeon. At the end of the immunisation protocol the majority of rodents will be humanely killed prior to tissue and blood collection. However, on rare occasions there may be situations where collection of samples in dead animal would not be compatible with the research objectives, or where this would increase the total number of rodents used on a project. In these circumstances, where there are reduction benefits, collection of bloods and/or tissues under terminal anaesthesia may be used. For example, where sterile collection of blood for polyclonal antibody production requires maximisation of total blood collection from each rodent, collection of blood under terminal anaesthesia at the end of the protocol will allow for a reduction in total number of rodents used. A small number of rodents may be shipped to a bone fide establishment inside or outside the UK for continued use for example where transit time for transportation of terminal samples could compromise the quality of the
	specimens.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No effective method for producing antibodies commercially without the use of animals is available. Cell culture techniques which allow the production of antibody fragments do exist; however such techniques are still experimental and have an uncertain yield, efficacy, and antibody function. Tissue studies and computer simulations can assist in scientific research, although these procedures do not produce the bio-active antibodies that can be used to detect a wide array of proteins. Consequently, animal use is essential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Working with customers and colleagues, we intend to continually assess the appropriateness of the techniques and equipment utilised for these protocols. Accurate, standard and concise collection of rodent information and program requirements with data will be sought from clients, suppliers and standard databases. Under this Licence, it is our responsibility to ensure that clients have given consideration to the use of non-animal alternatives and identifying the most appropriate reduction strategies for research work. This will be achieved through the regular assessment of web resources (for example http://www.frame.org.uk and http://www.nc3rs.org.uk/), review of relevant journals, client meetings, attendance at regular industry meetings and symposia (for example LASA) and through the review of internal data. We will continue to review our facilities, training and processes in response to information on best practice in the use of animals in scientific procedures.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will	Mice and rats will be used. Rodents are the lowest vertebrate group in which antibodies can be produced. Newer approaches, such as phage display libraries (the key advantage of phage display is that it provides a means to identify target-binding

take to minimise welfare costs (harms) to the animals.	proteins from a library of millions of different proteins without the need to screen each molecule individually) and transgenic mice, have proven much more successful in generating fully human antibodies, and there is every indication that continuing advances in these protocols will make the process more refined with improved outcomes. Working with customers and colleagues we intend to continually assess the appropriateness of the techniques and equipment utilised for these protocols. We will continue to review our facilities, training and processes in response to information on best practice for antibody production for continual refinement. Complete records of the health screening and welfare observations will be maintained. The quality will be assessed via retrospective review. Rodents will be observed appropriately to ensure they are maintained to humane endpoints as detailed in this licence. Our staff undergo their own continual professional development programmes and keep abreast of new developments which may facilitate refinement by attendance at meetings and conferences and by following the activities of organisations such as the NC3Rs.

Project	**13. Antibody production**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To raise polyclonal or monoclonal antibodies in camelids, cows, sheep, rabbits, guinea pigs, goats, chicken, mice, and rats that will provide benefits de-tailed in the box below. Antibodies produced will be available to academic collaborators and to industrial clients to further their work.	
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)?	Production of entirely new antibodies to scientific targets of interest that can be used as a) tools to ad-vance basic science, b) contribute towards new drug discovery and development, c) development of clinical diagnostic tools and d) provision of tools for industrial clients to benefit	
	UK economy	

What species and approximate numbers of animals do you expect to use over what period of time?	Within 5 years: Llama 80 Camels 20 Cow 200 Sheep 75 Rabbit 50 Goat 25 Guinea pig 25 Mouse 200 Rat 200 Chicken 100
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 injected with an immunogen via a peripheral site with optional booster injections and test bleeds to determine if antibodies have been produced before antibodies are harvested using a method appropriate to the species used (e.g. Schedule 1 termination, exsanguination under terminal anaesthesia, final blood sampling from a peripheral vein) Granulomas and sterile skin abscesses may occur but the protocol is designed to minimise this possibility (<1%). There is very small chance (<0.5%) that the immunogen may cause an adverse systemic effect. Overall, antibody production is of mild severity; if harvesting of bone marrow occurs, the protocols used have a moderate severity. Immediately at the end of antibody production, animals will be humanely killed by a Schedule 1 method with the exception of camelids, cows, goats and sheep that may be re-used or re-homed.
Application of the 3Rs	
1. Replacement	An intact immune system only available in a live,

State why you need to use animals and why you cannot use non-animal alternatives	host animal is required to generate highly specific antibodies, thereby necessitating the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals required to ensure successful production of an appropriate amount of antibody will be used. We have found that typically only one animal is required to successfully produce specific antibodies. Thus, only one animal will be used in each attempt to create a specific antibody before an additional animal is used if the initial attempt is unsuccessful. In the case of llama, cows, goat and sheep use, the long lifespan coupled with the mild nature of anti-body production supports re-use without compromising welfare which will reduce overall number of animals used.
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 all cases, the lowest order species possible for required use will be used. Suffering will be minimised by sequential injection of immunogen in successive animals in order to reduce the harm caused if any systemic adverse effect is caused. Careful consideration will be given to injection volumes used, needle gauges used and blood volumes drawn Strict criteria determining suitability for re-use of camelids, cows, goats and sheep will be adhered to. Clearly defined endpoints for all procedures prevent the possibility of undue suffering and minimises any necessary discomfort experienced by the animals. Once a previously unavailable antibody has been produced, the use of in vitro or synthetic techniques to produce further antibodies will be undertaken where scientifically possible.

Project	**14. Anticancer effects of naltrexone and cannabinoids**	
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?	Our overall aim is to improve treatments in cancer patients; to this end we have explored the ways that tumours can be killed by using novel therapies. Although these agents have activity given as single agents, studies have shown they can be used in combination with other therapies resulting in improved activity overall. Furthermore, the potential benefit of these combination approaches is maintaining overall activity but by using smaller amounts of each drug.	
Why is it important to undertake this work?	We believe that these studies will allow us an improved insight into how drugs such as low dose naltrexone can be employed in treatment regimens used to treat patients with cancer. In particular, understanding the way in which these agents can be employed most effectively in combination with other treatments is	

	particularly useful, as this will help in terms of developing novel therapy approaches.
What outputs do you think you will see at the end of this project?	We should better understand ways of altering treatment schedules to optimise the activity of drugs such as low dose naltrexone. In particular, the studies will help us identify new drug combinations involving low dose naltrexone. This would be brought about by our improved understanding of the mechanisms of action for naltrexone, which will help us determine drugs with complementary mechanisms of action. We expect to document these results and publish them for peer analysis. This should ultimately help bring to patients new treatment options.
Who or what will benefit from these outputs, and how?	We believe that these studies will help to identify the treatment schedules with greatest activity. These information would subsequently help in decision-making regarding the best ones to take to human trials. Once sucessfully completed, new treatment options may be made available to patients.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We will aim to publish results in peer-reviewed journals to disseminate our findings to the wider scientific and medical community. We also discuss work to the public in ocassional public seminars, to ensure that the work is shared.
Explain why you are using these types of animals and your choice of life stages.	Although some of these agents have previously been shown to reduce tumour size in certain xenograft models, these agents have not yet been studied in combination with standard anticancer therapies in syngeneic in vivo tumour models. Our in vitro data already shows potential synergistic interactions between these agents and conventional treatments, and for that reason we believe it is now important to study the same combination using in vivo models which will allow us to understand better the therapeutic benefits of these interactions. In addition, this mouse model would be useful for unravelling the toxicity of such drugs to normal tissues.
Typically, what will be done to an animal used in your	The model will involve implanting tumour cells into the flanks of mice. Once established, treatments can begin,

project?	and the approach will allow us to i) examine drug action in a cancer type expressing the proteins that we predict to be most receptive to the treatments, and ii) assess treatment in situ; something that cannot be done using in vitro models. Our intention is also to assess further the interactions underlying any improvements in these combination approaches. We will be able to track the growth of the tumour in situ, and assess the effects that the combination therapies may have on it. Analysis of the tumour post-mortem will also provide us with valuable information regarding the effects that treatments have on the molecular pathways that underlie the disease, and help guide further work into developing better combination therapies. We plan to inject tumour cell lines sub-cutaneously into a mouse, and after approximately 10 days, we will assess for the presence of a palpable tumour. In those mice with detectable tumours we will then deliver the treatment agent (e.g. low dose naltrexone) and monitor them frequently for any signs of distress, responding where necessary. A second round of treatment will also be included, which will allow us to assess the effect of combining another drug. After assessing any changes to tumour volume at the end of the experiment, mice will be humanely killed, and tumour material and organs will be removed for assessment of effects that the treatments have had on them. This will allow us to maximise results that we can get from each experiment.
What are the expected impacts and/or adverse effects for the animals during your project?	As with these models, the tumour that is implanted into the flanks of the animal may cause some discomfort; for this reason, we will be using as small a volume as possible, as specified by local rules. These volumes will not exceed 10 ml/kg. Although we do not anticipate any suffering as these methods have been used routinely in the past with minimum problems, we will monitor the animals for any undue signs of distress. Humane endpoints will be determined on the basis of adverse clinical signs. Any animal that shows deviation from normal health, such as piloerection, hunched posture, inactivity or inappetance, will be monitored more frequently, and supportive treatment supplied, such as warming or wet diet. Should signs persist for 24 hours, the animal will be humanely euthanised.
What are the expected severities and the	We do not expect to adverse effects; however, the severity of any would be moderate. We are aware that

proportion of animals in each category (per animal type)?	the a number of regulated procedures may accumulate resulting in the possibility that the severity taken as a whole is moderate. The possible proportion of mice experiencing these adverse effects would be <5%.
What will happen to animals at the end of this project?	used-in-other-projects, killed
Why do you need to use animals to achieve the aim of your project?	Our in vitro data already shows potential synergistic interactions between the novel agents we are studying and conventional treatments, and for that reason we believe it is now important to study the same combination using in vivo models which will allow us to understand better the therapeutic benefits of these interactions. In addition, this mouse model would be useful for unravelling the toxicity of such drugs to normal tissues.
Which non-animal alternatives did you consider for use in this project?	We have used a range of laboratory-based models, but these do not capture the complexity of tumour-microenvironment relationship seen in animals. These models have included combination models such as the median-effect analysis to try and understand the potential of drug-drug interactions. We have also sought to understand the effect that the drugs have on cell lines at a cellular and molecular level, and have identified key targets that are altered by treatment that can lead to enhanced anticancer action. We have used these to refine treatment schedules, however, it remains unclear what the drug-drug interactions will be like in an organism. Similarly, how these drugs interact with the microenvironment of the mouse is unclear. We understand how the drugs interact with immune cells through our in vitro bloo-based studies, but it is unclear how efficacious these treatments will be in a live organism.
Why were they not suitable?	The data generated from models in the lab lack the interactions between tumour and the microenvironment, thus, information is incomplete.
Enter the estimated number of animals of each type used in this project.	mice: 400
How have you estimated	The proposed work has been discussed by senior

the numbers of animals you will use?	members of the research team, and the numbers of experiments required to reach statistical significance has been discussed. Generally, the number of experimental groups has been kept low to ensure that any statistical calculations that are subsequently performed to understand our results are substantially significant. As different levels of signficance can be applied, we plan to use one that ensures any difference in the treatments examined is a result of a true effect rather than one that may occur at random. Exact numbers of animals for each group may vary; however, we will monitor these numbers on a weekly basis to ensure the lowest number of animals is used to draw adequate conclusions. For each and every experiment, as part of good laboratory practice, we write an experimental protocol that is discussed with members of the research team and animal care staff. This ensures that we work to specific objectives with clearly defined hypotheses. This ensures that only work that is necessary to draw conclusions are performed. The team also meets regularly, and experiments covering such matters as experimental strategy and analysis of results are discussed. We have also used the NC3Rs' Experimental Design Assistant to perform calculations to estimate the numbers of animals required for each experiment and treatment group.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	We have used the NC3Rs' Experimental Design Assistant to perform calculations to estimate the numbers of animals required for each experiment and treatment group. This ensures that the number of animals used in the current study is appropriate.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	Preliminary lab-based studies will allow us to establish the optimal concentration of each of the test agents to be used in our studies. Prior in vivo studies have already identified the optimum amount of cancer cell to be injected into the animals to maximise the chance that tumours will reach the desired sizes for our studies. These tumour sizes have to be big enough for changes to be detected that may be caused by the treatments studied. Ths size needs to be balanced by the possible discomfort animals may feel. These tumours are always used freshly resuscitated from frozen, and as tumour cells can alter their characteristics if left to grow in the laboratory for too long, they will be discarded after around 4-5 weeks. This ensures cells are consistent in

	character and are at ideal growth state, which increases the chances that they will be taken in the animals.
Which animal models and methods will you use during this project?	The experimental approach employed in this study relies on the successful formation of tumours, which allows for a more realistic assessment of whether the combination strategy will affect tumours in situ. For this reason, experimental models involving the implantation of human cell lines into mice will be employed to understand the effect of treatments in human tumours. We also plan to use other models that involve mouse tumours growing in mice. This is different to the earlier model described, as the study will involve animals with a competent immune system. This immune system may play a role in determing overall drug activity, and provides more clues as to the optimum way treatments may be improved. Two members of staff will be involved in the implantation of tumours to minimise animal suffering as well as employing precise, regimented introduction of the tumour cells to the animal which increases reproducibility and statistical significance. This will allow for minimal numbers of animals to be used. Through regular assessment of the tumour volume we will also allow be able to monitor tumour development and animal wellbeing and ensure animal suffering is kept to a minimum, this will be guided by ensuring the dimeter of tumours do not exceed 1.2 cm.
Why can’t you use animals that are less sentient?	The interplay/interactions between tumour, immune cells and the microenvironment means these models are the most appropriate.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Regular meetings with the research group as a whole, and discussions with the staff at the REDACTED. We also have access to the 3Rs’ newsletter and publications.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	Regular meetings with the research group as a whole, and discussions with the staff at the REDACTED.
What published best practice guidance will you follow to ensure experiments are conducted	We are supported by the staff at the REDACTED, and refer to the guidelines as published by Diehl in 2001 and Workman in 2010 (Diehl 2001: Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, van de Vorstenbosch C; J Appl Toxicol. 2001 Jan-

in the most refined way?	Feb;21(1):15-23; Workman 2010: Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA; Br J Cancer. 2010 May 25;102(11):1555-77.).

Project	**15. Anti-cancer therapy validation**	
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)	There are 360,000 new cancer cases in the UK every year. Advances in treatment, early detection and diagnosis have resulted in significant improvements in outcome for many cancer patients. However we still observe 164,000 cancer deaths in the UK every year and there remains a pressing need to develop new, improved treatments. Our research focuses on testing new cancer therapies that target the tumour microenvironment often in specific combinations where we hypothesise this would maximise benefit. Currently we lack understanding of how to effectively use these therapies to greatest effect because we do not understand the tumour biology underpinning response. We aim to evaluate approximately	

	10 new approaches for cancer treatment providing data that would enable decision to be made as to whether the approach is likely to be beneficial for patients. We will generate data to: 1. show how effective the treatment is against the cancer models used 2. understand how the therapy is working 3. identify potential ways to monitor response that could be used in patients 4. confirm that the benefits of the treatment would outweigh potential toxic effects
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. We will learn how new therapies work in models of cancer. 2. We will learn how we can monitor the effects of the therapies using approaches that can also be used in patients 3. We will be able to ensure that beneficial effects on tumours are not confounded by negative effects in other tissues that would affect quality of life in patients We anticipate that as a result of this work, 1-3 new strategies will progress into clinical trial and that we will also have provided sufficient information to stop progression of therapies unlikely to be of benefit in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	The studies will use mice. We anticipate the use of 3300 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the vast majority of studies, we will establish tumours within the animals. These may be implanted at superficial sites or may be introduced into specific organs to mimic clinical disease using surgical techniques. In the latter case we use refined, sterile procedures to reduce any chance of infection or poor recovery. We may track how the tumours are developing using imaging approaches that can also be used in patients. Cancer growth within an animal can cause detrimental effects. We monitor weight and specific behaviours in the animals that would indicate pain or distress and have well designed endpoints to minimise any suffering. Animals will be dosed with therapies, alone or in combination. There is a

	risk that we may observe toxicity with theses treatments. ,To mitigate this, we use therapies at doses that have previously been shown to be at well tolerated doses using the route of least severity and the minimum number of doses to produce an anticipated anti-tumour effect. We will monitor closely how well the therapy is working and whether it is causing any side effects. If the monitoring described above indicates toxicity, we reduce therapy dose and/or stop treatment. All procedures are moderate. We will not allow tumours to grow bigger than 1.25cm3. At the end of experiments animals are will then be humanely killed and tissues may be taken for further laboratory tests.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible, we gain information of how the therapy might be working using cell lines and/or computer aided approaches. However we are not yet able to model in the laboratory everything that can happen within the whole animal (person) when a cancer therapy is given. This necessitates the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	1 We use as similar animals as possible to reduce inherent variability, improve experimental consitency and confidence in outcome findings. 2. We start treatments when tumours are the same size which offers a substantial reduction compared to starting all treatments on a designated day with tumours of variable size. 3 We focus on experimental design to ensure that we have the best chance of gaining consistent data that we are confident in, using the lowest number of animals 4. We frequently use imaging that allows us to make multiple measurements in the same animal
3. Refinement Explain the choice of species and	1. We use rigorous monitoring processes to ensure we minimise welfare costs to the animals. We have developed health score

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.	systems over many years that provide a more holistic overview of potential harms we are causing and how and when to intervene such that harms are minimised. 2. We consistently refine our surgery techniques to aid recovery and have introduced revisions to pain management to improve welfare. 3. We will use behavioural testing which allows us to identify early detrimental changes in brain function which can occur as a consequence of some cancer therapies and is of huge burden within cancer patients.

Project	**16. Aquatic models of human 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
	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)	Motor Neuron disease (MND), multiple sclerosis (MS) are primarily adult onset neurological diseases that have devastating consequences on the life of patients suffering from these chronic lethal diseases. There is a great lack of knowledge on the causes and the mechanism of disease process and most importantly few disease modifying therapies to help these patients. Although MND and multiple sclerosis results in the loss of specific neurons, the exact mechanism or processes by which neurons die is unknown even where the exact genetic mutation that leads to the disease have been identified. Additionally, good models of disease that show many aspects of human disease and	

	that are appropriate for to study new treatments is lacking. The goal of this project is to develop fish models of disease that closely resemble the human conditions. The genetic models of human diseases developed will be used to determine how and why the disease forms. Additionally, we will use the zebrafish model to identify early changes in disease to help us understand what a healthy cell needs. The eventual goal of this study will be the use of these models to identify and develop drugs to treat these diseases.
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 fish models complement rodent models and provide a more rapid and high throughput system to study human neurological diseases. Due to their small size, transparency, rapid development and ageing, they can be used in screening drugs and treatments; in addition to uncover disease mechanisms. We will use advanced imaging and gene alterations to observe more easily small changes in the models of disease compared with the animals that do not show any signs of ill-health. This approach will provide an opportunity to develop a better understanding of disease and how we may treat to lessen the condition.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use Danio rerio (zebrafish) and to test the disease models and plan to use approximately 56000 zebrafish in our studies over a period of 5 years. Many of the animals generated for the study will be utilized just past protection (6-15 dpf) and a smaller fraction (20000) would be used for older age studies.
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 we are primarily studying ageing related diseases, many of the phenotype we observe would mimic ageing and thus of marginal consequence to the health and welfare of the animals. Most of the animals in these studies will have no visible symptoms with a fraction of older animals showing moderate symptoms with minimal impact on their ability to obtain feed and move around. When studies are completed on the animals, they are humanely killed.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Neurological diseases are complex diseases that require complex systems to study the disease process that occur during adult life. It is not possible to model the symptoms, pathology and behavioural changes observed in human disease in non-animal systems. Additionally, it is becoming clear that diseases such as ALS also are system wide disorders with changes in other organ systems such as the immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our goal in using fish in addition to studying disease process is to uncover early disease specific changes that occur prior to the onset of symptoms and hence, we try to identify early disease signs that can be analysed before they become protected (5.25dpf). This will allow great reduction in use of animals in research. Additionally, we use statistics to use the minimal number of animals to obtain robust data.
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 fish to understand the diseases that we are studying and where possible we use these at a very early age to minimise distress and to identify new drugs that can lessen the disease. Zebrafish at this early age are transparent and therefore, we can use this to our advantage using colour markers that switch on or off depending how they react with the new drug. We use appropriate pain relief and anaesthetics if required and again to minimise any discomfort for the fish.

17. Arginine methylation and tumourigenesis

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Arginine methylation, tumourigenesis

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

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?

Cancer is still a major cause of morbidity and mortality in the UK with more than 150,000 deaths a year. Therapeutic regimens have remained unchanged for decades, often involving the targeting of the hyper-proliferative cancer cell through inhibiting DNA and RNA replication. However this approach has many unwanted side effects as normal dividing cells are also targeted. Hence, there is still a continuing need to understand cancer at a molecular level.

Our laboratory is interested in a family of enzymes that have been highly implicated in cancer development and growth using cell culture based approaches. This is important, as these proteins could potentially be targeted for small molecule design in the development of novel anti-cancer therapies. We now want to understand the significance of these enzymes for cancer development, growth and metastasis in a living animal. To do this we will address the following objective:

We will determine the effect of altering enzyme expression on tumour development in a living animal, and the biological mechanism by which this occurs.
We will understand if and how these enzymes contribute to normal and cancer stem cells (CSCs) function.

We will determine how these enzymes contribute to metastatic disease.
We will establish if altering the expression of these enzymes synergise with known chemotherapeutic agents in reducing tumour growth in a living animal.

Potential benefits likely to derive from the project, for example how science might

be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

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

The primary potential benefits relates to new knowledge about the initiation and progression of tumourigenesis. This will be highly beneficial to both the academic and pre-clinical communities. To achieve this is a timely manner, we aim to disseminate our findings by publishing in academic journals and will do so according to the ARRIVE guidelines.

The enzymes we are studying have been recognised as potential drug targets. The knowledge gained in this project will therefore justify the further development of small molecule compounds that specifically inhibit their activity. In the long term, this could greatly benefit the lives of cancer patients.

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?

We will use approximately 10,000 mice over 5 years. Most of these will be in our breeding programme

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?

All experimental protocols have been prospectively assessed as being of moderate severity.

These include:

Injection of substances that promote gene deletion, enable imaging and measurement of proliferation, or chemotherapeutic agents

Blood sampling

General anaesthesia for imaging and surgical procedures.

Administration of hormones through the surgical insertion of a small pellet or a slow release device.

Engraftment of human and murine cancer cell lines

Surgical removal of the mammary fat pad under general anaesthesia

Some of the genetically altered mice we will breed will spontaneously generate solid malignancies and/or leukaemia's/lymphomas. However, as these are well-established strains, we will know from previous published studies the expected age of tumour development. To minimise adverse effects, animals will be closely monitored for signs of distress and killed immediately if this occurs.

We will end experiments at the first possible humane endpoint that enables the object of the experiments to be achieved with the least possible suffering. We will use suitable anaesthesia and analgesia under veterinary guidance. When required, we will conduct small pilot experiments to obtain information that will minimise the suffering of larger cohorts of animals.

After the animal has been killed, we will dissect all relevant tissues and perform extensive biological analysis. Tissue that has not been immediately analysed will be archived for future use by others and us.

Replacement

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

Genetic analysis of gene function has been essential for the development of novel therapies for disease, and generally involves the genetic modification of mice. The development of new technologies is now enabling gene editing of human cancer cells in vitro. However, normal development and the pathogenesis of cancer is incredibly complex involving the integration and interplay between numerous cell types. Very few, if any, in vitro cell culture based models are able to recapitulate this interactive environment. Hence, studies using murine models of cancer have been fundamental in enabling a better understanding of the processes that lead to cancer that could not have been achieved otherwise. As a direct consequence of this, new drug targets and insights into the molecular mechanism of cancer establishment and progression have come to light.

Reduction

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

In all experiments, appropriate animal cohort size that will enable the generation of statically significant results will be calculated through consultation with a statistician. When possible, experiments will involve a factorial design that will maximise the information obtained from a minimal number of animals. For example, non-invasive imaging will enable multiple measurements on the same animal over a period of time.
All strains not immediately required for scientific study will be cryopreserved as embryos.

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 are using mice to enable a genetic approach to understand how cancer develops and

thus identify novel targets for therapeutic intervention. Mice are the species of choice because there are a large number of widely available genetically modified strains that means that the function of most genes of interest can be studied. In most cases we use a system whereby we can study our gene of choice within a specific tissue through a process called “conditional genetic alteration”. By doing so, we minimise the effect of genetic alteration on the animal.

A second reason we have chosen to use mice is that the cancer models we will be using have been extensively characterised previously, and develop tumours with similar etiology and molecular profile as that observed in human disease. Moreover, tumour growth rate is predictable, thus fewer mice are required to be used for each experiment. Some of our studies will involve the generation of novel genetically modified animals, however during this process, all animals will be closely monitored for signs of distress and handled in the appropriate manner.

In experiments where we transplant cancer cell lines into recipient mice to determine metastasis formation and tumour growth after gene alteration or drug treatment, we will first manipulate cells to express a protein that permits non-invasive imaging. This will enable continuous monitoring of tumour progression in living animals, and hence reduces the number of animals we need to use. Moreover, this design offers the advantage of determining significant differences between tumourigenic growth potential of cell lines before the tumour volume exceeds the limiting size.

18. Arginine methylation and tumourigenesis

Project duration

5 years 0 months

Project purpose

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

Key words

No answer provided

Animal types	Life stages
Mice	adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

Arginine methylation is catalysed by a family of enzymes called PRMTs. PRMT expression and activity is elevated in cancer and interest in drug targeting PRMTs for cancer treatment has been gaining momentum. Understanding the genetic and pharmacological role of PRMTs and their methylated substrates for cancer development, progression and drug resistance is therefore required for future therapeutic advancements to become clinically viable.

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

Why is it important to undertake this work?

Every day, 31 people die of breast cancer in the UK equating to 11,400 deaths per year. Accordingly, breast cancer is the 4th most common cause of cancer death in the UK, accounting for 7% of all cancer deaths. Breast cancer is a highly complex disease with many subtypes that each require specific drug targeting. In many cases, therapeutic

regimens have remained unchanged for decades, often involving the targeting of the hyper-proliferative cancer cell through inhibiting DNA and RNA replication. However, this approach has many unwanted side effects as normal dividing cells are also targeted. The discovery of oncogenes and tumour suppressor protein and the concept that cancer arises from aberrant activity or expression of such proteins has led to a more targeted approach to rational drug design. This strategy has only been made possible by our increased understanding of the molecular mechanisms underlying tumourigenesis. Despite promising advances and the implementation of targeted based therapeutic strategies, cancer cells often develop resistance leading to relapse and mortality; therefore, there is still a continuing need to identify novel therapeutic targets for rational drug design.

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

The principle outputs from this project will be in the form of new information that will be disseminated to the pharmaceutical and academic community via high impact publication and presentations at conferences. We expect to show that genetic and pharmacological disruption of PRMTs, or their methylated substrates, alters mammary cancer growth providing compelling evidence that drugging PRMTs is a novel strategy for breast cancer therapy. We will understand which oncogenes co-operate with PRMTs in the tumourigenic process that will provide a molecular mechanism by which PRMTs facilitate cancer formation, and the significance of PRMTs in cancer stem cell biology. Given our already established links with pharmaceuticals that have led to active collaborations, our results could lead to phase I clinical trials using PRMT inhibitors for breast cancer treatments. Finally, we expect to validate the use of CRISPR/Cas9 technology as refinement in the conventional germline approach that enables genetic model of breast cancer in mice that will greatly decrease the number of animals wasted through complex breeding strategies, and make murine breast cancer modelling more readily available to the scientific community.

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

This project will greatly advance the biological understanding and impact of PRMTs on disease processes, and is most likely to be of interest to pre-clinical scientist interested in tumour biology. A second benefit will be the validation that modelling breast cancer in mice can be achieved with a much less complex approach, significantly facilitating the ability for researchers in the breast cancer community to investigate their gene of interest in a humane and cost-effective way. The third benefit is the pharmaceutical sector that are developing small molecule compounds targeting PRMTs. The forth benefit is to breast cancer patients. Our demonstration that genetic and chemical modulation of PRMT activity restricts tumour growth, in combination with a way in which to stratify which patients will respond to treatments, could lead to a change in clinical practice. Hence in the long term, a significant output could be the increased survival rate of breast cancer patients.

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

We are already collaborating with a number of prominent academic and leading industrial collaborators that will help facilitate the dissemination of our findings. We will present our findings in a timely manner at conferences and symposiums. If we are successful in modelling breast cancer using CRISPR/Cas9 technology, we will request funds to hold a workshop to demonstrate this technology to others that are interested. I am also actively involved in disseminating our research to supporters of CRUK through presenting our research and hosting lab tours, and whilst talking about animal experimentation can be a

contentious subject, I do believe that this provides an excellent opportunity to discuss the 3R principle and scientific studies involving animals in the UK to broaden perspective and challenge preconceptions.

Whilst it is still challenging to publish unsuccessful approaches, it is important that this information is disseminated to prevent serendipitous repetition. PLOS One is one respected journal that is highly read and will consider publishing negative results, and we will attempt to pursue this route if required.

Species and numbers of animals expected to be used

- Mice: 7600

Predicted harms

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

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

We use mice to enable a genetic approach to understand how cancer develops and thus identify novel targets for therapeutic intervention. Mice are the species of choice because there are a large number of widely available genetically modified strains that means that the function of most genes of interest can be studied. Moreover, they are able to grow human tumours as xenografts enabling drug testing and *in vitro* genetic manipulation before implantation. This enables us to mechanistically interrogate tumour growth, and hence identify new way in which to drug target.

We are predominantly using mice strains that are conditionally altered for our genes of interest. This means that genetic events will only occur in a specific tissue minimise the effect of genetic alteration on the animal. Whilst some Cre lines are predominantly active in adult tissue, some are switched on during embryogenesis, and this can lead to embryonic lethality if a gene of interest is critical for this process. To overcome this, we have developed a way of inducing genetic recombination in the adult mouse through intraductal injection of viruses.

A second reason we have chosen to use mice is that the majority of cancer models we will be using have been extensively characterised previously and develop tumours with similar aetiology and molecular profile as that observed in human disease. Tumour growth rate is predictable, thus fewer mice are required to be used for each experiment. Some of our studies will involve the generation of novel genetically modified animals, however during this process, all animals will be closely monitored for signs of distress and handled in the appropriate manner.

A number of our approaches require the growth of human cancer cell lines or PDTX, and this has to be conducted in immune compromised mice to prevent human cell rejection. We choose to use adult mice of a specific age range (5-7 weeks of age) to minimise variability.

In some cases we need to isolate cells that have undergone genetic manipulation, or can be induced to alter gene expression *ex vivo* through administration of 4-hydroxytamoxifen, for biochemical analysis. In this case, embryos are isolated from E13.5 enabling the

generation and culturing of mouse embryonic fibroblast. This provides us with a source of genetically altered cells in which we can conduct in vitro experiments, including mechanistic studies.

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

Many of our animals will be set up in breeding pairs enabling the generation of mice with specific genetic events that enables us to monitor tumour progression. Mice will undergo a maximum of 6 breeding cycles. In some cases gene deletion needs to be induced in the adult mouse, which can be achieved through administration of tamoxifen in case of the CRE-ERT2 system or via intraductal delivery of viruses. Once genetic recombination is induced, animals will be left to age and tumour development monitored closely. 1-3hrs before culling, administration of BrdU will enable proliferation rates of tumour to be determined *ex vivo*.

To determine the effects of genetic manipulation on normal mammary stem cell function, after genetic engineering, female mice will be inseminated through natural timed breeding and dams culled at specific time points.

To be able to switch on inducible genetic elements in cells that have been engrafted into mice, we will need to feed animals doxycycline food.

To generate a new way in which to model breast cancer, mice will undergo intraductal delivery of small guide RNAs/Cas9 that will enable CRISPR-mediated gene editing in the adult mammary gland.

When using human cancer cell lines or patient derived tumour material, we will need to implant these cells into immune compromised mice to allow tumour growth. If we are using an ER+ breast cancer cells then mice will require a minor surgical procedure to implant a slow release hormone pellet/minipump enabling cancer cell growth. In some cases, when studying breast cancer stem cells (BCSCs), we will need to conduct IVIS imaging to analyse the fate of BCSCs, involving IP injections of luciferin and general anaesthesia for imaging. To determine the role of the DNA damage response in our cancer cell, mice will undergo whole body irradiation and culled within 24 hrs.

An important part of this project is the assessment of small molecule inhibitors to target proteins, either alone or in combination with DNA damaging chemotherapies. Here, the route of drug administration depends on the specific agent, but could include multiple rounds of IP, intravenous or subcutaneous injection, or by oral gavage, or through the addition to the diet through drinking water or food. To check that chemotherapies are not adversely affecting mice, we will take tail vein blood samples to test for anaemia and leukopenia. In some cases, after a course of treatment, some animals will be allowed to age to see if tumours grow back.

For many of our experiments, 1-3hrs before culling, mice will be IP injected with BrdU enabling proliferation rates of tumour to be determined *in vitro*.

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

As many of the mice we are using will be genetically predisposed to generate tumours, or we will be engrafted with human/nouse cells that will form tumours as a xenograft, the

main adverse effects will be related to this. Potentially, this could include weight loss (maximum of 20%), a reduction in BC score of <2, reduced activity, failure to respond to gentle stimulation, lethargic, abdominal distension, jaundice, piloerection, intermittent hunched posture, diarrhoea, or intermittent laboured respiration. However, animals will not experience these effects for long, as when any of these signs is present, the animal will be humanely killed immediately.

It is possible that delivery of chemotherapeutic drugs will cause adverse effects. As the drugs we will use as well reported in the literature, we will be able to pay close attention to the development of any signs of distress. For example, anaemia and leukopenia are common side effects of chemotherapy, and we will be able to monitor the extent of this by tail vein blood analysis.

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

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

GEM: The majority of our strains are conditional; hence mice will have no detectable phenotype or severity. Mice used for breeding that are genetically predisposed to develop tumours will only be used for limited periods and at a young age to minimise tumour development. Together, we predict that only 5% of breeding animals will reach a moderate severity banding. In all of our tumour experiments, control mice are expected to generate tumours as we need to be able to compare our genetic events with that of a standard. We are expecting that genetic deletion of PRMTs will slow down or prevent tumour growth, however it is unlikely that this will happen in all animals. Animals that do not develop tumours will be classified as mild (as they have undergone at least one procedure), animals that do develop tumours will be classified as moderate.

Xenograft models: In all of our tumour experiments, control mice are expected to generate tumours as we need to be able to compare our treatment/genetic events with that of a standard. We are expecting that genetic deletion of PRMTs, or inhibitor treatment will slow down or prevent tumour growth, however it is unlikely that this will happen in all animals. Animals that do not develop tumours will be classified as mild (as they have undergone at least one procedure), animals that do develop tumours will be classified as moderate. All animals that are implanted with an ER+ xenograft will be classified as moderate as implantation of hormone pellets/mini-pump is a surgical procedure. Mice that receive cancer cells that do not require hormone supplements will only require a subcutaneous injection, which is classified as mild. However, as all mice are expected to develop tumours the overall severity banding will be moderate.

Intraductal injection: Animals will be classified as moderate as this procedure involves anaesthesia with recovery.

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

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you

have considered and why they cannot be used for this purpose.

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

Genetic analysis of gene function during the tumourigenic process has been essential for the development of novel therapies for cancer, and generally involves the genetic modification of mice. Very few, if any, *in vitro* cell culture based models are able to recapitulate the complex interplay between cancer cells and the tumour microenvironment. Indeed, studies using murine models of cancer that enable the modelling of disease within a complex multicellular living organism have been fundamental in enabling a better understanding of the processes that lead to malignancy that could not have been achieved otherwise. As a direct consequence of this, new drug targets and insights into the molecular mechanism of disease have come to light.

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

In vitro data obtained from cell culture approaches will always guide *in vivo* studies, and we always conduct cell culture based experiments to justify the need to use animals. These system include culturing cells in monolayer as a homogeneous population on plastic, and the more sophisticated 3D culturing of either cells lines grown in matrigel (as acinar) or mouse/human derived tissue as organoids. However, these models are not suitable to conclusively establish the importance of arginine methylation in tumour biology as mentioned in the above and below section.

Why were they not suitable?

There are significant limitations of basic culture conditions using cells gown in isolation as a monolayer on a piece of plastic as this is not an accurate representation of what occurs within a patient. Indeed, the reason why many new drugs fail between cell culture and *in vivo* studies is in the inability to full recapitulate the *in vivo* environment. Technologies are being developed to address this gap, including the development of 3D cultures (acinar cell line cultures and organoid mouse/human derived tissue). However, none of these model systems are yet able to phenocopy the integration and interplay between the numerous cell types that constitute the tumour and its microenvironment, or the fact that tumourigenesis occur in and is influenced by biological systems (such as the immune system).

Moreover, genetic manipulation of organoid cultures is still technically challenging.

Modelling cancer in mice is thus still required to fully understand disease progression and identify novel therapeutic avenues.

Reduction

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

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

When possible we will always use power calculations to determine estimate number of animal that should be used. For most quantitative experiments, animal cohort size will be calculated via two-tail power analysis (assuming alpha = 0.05 (probability of a type I error), beta= 0.2 (probability of a type II error). Expected effect size will be determined through consultation of the literature, cell culture based *in vitro* analysis or through small pilot experiments when possible.

Power calculations that we have already conducted equates as follows:

PRMT1 and breast cancer pathogenesis using GEM models: By analysing the effect of PRMT1 disruption in a Her2 (ErbB2) mouse model of breast cancer we have determined our effect size on the basis that 56.3% of ER negative human breast cancers display high levels of PRMT1 expression . Two-tail power analysis assuming alpha = 0.05 (probability of a type I error), beta = 0.2 (probability of a type II error) equates to a size of 20 experimental animals per genotype. This number is similar to that used by our collaborator examining the genetic requirement of other pro-tumourigenic proteins to statistical significance on the background of the NIC mouse (Lahlou et al. Breast Can Res 2012; Huck et al. PNAS 2010).

Effects of PRMT5 inhibitor on tumour growth: We have shown that shRNA-mediated depletion of PRMT5 in an established xenograft model results in the cessation of tumour growth (n=4) . We therefore predict that a similar number of animals will be required to see an effect after PRMT5 inhibition.

Limiting dilution analysis: Five different dilutions of unsorted cells (ranging from $5x10^3$ – $5x10^5$ cells) will be injected into six animals per group. Frequency of tumour initiatingcells will be determined by LCalc software.

CRISPR/Cas9 model: We will be the first to generate a murine model that uses AAV-mediated delivery of guide RNA sequences intothe adult mammary gland, however given that our model is based on the genetics of the *Blg-Cre;Brca1$^{f/f}$;Tp53$^{+/-}$*strain generated by Co-Investigator, we have determinedthe effect size to be 0.70 (based on the proportion of *Blg-Cre;Brca1$^{f/f}$;Tp53$^{+/-}$* alive after 365 days being 30%). Two-tail power analysis assuming alpha = 0.05 (probability of a type I error), beta = 0.2 (probability of a type II error) equates to a size of 19 experimental animals per virus injected, but predict that 22 animals per group is required to allow for unpredictable husbandry issues (e.g. overgrown teeth). To minimise the number of animalsthat are required, we will inject both the left and the right 4th inguinal gland of each mouse (with the same type of AAV-sgRNA), hence animal numbers will be 11 mice per group.

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

If suitable, we will use EDA in conjunction with a local statistician to assist with experimental design to reduce animal numbers, methods to reduce subjective bias, and appropriate statistical analysis without compromising the scientific objectives. When possible, experiments will involve a factorial design that will maximise the information obtained from a minimal number of animals. For example, non-invasive imaging and quantification techniques of transplantations will enable multiple measurements on the same animal over a period of time. In such cases, ANOVA will be utilised for statistical analysis.

When conducting xenograft experiments, we routinely inject two contralateral flanks of the mouse, thereby reducing the number of animals being used by a half.

Our approach of modelling breast cancer using CRISPR/Cas9 will substantially reduce the number of animals culled because of "wrong genotype" that occur from complex breeding strategies involving conventional GEM approaches of floxed alleles. For example, our new approach requires just two breeding crosses with a 1:2 and 1:8 chance of female animals of the correct genotype being generated that will enabling modelling of basal-like breast cancer and the role of a candidate gene. In comparison conventional GEM approaches requires three crosses In just one breeding cycle that would generate one animal for experimental analysis, our approach would result in 75 animals being spared. We have calculated that 19 animals will be required for power (effect size to be 0.70 based on the proportion of *Blg-Cre;Brca1$^{f/f}$;Tp53$^{+/-}$* alive after 365 days being 30%. Two-tail power analysis assuming alpha = 0.05 (probability of a type I error), beta = 0.2 (probability of a type II error)), results in 1425 animals being spared per cohort (2850 mice spared in an experiment comparing tumour development with and without PRMT deletion).

To calculate frequencies of stem cells in cancer cell populations, the L-Calc program is used . The minimum numbers required to obtain estimates of stem cell numbers with this program are data from three different cell dilutions, each of which was transplanted into five different animals. However, five dilutions into five animals are required for robust data with smaller 95% confidence intervals.

Where possible, we will use live imaging to track tumour development longitudinally. Not only does this mean less animals are needed overall as there is no need to cull at each time point, but it also reduces variation and so improves quality of the data produced.
All strains not immediately required for scientific study will be cryopreserved as embryos.

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

We always strive to generate the most effective breeding strategies to ensure that we obtain mice of the desired genotype with minimal animal wastage. If we are unable to estimate an effect size from our *in vitro* data, the literature, or our collaborators, we will conduct small pilot experiments.

All tissue surplus to requirement will be deposited into SEARCHBreast (https://searchbreast.org), a resource to facilitate sharing of archived material derived from in vivo breast cancer models.

Refinement

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

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

Mice are the species of choice for a genetic approach to the analysis of biological phenomena. In particular, the wide availability of genetically modified mouse strains and mouse ES cells means that the function of most genes of interest can be studied in the tissue of choice.

We have chosen genetic mouse models that will develop spontaneous tumours that have been extensively characterised, and develop tumours with similar aetiology and molecular profile as that observed in human disease. Hence, we will be aware of potential adverse effects and monitor for them appropriately. In line with this, we will use tailored welfare sheets that have been developed based on extensive experience of these models that will inform of adverse effects in the quickest possible manner. Indeed, as tumour growth rate is predictable, fewer mice are required to be used for each experiment. The phenotype of any novel genetically modified strain that we generate will be closely monitored for signs of distress and handled in the appropriate manner and a tailored welfare sheet developed. When using spontaneous mammary tumour models, we avoid using females for breeding as pregnancy-induced hormonal surges will decrease tumour latency. Male mice will also develop tumours, however we only use young male mice for breeding and in our experience, they rarely develop a tumour in their lifetime.

For human xenografts models we will use immune compromised mice, hence heterologous cells will be more easily engrafted. Imaging of engrafts will be conducted through the use of non-invasive techniques that will enable us to monitor tumour progression in living animals throughout the experiment, and will therefore reduce animal numbers. Here, the tumour volume as determined by non-invasive calliper measurements or bioluminescence imaging will be plotted against time. This design offers the advantage of determining significant differences between tumourigenic growth potential of cell lines before the limited tumour volume is reached.

A significant refinement we have made during our last Project Licence was the establishment of intraductal delivery of viral particles enabling mammary-specific gene recombination. We developed this procedure as we found that the MMTV promoter from the MMTV-Neu2-IRES-Cre (NIC) strain is expressed during embryogenesis (a fact that has yet to be published). This was a major issue for our experiments because homozygous deletion of *Prmt1* or *Prmt5* is early embryonic lethal, hence we were unable to generate animals of the desired genotype ($NIC^{tg/+}$; $Prmt1^{f/f}$). By developing this procedure we can now apply it to other Reduction (CRISPR project) and Refinement (MIND model - see below) aspects, thereby enhancing the welfare experience of mice used during our experiments.

In some cases, we will analyses tumour growth using the MIND (**m**ouse **in**tra**d**uctal) model. Here, cell lines are injected into the mammary ducts leading to the development of ductal carcinoma in situ, invasive disease that retains the molecular characteristics of the original cell line. It also offers refinement in the study of ER+ breast cancer as tumours will grow in systemic levels of estrogen, elevating the need of surgical implantation of hormone pellet/mini-pump and the adverse effects associated with excess estrogen levels.

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

We need to study tumour development in adult mice as this is what most closely resemble what occurs in humans. We have chosen to use mice over other less sentient species such as Danio Rerio (zebra fish) and drosophila melanogaster (the fruitfly) as mice and humans share 97.5% of their coding DNA sequences. In comparison, the drosophila

genome is only 60% homologous to that of humans and only 75% of the genes responsible for human diseases have homologs in flies. Mice are also a more appropriate for studying complex biological systems found in humans as they possess immune, endocrine and nervous system. Consequently, like humans, mice naturally develop diseases, including cancer.

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

When conducting a surgical procedure, we ensure that analgesia is administered 30 mins before the start of the procedure. Mice are allowed to recovery from the surgery by being housed in a warm cage and observed by a member of my team until animal has fully recovered and are mobile. Mice are check again for wellbeing and wound closure 4hrs later. We have found that by placing the wound towards the bottom third of the spine with a combination of suturing and glue, mice are less likely to bit and cause reopening. Once mice start to develop tumours, they are monitored at least twice a week, and more so if tumour growth develops rapidly. Tumours that developed in the mammary gland are carefully monitored for signs of ulceration, and hard housing replaced by cardboard housing and additional bedding if this occurs.

We are highly trained in intraductal injections, and members of my team undergo extensive training on dead animals and require to be authorised by BMSU before being allowed to perform a procedure on live mice. We have refined this technique and now use hair removal cream rather than shave the mouse as the latter resulted in removal of the nipple. We also generally prefer to inject the 4th gland as this has longer nipples than glands 1-3 and is thus less likely to result in a failed injection.

Our approach to model breast cancer through CRISPR/Cas9 gene editing in the adult mammary gland is a refinement on conventional GEM breeding approaches as it significantly reduces the amount of breeding that is required to generate knockout animals.

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

Guidelines for the welfare and use of animals in cancer research. Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA; Committee of the National Cancer Research Institute. Br J Cancer. 2010 May 25;102(11):1555-77. doi: 10.1038/sj.bjc.6605642.

RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.) Jones HRP, Oates J, Trussel l BA (1999) An applied approach to assessment of severity. In: Humane End points in Animal Experiments for Biomedical Research (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 40±7.

We will publish in journals that support the ARRIVE guidelines and conduct our experiments with advice from the PREPARE publication (PREPARE: guidelines for planning animal research and testing. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. Lab Anim. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823. Epub 2017 Aug 3.PMID: 28771074).

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

I have acted as a panel member for NC3Rs and my lab will comply with the ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments; www.nc3rs.org.uk/arrive), a NC3Rs-developed checklist of the essential information that should be included in publications reporting animal research. ARRIVE has now been endorsed by more than 400 journals including the Nature group, PLoS, and Cell, as well as funders, universities, and learned societies.

I also follow NC3Rs twitter account, so will be made aware of any notification via social media.

Any new advancements will be made clear to members of my team through our weekly lab meetings.

Project	**19. Artificial intelligence assisted pharmacokinetic characterisation of novel therapeutic agents**	
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 find out how well a new drug enters the body after dosing and if it gets to the relevant organ or tissue at a level that will cause a therapeutic effect. Compounds that have the right properties will progress to pre-clinical development with the aim of treating diverse diseases with significant unmet medical need such as amyotrophic lateral sclerosis, Parkinson' s Disease, Glioblastoma, inflammation and age related diseases. The project will be supported by Artificial Intelligence and Machine learning to ensure only the most promising compounds are	

	synthesised and screened in vitro before moving forward into animal testing. Data will be generated by dosing animals, usually orally and intravenously, with test compounds and assessing levels of the compound in blood and tissue over time. Initial experiments will involve dosing once only at a low concentration. Only when a compound is deemed to have the correct profile in simple studies will we progress to more complex studies involving multiple doses and higher, therapeutic, doses. In the more complex studies animals may be kept in metabolism cages to collect excreta for the analysis of drug metabolism.
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 the short term this project will enable decisions to be made as to which compounds should move forward for further development such as testing in disease relevant animal models. Data generated will also help to inform Artificial intelligence and Machine Learning so that better compounds are designed, meaning fewer animals are needed for research. The long-term benefit of this project is compounds moving forward into pre-clinical development and ultimately clinical testing in humans
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use adult rats and mice in this project as their physiology and translatability to humans is well understood. Over the 5-year course we plan on using 15000 rats and 6000 mice. This is based on running 8 non-surgical and 8 surgical Rat PK studies per month and four Target engagement studies per month supporting 3 to 4 projects..
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The experiments are designed and conducted by highly trained personnel to ensure animals suffer the minimum amount of distress. Animals will be dosed with compounds (usually once but at times repeated dosed may be required) followed either by collection of blood and tissue post mortem (or under terminal anaesthesia) or serial bleeding from implanted cannula or peripheral blood vessels. This is minimally invasive, and animals will be monitored for signs of discomfort or distress. Low doses of compound will be dosed initially and animals will be closely monitored for adverse drug reactions.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Initial *in vitro* assays will be used to screen out compounds that do not have the correct profile to likely succeed in *in vivo* studies. however, we need to use live animals as current cell-based assays and computer modes do not fully represent the complex nature of whole animal biology e.g. metabolism and complex organ systems. We can only gain this information using whole animals. By testing in animals, we can make sure compounds that are intended to treat diseases in patients can be dosed safely and effectively. At present there are no alternatives to using live animals for this purpose.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The machine learning platform used will ultimately reduce the total number of studies required to run a successful drug discovery, reducing animal numbers significantly. Where possible animal use will be reduced by using serial blood sampling where smaller blood samples are taken on several occasions from a single animal. Furthermore, biological processes involved in pharmacokinetics are reproducible between animals and so a low number per treatment will be used, usually n=3. However, where data suggest there is reduced variability within a study design n=2 will be used. Over the 5-year course we plan on using 15000 rats and 6000 mice. This is based on running 8 non-surgical and 8 surgical Rat PK studies per month and four Target engagement studies per month supporting 3 to 4 projects. At the end of each study animals will be humanely euthanized to provide terminal blood and/or tissue samples to maximise data acquired from each animal
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 and mice share many common features with humans and so are an appropriate species. Their biology and relevance to humans has undergone extensive research. All animals are housed in modern, purpose built, pathogen free facilities. All animals will be cared for by highly trained staff and have access to food, water and environmental enrichment. Adult rats and mice will be used in this project as their translatability to human physiology is well understood compared to less sentient species. Due to the effect of anaesthetics on

	animal physiology e.g. plasma pH, metabolism etc, awake animals must be used to ensure accurate, reliable and translatable data is produced. Throughout experimental procedures animals are closely monitored by highly trained staff to ensure stress and suffering is reduced to the absolute minimum. Surgical procedures are carried out by highly trained, experienced scientists, pre-operative analgesia is always used to minimises pain and suffering and close post-operative monitoring is used to ensure distress free recovery. All experiments are designed and carried out under the principles of PREPARE and ARRIVE guidelines.

Project	**20. Assessing alterations in specific metabolic pathways in vivo using hyperpolarised 13C MR**	
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)	Hyperpolarised MRI is a novel technique that allows performing real-time metabolic imaging in a non-invasive manner. Applications are, however, severely limited by the current technology. The aim of this project is to address these limitations to improve the assessment of metabolic disorders in cancer and to expand the field of applications of hyperpolarised MRI to the investigation of brain metabolism and diseases such as diabetes, obesity, and to detect immune response. More specifically, we will develop new hyperpolarised substances and methods to detect	

	changes in metabolic pathways involving for instance glucose and lipids, which are potentially useful in the clinic to guide treatment and disease management in individual patients.
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 hyperpolarised substance pyruvate has already been translated from research laboratories to the clinics and it is now used in 7 different hospitals across the world. Although several trials have been launched, the two main current targets are the diagnostics of patients with prostate cancer and the assessment of the myocardium in patients with heart failure. We expect that some of the methods and associated substances developed within the framework of the present project will open new opportunities for clinical diagnosis and help understand some of the metabolic disorders associated with various diseases. In particular, we hope to provide new ways to differentiate between fibrosis and more advanced stages of non-alcoholic fatty liver disease, which currently can only be done with liver biopsy, having a huge impact on the clinical management of the disease and the patient's quality of life. We would also like to develop methods to assess cerebral metabolism and that could be used to better detect and evaluate dysfunction associated to neuro-degenerative diseases or brain cancer using hyperpolarised MRI. Finally, we aim at identifying hyperpolarised substances that specifically probe the activation of the immune system in order to possibly image immune response in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	The most appropriate animal model for testing hyperpolarised MRI is the rodent model, namely rats and mice. We anticipate that 430 animals will be necessary to complete our 5-year 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?	Animals will be anaesthetised during all MRI sessions, including the placement of a tail vein catheter that will be used for the administration of the hyperpolarised substances. Physiological parameters (temperature and breathing) will be monitored throughout each MRI experiment. A limited number of experiments will require animals to be awakened to perform longitudinal experiments to assess disease progression or treatment response. For example, when studying

	liver diseases, the foreseeable adverse effects will then be additional distress due to the consecutive anaesthesia. To study liver diseases, some animals will be given a high-fat diet or administered streptozocin, which will lead to moderate weight gain, increased glucose levels in blood and diabetes. The level of severity will nevertheless remain moderate because we will be studying only the initial stages of the diseases. Some animals will undergo immune stimulation via subcutaneous injection in order to assess the sensitivity of hyperpolarised MRI to probe immune response. These animals are expected to develop localised inflammation but they will suffer no discomfort because all the procedures will be carried out under general anaesthesia. At the end of each protocol, animals will be humanely killed using a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because metabolic response to the hyperpolarised substance depends on complex interactions within the entire organism, it is crucial to evaluate their suitability *in vivo* in animal l models. Rodents are considered standard models for preclinical metabolic studies involving the injection and uptake of hyperpolarised substances. This is because rodent metabolism is similar to human metabolism. In addition, the high cardiac rate leads to fast delivery of substances to organs, which is important in the case of hyperpolarised substances. However, *in vitro* models (e.g. cells growing in culture) will be always used first to characterise and evaluate the suitability of the hyperpolarised substance of choice. Substances with poor *in vitro* performance will not be used *in vivo*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary studies will be done on small cohorts of animals to determine whether statistical significance is obtained with a specific hyperpolarised substance in the selected animal model and to evaluate toxicity of any new hyperpolarised substrate. Then, statistical tests will be used based on previous studies to determine the number of animals required to achieve meaningful results with our study.

	Conversely, if the MRI signal is too low and we cannot detect any meaningful/statistically significant results with the proposed hyperpolarised substance, the study will be terminated at this stage.
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 technique used in this project is hyperpolarised MRI, a non-radiative imaging technique based on the use of endogenous contrast agents. The animals will be under general anaesthesia during the entire duration of MRI sessions to keep them still. We will ensure that any distress, pain or discomfort that the animal may experience will be minimised by constantly monitoring their physiology (temperature and breathing) throughout the entire length of the MRI sessions. If any abnormality in the vital parameters is observed during the course of the experiment, it will immediately be terminated and the animal will be euthanised. When the experiments permit it, the animals will be killed while under anaesthesia on the last MRI session of the experiment. We will use a rat model with high-fat diet induced fatty-liver disease approximates to the human condition and is well documented in the literature. This dietary model causes a progressive weight gain as well as progressive damage to the liver, with minimal discomfort to the rat. The experiments will be finalised before any weight gain can cause pain or discomfort to the animals. All the immune response experiments will be performed using wild-type animals, which have no aberrant phenotype to minimise suffering, and under terminal general anaesthesia.

Project	**21. Assessing sensitivity and responsiveness of a compact, portable, non-invasive glucose sensor**	
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 study aims to test a newly developed prototype device which will attach to skin and measure changes in blood glucose levels accurately, in real-time and without the need to draw blood.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	Studies have shown that more frequent monitoring of blood glucose can improve management of diabetes, but the pain and inconvenience that comes with drawing blood to test your own blood glucose level can lead to	

benefit from the project)?	measuring this less frequently. A device that can measure blood glucose through the skin, without drawing blood, could help millions of people with diabetes all over the world manage the disease better, improve their quality of life and even prevent serious complications that can arise from diabetes. This particular study will generate data which can be used to improve the design of the device and the accuracy of the measurements, in order to start trials on humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Maximum of 30 pigs 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 most likely adverse events are physiological distress during the anaesthesia. Animals will be killed at the end of the study whilst under general anaesthesia, or earlier if signs of physiological distress whilst under anaesthesia are evident which cannot be alleviated by adjusting any of the available means (for example, adjustment of anaesthetic concentration or flow rate, ventilation rate, or providing additional injectable sedation, relaxant or anaesthetic).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The level of glucose in the blood is dependent on multiple processes acting simultaneously in the body, which is difficult to mimic in laboratory bench tests or computer models. The function of the device has been demonstrated in a human trial, but generating enough data to achieve the stated objectives in a controlled environment requires multiple manipulations of the blood glucose via infusions. It is also important to demonstrate that the sensor can detect low levels of blood glucose, which is usually the most difficult to detect. To replicate these conditions in humans would be more difficult and risky.
2. Reduction Explain how you will assure the use of minimum numbers of	Animals will be obtained from a single supplier to minimise individual variability as far as reasonably possible. We will not use genetically modified animals.

animals	By taking repeated blood samples during the experiments, blood glucose levels can be compared to the initial parameters for each individual animal. This means each animal provides its own control data, meaning there is no need for an extra control 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 pig is considered a good and well-established model for studies of diabetes management: they have a similar omnivorous diet, can develop obesity, have a similar respiratory and circulatory physiology to humans and a similar metabolism. They have a larger blood volume than smaller mammals (e.g. rats or mice) for analysing blood chemistry, and pig skin is also a suitable and frequently used model of human skin. Pilot experiments (up to 2 animals) may be used to refine points in the experimental procedure; if there are no adjustments to be made, the data from these animals may be included in the experimental group. A blood sample may be taken from some animals before the main experiment, otherwise the main experimental procedures will be carried out while the pig is under non-recovery general anaesthesia. The animal will be continuously monitored by attending anaesthetists and scientists throughout those procedures.

Project	**22. Assessment of drugs with activity in the CNS: determination of novel targets, efficacy and safety**	
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)	Mental health problems are a primary cause of the overall disease burden worldwide. Drugs currently available to treat CNS disorders are often limited in their effectiveness and may be associated with unacceptable side effects. As a result, more efficacious and safer medicines to treat CNS disorders are urgently required. The overall project aim is to provide highly specialised preclinical services to the pharmaceutical and biotech industry to evaluate the efficacy, mode of action and side effects of novel drugs for the treatment of CNS disorders. These specialised techniques may	

	occasionally be employed to evaluate centrally-mediated side effects of drugs developed to treat other conditions.
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 evaluation of novel drugs provided by clients in suitable tests/models in this project is expected to facilitate decision making by the client in regard to the progression and development of a compound as a treatment for a CNS disorder. Accordingly, experiments performed in this project are expected to expedite the development of better drugs to treat CNS disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	Based on experience, up to 9,500 rats and 4000 mice may be used on the project over 5 years. The exact number of animals used will be dependent upon external factors such as the number of clients and the success of those clients in designing suitable drugs, and the models and protocols used. Accordingly, numbers may be less than the above approximation.
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 studies will involve the administration of centrally-acting drugs (usually orally) with blood or tissue sampling or behavioural/physiological testing. Drug treatment might be once or repeated. Most of the procedures are anticipated to be mild. Some drugs will have been tested in vivo prior to being sent to us for assessment in assays the clients do not have validated. In such circumstances no adverse effects are expected. Occasionally, substances will be evaluated which may not have been tested in animals and unexpected toxic effects might arise which could cause pain, suffering, lasting harm or in extreme cases death if humane end points were not applied. Occasionally, drugs will be given centrally or by infusion via small pumps implanted under the skin (so avoiding daily dosing). Such procedures will involve anaesthesia. In some cases, the animal models employed may involve induction of specific pharmacological responses and/or involve training in specialised equipment which may produce transient discomfort/stress. In some instances, animals that are fully recovered at the end of procedures may be kept alive at the establishment (with the agreement of the vet), with a view to their re-use on procedures if appropriate and licensed. Upon completion of procedures animals will be

	killed. For these reasons, the likely/expected level of severity of the licence is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no alternatives to the models employed as they are used to assess the integrated behavioural and/or physiological/pharmacological responses of the whole animal to different treatments. Although the decision to test compounds in the project is generally based on responses in cell lines, cells and tissues, such assays cannot replace *ex vivo* or *in vivo* testing. Such animal testing is a fundamental requirement for progressing novel agents in man.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Qualified and highly experienced biostatisticians advise on experimental design and ensure that the correct number of animals is used to produce meaningful statistical comparisons. Animals will also be minimised by measuring several parameters in the same animals where possible (where animal welfare and experimental data will not be compromised). Where animal welfare is not compromised animals may be re-used in certain tests (on up to 8 occasions).
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 and mice will be used since their central nervous systems are well documented and they are the lowest form of mammal that can provide meaningful data about man. All studies will employ healthy adult animals. A variety of established, fully-validated animal models and assays will be employed. These have been widely used by the pharmaceutical industry to predict the effects of drugs in man. Where substance classes have not been given to animals before, pilot studies will be performed. Where substances are given as part of the procedure to induce an animal model of a CNS disorder, or a specific pharmacological response, doses will be carefully chosen and experiments designed so that any adverse effects and/or the duration that animals are exposed to the adverse effects are the minimum required for the scientific objectives to be met. Surgical procedures will only be used if alternatives are not available. Anaesthesia will be maintained at a suitable depth to avoid the animal feeling pain. Aseptic operating

	procedures, topical application of antiseptics and dressing will be used to reduce the possibility of infection. Post-operative analgesia will be used as advised by the vet to reduce pain and suffering. All animals will receive the highest possible standard of post-operative care. The project is supported by a dedicated animal husbandry and technical support team. Studies will be conducted by staff highly experienced in animal handling.

Project	**23. Assessment of fish stocks and impacts of environmental and anthropogenic factors**	
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
	X	Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To generate reliable and objective scientific evidence on fish populations, their ecology and the impact of selected environmental and anthropogenic (man-made) factors. This will support specialist advice provided to stakeholders, national and international governments and other international organisations on the conservation and management of fish stocks. Primarily, the work will focus on diadromous fish species that may use freshwater, estuarine and marine habitats during their life cycle, but will also include exclusively freshwater, marine and non-native	

	fish species. Effective management of freshwater, diadromous and marine fish stocks and the fisheries dependent upon them requires reliable information on the status of stocks, patterns and levels of exploitation and on a range of factors, both natural and made-made, which can impact on fish at different times in their life-cycle. Furthermore, research is required to be carried out in order to understand the abundance, distribution, behaviour, movement and diet of these fish and stocks, and the effects of man's influence on these populations through impacts on the aquatic environment (e.g. climate change, artificial night light, water abstraction fish stocking and introductions) to both manage and conserve these species.
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)?	Information on all aspects of fish ecology including their abundance, distribution and migrations under a changing environment, and the impact of introduced fish species, will permit better advice to Government departments and agencies, the International Council for the Exploration of the Sea (ICES) and North Atlantic Salmon Conservation Organisation (NASCO) on the conservation and management of fish stocks. The information will also contribute to the development of methods for assessing salmon, sea trout and European eel stocks and improve management of fisheries.
What species and approximate numbers of animals do you expect to use over what period of time?	This licence will use fish. There is no alternative to the use of living animals, as the principal aim of the work is to describe the ecology and behaviour of fish in relation to changes in their aquatic environment to conserve and manage populations. Consequently, a range of fish species (including salmon, sea trout, European eels, coarse fish, lamprey, shad and smelt) will be studied over the course of 5 years to provide relevant and meaningful data on which decisions are made. In developing the project, advice has been obtained from a statistician experienced in animal research, regarding animal numbers and design. The numbers used will range between protocols from 500 to 30 000, depending on the procedure used. For example, large numbers of

	fish will be used solely for sampling and tagging procedures, with mild levels of severity.
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 will involve mild or moderate levels of severity, apart from terminal sampling. However, it will be ensured that smaller numbers of fish are used for those procedures when possible. At the end of each procedure, if fish are to be released to the wild, they will be assessed by appropriately trained and qualified individuals to ensure that the fish are fit and their welfare is protected. Alternatively, they will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The principal aim of the work is to describe the ecology and behaviour of fish in relation to changes in their aquatic environment to conserve and manage populations. Therefore, there is no alternative to the use of living animals. However, where appropriate, environmental DNA sampling is being used as a non-invasive means to sample waterbodies to determine the presence or absence of fish species. This is a developing tool and has further potential to replace fish capture in some circumstances.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experimental work will use the peer-reviewed scientific literature, discussions with professional statisticians and experience by the Project Licence holder and colleagues who undertake similar work to ensure that the minimum number of animals are used that will permit a robust statistical and meaningful analyses of the results. Statisticians will provide statistical support to all aspects of the research, from designing the experimental approach to conducting and reporting the analyses.
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 purpose of the work is to provide advice on the conservation and management of fish stocks. Therefore, a range of fish species need to be studied to produce adequate data on the impacts on fish populations from a wide range of environmental and man-made changes to the aquatic environment. The methods chosen are based on previous experience and research and

to the animals.	will provide evidence that will form the basis of suitable advice to Government on the factors affecting fish populations and recommendations for suitable mitigation. Where fish undergo a procedure and recovery, they will be monitored for a suitable period of time in order to assess any adverse impacts and ensure a minimum of suffering.

Project	**24. Assessment of immunogenicity of and protection induced by an oral inactivated vaccine**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	1 Year 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)	UK dairy farmers are currently under considerable pressure to minimise antimicrobial usage, specifically to prevent secondary bacterial infections after primary viral infections. This might be achieved through increased number of vaccinations. However, for many of the vaccines given systemically, the crucial cut-off is that these do not work (well) in the presence of maternal, colostrum-derived antibodies. Here, mucosal vaccination strategies might provide a way forward. Through the stimulation of the common mucosal system, it should be possible to reduce	

	pathogen exposure on these ports of entry. However, for ruminants, oral vaccination may provide a challenge due to the development of the rumen within the first 2 months of life. The overall aim of the project is to increase our understanding whether oral vaccination is possible to achieve using a yeast additive, and to assess its immunogenic effects in mounting a humoral and cellular immune response. This will be assessed by testing for pathogen specific systemic and mucosal immune responses . We will also study the ability of the yeast construct expressing Bovine Viral Diarrhoe Virus proteins. This is endemic viral diseases effecting dairy and feedlot animals world-wide, leading to immunosuppression, thus enhanced occurrence of secondary bacterial infections as well as reduction in production parameters, such as milk production and increase inter-calving intervals
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 will help us to understand whether it is in principal possible to orally immunize suckling calves, similar as it has been described for mice, chicken and pigs. Overall we are working closely with a veterinary pharmaceutical partner to ensure a potentially fast transfer of this strategy into the real world. This will benefit animal health, consequently improving cattle longevity and welfare.
What species and approximate numbers of animals do you expect to use over what period of time?	The work will involve neonatal calves. Based on own experiences, we require a total of 9 animals for this proof-of-concept study.
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?	All of the work will use calves kept according to normal husbandry procedures on dairy farms. Occasional blood samples will be taken for analysis. This is a standard procedures and the severity throughout will be mild. The animals will remain on the farm after use.
Application of the 3Rs	
1. Replacement	We have performed already all necessary in-vitro experiments to assess the response generated to

State why you need to use animals and why you cannot use non-animal alternatives	the yeast construct. However, we need to assess whether this construct is actually immunogenic in real animals to identify future directions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our sampling strategy is based on previous experience to target the key time points. Individual blood samples will be split so that each can be used for a variety of tests.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The project is only relevant to cattle. Animals will be exposed to normal husbandry procedures as used on UK farms. Only mild protocols will be used.

Project	**25. Assessment of metabolic status in dairy cattle**	
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)	Correct nutrition in dairy cattle is an essential requirement for milk production, in order to meet the high nutritional demands of lactation. For example an average dairy cow giving 30 litres of milk will be working at over three times her basic maintenance energy requirements, and a cow giving 50 litres (not uncommon in modern Holstein dairy herds) will be working at nearly five times her maintenance energy requirements. This places significant metabolic stress on the animal, which can be harmful for cow health, productivity and future fertility. In order to try and meet the high demands of lactation and reduce the metabolic stress on the	

	cows, they are often fed high energy diets supplemented with concentrate feedstuffs such as wheat, maize or molasses. However high levels of fermentable carbohydrates can result in a lowering of the pH in the rumen of the cow, which can also have harmful effects on cow health. This project seeks to assess nutritional status in dairy cattle with the aim of quantifying the effects of different nutritional strategies for the reduction in metabolic disease, and effects on cow behaviour.
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 welfare of the high yielding dairy cow is coming under close scrutiny, and there is increasing evidence that the high metabolic demands of lactation predispose cows to increased levels of disease such as fatty liver, mastitis and reduced fertility. This work will seek to more objectively assess nutritional status in dairy cows compared to traditional methods such as body condition score and weight changes, with the aim of monitoring and reducing the incidence of disease and improving cow health. In addition it will aim to develop existing models of dairy cow nutrition to improve the accuracy and precision of feeding dairy cows. It will also enable us to trial various nutritional supplements that are promoted to potentially reduce the effects of metabolic disease
What species and approximate numbers of animals do you expect to use over what period of time?	We will use adult dairy cows kept on a commercial dairy farm under conditions that are commonly encountered on UK dairy farms. Over the 5 year course of the project, we expect to use 200 cows in the experimental 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?	We require to blood sample the cows on a weekly basis, to be able to analyse metabolites in the blood for the assessment of metabolic status. Any adverse effects will be mild, limited to local irritation from the needle puncture required to obtain the samples. At the end of the experiment, the animals will be returned to the commercial dairy herd to continue with their productive life.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Feeding the dairy cow is a very complex science, as she effectively contains a “fermentation vat” in her rumen that digests plant cell wall material to obtain nutrients. Non-animal models cannot replicate complex cow behaviours that affect the rumen environment such as feeding, feed intake and rumination activity, nor the holistic view of cow health, productivity and reproductive function that are affected by nutrition of the dairy cow, nor the complex pathways that determine energy balance in a high producing dairy cow. Dairy cattle are therefore the only animals that can be used in this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of cows required will be based on extensive experience of feed trial work carried out previously, and other peer-reviewed research that has been published. Previous work has utilised similar number of cows to assess the efficacy of dietary supplements on milk production, rumination behaviour and rumen pH measurements. Through the use of good statistical methods we will minimise the number of animals required in our experiments.
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.	This project will seek to obtain information that will be applied to dairy cattle, and therefore this is the species and type of animal of choice. The methods used will seek to obtain data on nutritional status in dairy cows, using well established methodology for the assessment of nutritional status such as body condition scoring and measurement of blood metabolites to assess metabolic status. The procedure involved (blood sampling) is classified as mild, and are designed to obtain samples with minimal suffering in order to assess metabolic status

Project	**26. Assessment of novel entities for the treatment of metabolic disorders**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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)	Metabolic diseases, in particular, obesity, diabetes and its associated co-morbidities including diabetic neuropathy and cardiovascular-renal diseases, non-alcoholic fatty liver disease (NASH), kidney disease and metabolic syndrome are emerging as the epidemic of the 21st century. The global prevalence of obesity almost doubled between 1980 and 2014 so that approximately 40% of adults are overweight world-wide and 11-15% are clinically obese. According to the International Diabetes Federation (IDF), the worldwide prevalence of Type2 diabetes (T2D) is 371 million, estimated to rise to 552 million by 2030, closely paralleling the rise in obesity. Non-alcoholic fatty liver disease (NASH) and hyperlipidemia are considered by some to be	

	the hepatic manifestation of obesity and metabolic syndrome. Staggering healthcare costs are associated with treating these diseases. In obesity, pharmacotherapy intervention is hampered by the small number of prescription drugs available and limited efficacy. Whilst there have been significant improvements in the management of T2D with the launch of several new prescription drugs the treatment of diabetic neuropathy and cardiovascular-renal diseases remains poor. There is currently no prescription drug available for the treatment of NASH. Thus, new treatments based on novel drug classes are urgently required for better management of metabolic diseases. The overall project aim is to provide highly specialised preclinical services to the pharmaceutical and biotech industry to evaluate the novel compounds for the treatment of metabolic disorders. This is achieved by the following three objectives: 1. To evaluate the pharmacokinetic profile of novel compounds including pilot studies in order to allow clients to decide whether to progress into efficacy studies. 2. To evaluate the potential of novel drug candidates and pharmacological approaches to treat metabolic disorders, including efficacy, mode of action and side- effect studies. 3. To evaluate the potential of novel drug candidates irrespective of primary therapeutic indication to produce metabolic effects which may be considered of benefit (secondary indication) or to be a potential side-effect. The fourth objective is aimed at evaluation of various handling techniques, in particular in mice, in order to attempt to further minimise stresses associated with handling/dosing.

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)?	It is anticipated that studies performed under this Project Licence will assist sponsors to develop new and improved drugs to treat metabolic disorders. Data provided allows the client to answer the following questions: does the compound exhibit efficacy in a relevant model, is the compound advantaged over existing products, were unacceptable side-effects or pharmacokinetics observed. The studies and advice we give will assist the sponsor to move rapidly through the pre- clinical development programme and ultimately into man or stop the development of a particular compound or even programme. Wherever possible, information will be disseminated into the scientific community. This will further knowledge about novel molecular targets in metabolic disorders and the efficacy and/or safety of new drugs to treat metabolic disorders. In addition, data on model development and refinements will be published in order to improve animal welfare.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 9,420 mice and 5,270 rats over 5 years. The exact number of animals used will be dependent upon external factors such as the number of clients and the success of those clients in designing suitable drugs.
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 studies will involve the administration of novel compounds in models of metabolic disorders, such as obesity, type 2 diabetes, NASH and kidney disease. Novel compounds are predominantly administered orally but occasionally by the ip, sc and iv route and very occasionally by pump for measurement of body weight, food and water intake, body fat (DEXA), collection of blood samples (eg during an oral glucose tolerance test), urine samples and terminal blood and tissue samples. Drug treatment might be once but more likely to be repeated. Procedures such as dosing and blood sampling are anticipated to be mild. Some compounds will have been extensively evaluated prior to assessment. In such circumstances no adverse effects are expected. Some compounds may not have been tested in animals or there is limited data and unexpected toxic effects might arise which could cause pain, suffering, lasting harm or in extreme cases death if humane end points were

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project objectives each require investigation of candidate substances to be tested in an integrated behavioural /physiological/pharmacological model that requires the whole animal which cannot be replaced by in vitro or ex vivo studies. Prior to evaluation, many test compounds will have been selected on the basis of some efficacy data but compounds in early stage discovery will have only *in silico*, *in vitro* and/or *ex vivo* data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The majority of models detailed in this Project Licence have been extensively use. These data have been extensively reviewed by our in-house statistician and Study Directors in order to produce the most scientifically valid design using the minimum number of animals to achieve high quality data. For each and every experiment a Study Plan is written which includes: ● a statement of the objective(s) ● a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material ● an outline of the method of analysis of the results, 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 the treatment differences that are to be estimated. However, once the data are available it is analysed in the most appropriate way by in-house statisticians who have over 20 years' experience of analysing data from studies included in this Project Licence. Studies are appropriately designed using the input of statisticians. Experiments include appropriate controls, treatment groups and reference comparators to allow relevant statistical comparisons to be performed. The design of some studies is improved by allocating animals into balanced treatment groups using appropriate factors to reduce variability. For example, animals in feeding studies will be allocated to a study by

	statisticians in a way that body weight, food and water intake are similar across groups. All data are analysed by Statisticians and a Statistical report for the study made which is sent to the client.
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 and mice will be used since their endocrine and central nervous systems are well documented and they are the lowest form of mammal that can provide meaningful data about man. Typically, studies will employ healthy adult animals. Occasionally, genetically-altered animals will be used to model specific metabolic disorders. Animals displaying adverse phenotypes will not be used. A variety of established, fully- validated animal models and assays will be employed. These have been widely and successfully used by the pharmaceutical industry to predict the effects of drugs in man. Where substance classes have not been given to animals before, pilot studies will be performed. Where substances (e.g. streptozotocin) are given as part of the procedure to induce an animal model of a disease (e.g. diabetes), or a particular pharmacological response, doses will be carefully chosen so that adverse effects, and their duration, are the minimum required to enable scientific objectives to be met. The majority of models detailed in this Project Licence have been established, used and refined over the past 15 years. They have all been validated using suitable compounds and widely used by the pharmaceutical industry. In addition, over the last 10 years we have developed and validated a handling and baseline dosing protocol for rats and mice which ensures little or no weight-loss upon the start of compound dosing. The handling technique utilised differs from that proposed by the NC3Rs committee which has been developed and validated in particular for mouse behavioural studies. We propose to compare the handling methodologies employed over the past 10 years with those recommended by the NC3Rs committee with a view to employing the most refined handling methodology for the studies detailed in this Project Licence in particular for mouse studies. Rats and mice are social animals and they will be group housed unless single housing is required for scientific reasons, for example, in feeding studies where accurate measurement of food and water intake is required to be collected for individual

	animals. For all NASH studies mice will be singly housed as C57Bl6J mice are aggressive so group housing even when it is limited to litter mates and involves weight matching is often unsuccessful (with between 25 and 50% of mice in a study having to be singly housed due to aggressive behaviour). Also as the mice are exposed to a diet either lacking in a nutrient or high fat in nature if the mice are group housed we get a lot of variation in the level of fibrosis compared to when mice are singly housed and this impacts on the validity of the data and reproducibility. When individually housed mice are provided with a red house, nestlet, sizzlenest, red tunnel and chew stick. Animals will be singly housed in metabolism cages (e.g. for urine collection) and food (but not water) may be withdrawn. Accurate measurement of total urine volume is essential in order to calculate total glucose or albumin. Calculation of concentration alone is of limited utility and, accordingly, the use of products such as hydrophobic sand do not act as a refinement since total urine cannot be accurately determined. Animals may be fasted for a maximum of 16 h (lean mice) or 24 h (rats; diabetic or obese mice) during collection. Food removal is to limit urine contamination especially relevant to urine collections undertaken to assess kidney function. Food contains protein and so effects urine protein levels. Food present in the urine also makes accurate collection of the urine difficult: not only the collection of a large enough sample to analyse but also in obtaining an accurate final volume (often determined by weight) so that total protein, albumin, creatinine can be calculated. Food restriction duration and urine collection times are strain and study-dependent but are kept to an absolute minimum and are based on in house experience and the experience of clients. During the validation of the Adriamycin (ADR) model in BALBc mice we initially utilised 24 h urine collections in freely- fed animals. However, we found that no urine could be collected from control mice or from many of the Adriamycin- treated animals. To try and resolve this issue, wet mash was provided during the collections and we trialled labsand. None of these resolved the problem. The fasting of animals during collection was introduced on the recommendation of one of our clients. Initially mice were placed in metabolism cages for 8 hours. Urine could not be

	obtained from 7 out of 18 mice. In the same study at a later time point a 16 hour collection obtained suitable volumes and quality of urine in all but 2 mice. Accordingly, in such protocols 16 h fasted collection periods are believed to be the minimum necessary to generate suitable urine data to meet study endpoints. New metabolic cages have also been purchased which are clear Perspex so the mice can be clearly monitored, a red house and plastic chew stick are placed in the metabolism cage and the room temperature is elevated to protect against any deficits in thermoregulation that the mice might experience during the fasting period (e.g. 23– 24ºC). Wet mash will be presented prior to and subsequent to the collection to minimise weight-loss and promote rapid regain. In addition, our statistician has evaluated all the data we have collected to assess the body weight-loss of each mouse whilst in the metabolism cages. If the body weight of the mouse is predicted to exceed 25% from day 1 the mouse is not placed in the metabolism cage. Importantly, animals will not always be fasted in the metabolism cages (food is present during urine collections using diabetic animals). Studies undertaken in this project may involve the use grid floors (only in rats). These steps are to ensure accurate food intake measurements especially relevant where foods are provided in a powdered form. They are also essential in studies where faeces and urine are to be collected. In the case of using grid floors, polypropylene trays will be placed below each cage to detect any food spillage. Where grid floors are used, animals will have access to sufficient paper bedding in order to provide a refuge from the gridded floors unless it is found to interfere with the experimental results (e.g. pica behaviour directed at the bedding) and a red tunnel or platform. However, we are making every effort to move away from the use of grid floors and this type of housing will only be used when absolutely necessary, for example, we have moved away from using powdered diet to pelleted diet for rat high fat studies and so the use of grid flooring is no longer required.

	Animals may be maintained on reverse-phase lighting during studies in the project since they are nocturnal and it may be relevant to organise this period of feeding behaviour so that it occurs during the normal working day. Where placed on reverse-phase lighting, animals will typically be acclimatised to these conditions for at least two weeks before experimentation. Such conditions have been used routinely for over 10 years and no adverse events have been observed during such studies. The majority of studies conducted under this Licence will involve dosing by the oral, intraperitoneal, subcutaneous or intravenous route by gavage (oral) or injection. This will be performed by highly-skilled staff, using appropriate dosing techniques (eg http: //www.Procedureswithcare.org.uk/administration-of-substances/ and dose volumes (LASA guidelines) to minimise any stress and discomfort to the animals. Appropriate sized needles will be used. Oral administration to rats will normally be by gavage using flexible catheters to minimise oesophageal trauma. Short dosing needles are typically used for mice which are likely to bite the flexible tubing. A single-use needle approach has been adopted. When intraperitoneal and intravenous dosing is performed in mice we now use U-100 Insulin syringes (0.5 ml: 30G x ½" (0.3mm x 12mm); 1 ml 29G x 12.7 mm) which are smaller gauge than those used historically and so are associated with less discomfort. Any tissue damage or discomfort due to multiple subcutaneous, intraperitoneal or intravenous injections will be minimised by using appropriate sterile techniques, solutions at suitable pH and different sites of administration. Where appropriate, the use of topical anaesthetics will be considered and implemented according to NVS advice. For measurement of rectal temperature a suitable lubricants will be used and the number of measurements will be limited to reduce the possibility of any pain or discomfort to the animal. Implantable recorders are available (e.g. http://www.staroddi.com/Home/PharmaceuticalResearch/ Biomedical-Research/) the fact that these have to be implanted (ideally after laparotomy) is believed to outweigh any adverse effects of transient insertion of a lubricated probe in the

	present project. The choline deficient (MCD) diet model of NASH is associated with marked weight-loss (35-40%) which occurs over a 2-3 week period. Although the MCD diet model of NASH has been viewed as the gold standard there are a number of other models emerging. We have developed and validated the choline-deficit (CD) diet model of NASH. This model is run over a similar timeframe to the MCD diet model but has the clear advantage that the weight-loss observed is around 12- 15%. This model has replaced the MCD diet model of NASH. Rigorous body condition scores in addition to weight-loss are now used to assess health status and establish endpoints (for example: when seeing weight loss in studies either due to drug treatment (often an expected result in the project) or diets used in NASH studies or adriamycin studies. Rat and mouse grimace scoring for pain has recently been introduced and will be used when appropriate. All staff have received the appropriate documentation to read and review and this will be placed in their training files. Posters will be available to aid scoring Surgical procedures will only be used if alternatives are not available. Anaesthesia will be maintained at a suitable depth to avoid the animal feeling pain. Aseptic operating procedures, topical application of antiseptics and dressing will be used to reduce the possibility of infection. Post-operative analgesia will be used as advised by the vet to reduce pain and suffering. All animals will receive the highest possible standard of post-operative care. The project is supported by a dedicated animal husbandry and technical support team. Studies will be conducted by staff highly experienced in animal handling. The taking of blood samples (anticipated to be primarily from the tail vein) is likely to be a frequent procedure undertaken under this licence. Heated chambers may be used to aid blood sampling. Restraint in a suitable chamber may be used to facilitate the procedure. Temporary cannulation may be used in rats to avoid multiple needle entries over short periods of time. The use of a topical local anaesthetic to reduce any pain and discomfort during blood sampling will be considered and implemented according to NVS

	advice. Temporary cannulation is currently not a suitable method for mice as it would not have any advantages over the current technique. Sampling in mice will be by section of the lateral tail vein. When repeat samples are collected eg in an OGTT the additional sample are collected from the initial section in mice. Repeated venepuncture is not used in mice. In both rats and mice, the lateral tail vein is usually accessed approximately one-third along the length of the tail from the tail tip, moving towards the base of the tail for multiple samples. Blood samples will only be taken from the base of the tail if no vein is visible elsewhere since taking the first sample/s from the proximal end of the tail can result in a perivascular clot and inflammation that significantly reduces blood flow to the distal portion of the vessel (source www.nc3rs.org.uk). For all blood sampling, the use of a topical anaesthetic cream (e.g. EMLA) will be considered. Should we encounter issues with current methods for the taking of blood samples (or associated with warming/restraint) alternatives will be considered. Microsampling (collection of blood samples of less than or equal to 50µl) will be utilised wherever possible. The size of the blood sample required is based on the plasma requirement of the assay. For the assay of test compound levels (PK) it is our experience that very few of the currently utilised assays employed by either the client or specialist CROs which run these assays can be performed with such small plasma sample volumes, in particular in the rat. However, we raise this with every client who initiates a study with us and collect the minimum volume possible. The loss or damage to samples in transit has never occurred at REDACTED as we use a Company for transportation of samples that have an outstanding reputation in this area. However, we will continue to strive to keep blood volumes to a minimum and wherever possible introduce microsampling. The blood volumes (source: LASA) are expressed per unit body weight (Mouse: 78 ml/kg). Accordingly, the total blood volume for each animal is adjusted for weight and lighter animals will be calculated as having lower blood volumes and those limits followed. Accordingly it is not the case that an “underweight” mouse is treated as having the same blood volume as a heavier animal. Even when running GTTs it is the case that on the

	morning of the test mice are weighed and, if required, lighter mice may need to have fewer samples taken. As an example: an OGTT protocol may require 6 blood samples (30ul) to be taken from lean mice over a 2h period for glucose and insulin measurement. As a maximum of 10% of circulating blood volume can be removed on such a single test occasion (and no more than a further 5% in the forthcoming 28- day period), any mouse under 24g would not undertake all samples in the OGTT. In the project plan the minimum (eg 8) and maximum (eg 10- 12) number of animals used on a study have been included. However, for every study we do we determine what is the most important parameters a client wishes to look at and do a power calculation on what we feel is a realistic change to be significant for example: In an OGTT in a chronic study in the DIO mouse, where fasted glucose and insulin were measured at baseline (i.e. prior to Day 1), we wish to be able to detect a 12.5% reduction in AUC glucose and a 25% reduction in AUC insulin with 80% power. In our example, glucose was not transformed, the vehicle mean was 15.51 mM and the mean squared error (MSE) was 2.35136. A sample size of 10 was required to detect a 12.5% reduction with 80% power. Insulin was log transformed. Using a log10 transformation, the MSE was 0.00841. A sample size of 10 was required to detect a 12.5% reduction with 80% power.

Project	**27. Assessment of nutritional and immunological status in sheep**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 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 overall aim of this project is to improve newborn lamb health and welfare. This will be achieved by understanding how the nutritional status of the ewe impacts on newborn lamb immune status, disease and mortality. It will also involve developing field tests to allow the immune status of newborn lambs to be determined by veterinary surgeons on commercial farms and an understanding as to how the lamb's immune status impacts on its risk of disease and/or death.	
What are the potential benefits likely to derive from this project (how science could be advanced	The potential benefits of this project involve better monitoring of ewes and lambs on commercial units to ensure that action can be taken to	

or humans or animals could benefit from the project)?	improve nutrition and husbandry during early life to reduce disease and mortality in newborn lambs. This will translate through to improved animal welfare due to less disease, whilst also improving profitability for farmers. It should also result in more efficient lamb production, hence reducing carbon-dioxide production per kilo of lamb produced. Improved health will also reduce antibiotic use in sheep production and hence reduce the risk to human, animal and environmental health from the development of antimicrobial resistance in livestock farming.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use all the ewes and lambs from a 350 ewe flock during lambing time. Lambing typically lasts around 1 month.
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 animals will be managed under conditions that are typical of commercial sheep farms in the UK. The main difference to normal farming practice, will be that all the animals in this study will be blood sampled. Blood sampling is a mild procedure and any adverse events, such as irritation around the sampling site, are expected to be rare and mild. At the end of the study, sheep will either go for human consumption or return to the breeding flock.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project relates to commercial sheep production in the UK. It is therefore necessary to use sheep managed under commercial conditions to achieve the project objectives. As the only regulated procedure in this project is blood sampling, we did explore undertaking this work in the field. Our ethical review committee considered the number of animals to be sampled to exceed standard agricultural practice, hence necessitating that this work be undertaken under Home Office Licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	There is limited data available in sheep to guide the appropriate number of animals to use in this study. Similar work in cattle suggests that immune status in newborn calves is highly variable between herds. To reduce variability and hence the number of animals involved in the

	study, we have chosen to undertake this study in a single flock. The entire flock will be enrolled in the study because under standard management conditions, it is impossible to predict which ewes are under the most metabolic stress or which lambs will fail to receive sufficient antibodies from the ewe shortly after birth. Capturing the full distribution of nutritional and immune status within the flock is an important component to the success of this 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) to the animals.	Sheep are chosen for this project as the project aims and objectives directly relate to commercial sheep production. Animal suffering will be minimised by ensuing that blood sampling (i.e. a mild procedure) is the only regulated procedure undertaken in this project.

Project	**28. Assessment of the safety and efficiency of a 'delivery of anaesthesia device' (dad)**	
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 ultimately aims to develop a small, portable device for the safe, controlled delivery of volatile sedation and/or anaesthesia, which will be suitable for use by a wide-range of clinical staff, and be lightweight and portable enough to be used in clinical and "in-the-field" environments, including in the developing world. The aim of the work described in this application is to provide pre-clinical information to demonstrate the safety and effectiveness of this device and the modified anaesthetic formulation it delivers, in order to support licencing the device as a product and beginning human clinical trials.	

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)?	A wide range of medical procedures and interventions are carried out under sedation, and in even the most difficult situations the delivery of combined anaesthesia and analgesia is mandatory for surgical procedures. This is difficult to achieve with intravenous agents which are hard to adjust to response and can easily accumulate. The use of breathable (volatile) sedation agents which are rapidly breathed out by patients would be an excellent and responsive way of delivering sedation in challenging circumstances. Availability of a simpler to use technology, that would allow patients to receive volatile anaesthesia/sedation in a much wider variety of places, therefore offers significant patient benefit via the possibility of pre-hospital medicine, local clinic based treatment, more rapid sedation recovery times and therefore higher patient throughput. The data generated by the experiments in this application will be used to support licencing the device as a product and beginning human clinical trials.
What species and approximate numbers of animals do you expect to use over what period of time?	Maximum of 30 pigs 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 most likely adverse events are physiological distress during the anaesthesia, slow recovery from anaesthesia or infection of the cannulation site (which is needed for blood sampling during the recovery period). Distress or slow recovery from the anaesthetic can be moderately severe; infection of the cannulation site would likely be mild. These events are unlikely as the surgery is performed under sterile conditions, and the animals are monitored frequently during the anaesthesia and during the recovery, so they would be recognised promptly. Animals will be killed after up to 96 hours recovery from the anaesthetic, or if signs of distress, pain or suffering cannot be alleviated by treatments such as analgesics or antibiotics.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Laboratory tests and patient simulators have demonstrated that the device delivers an adequate concentration of a modified formulation of anaesthetic which does not affect the viability of lung cells, and that other substances in the delivery gas are “carried-over” in very low concentrations. However, as anaesthesia involves multiple processes in the body acting simultaneously, the next step is to assess the device in a live multi-organ model which can be extrapolated to humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals will be obtained from a single supplier to minimise individual variability as far as reasonably possible. We will not use genetically modified animals. By taking repeated blood samples during the recovery phase of the experiments, blood chemistry and metabolite levels can be compared to the pre-anaesthetic measurements for each individual animal. This means each animal provides its own control data, meaning there is no need for an extra control group or interim termination timepoints.
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 pig is considered a good and well-established model for anaesthetic studies, and has a similar respiratory and circulatory physiology to humans and a larger blood volume than smaller mammals (e.g. rats or mice) for analysing blood chemistry and metabolites to investigate any toxicity. Animals will be acclimatised to their surroundings for at least 2 weeks before the experiments and will be given enrichment for their environment. Repeat blood samples during the recovery phase will be taken from a surgically implanted cannula, which will ease suffering and distress during blood sampling.

Project	**29. Autonomic control of cardiac excitability**	
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)	Many cardiovascular diseases (eg heart failure, high blood pressure, heart attacks) are also diseases of the nerves that speed up and slow the heart. during times of stress and rest, however, their impairment can trigger abnormal cardiac rhythm and cause the heart to stop beating. In particular, sympathetic activation, high levels of circulating adrenaline (which cause the heart to beat faster), and impaired cardiac vagal activity (which slow the heart down) are all poor indicators that increase the likelihood for sudden cardiac death. Yet the communication between the neuron and target heart cells is still poorly understood and remains	

	a major therapeutic target. Our research aims to understand how the chemicals released from these nerves control the rhythm of the heart during normal and abnormal activation. Specifically, we are interested in the intracellular chemical messenger systems in the nerves that control the release of neurotransmitters that speed up and slow down the heart. In particular we want to establish whether a fast acting chemical messenger is important in initiating a cascade of chemical events that regulate the activity of small pores on the cell membranes of nerves that affect the excitability of the heart. Importantly, we also want to establish where these changes occur, since both central (brain) and peripheral neural sites (close to the heart itself) have been implicated in cardiovascular disease.
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)?	If we can identify the key target proteins in the nerves from diseased animals then we can target the genes that encode these proteins with a gene transfer strategy. Current therapy on the heart cells themselves is still not particular effective. By controlling the release of chemical transmitters released from the heart nerves we may be able to minimise their ability to trigger arrhythmia in patients with cardiovascular disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the period of five years we expect to use 2000 mice, 3000 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?	We propose to maintain animals up to 12 months duration with minimal impact on animal welfare. All animals will be killed humanely by approved methods and tissue taken for laboratory analysis. The stress of handling the animal during killing is minor. We have refined our techniques to in vitro models where we have now replaced the need for in vivo work that has resulted in a reduction in regulated procedures, thus we believe this PPL has aspired to the best practise of the 3Rs.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To study the consequences of heart disease we need animal models. But we have developed *in vitro* studies that mimick the complexity of the disease state found *in vivo*. Taking tissue from animals is therefore essential for our understanding of the chemical components in the cell that lead to abnormal chemical transmission as we relate this back to the whole organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals required has been carefully determined based on previous experiments, and is the minimum required to achieve statistically significant results in our investigations. Everything has been done to ensure that the numbers of animals used are as small as possible. Through years of basic research experience, the applicant and his collaborators are familiar with the use of adequate statistical tests to ensure that a viable minimum is used. Power calculations are routinely performed by the applicant when experiments are designed. The minimum numbers of animals required have been carefully reviewed by the funding agencies and have been based on power calculations.
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 and RAT are the best models for studying autonomic phenotypes. Our major refinement is that we can now study phenotypes in a 'dish' and mimick the *in-vivo* setting thereby not require the use of regulated procedures. All animals will be studiedbefore clinical symptoms of the disease are present. That is for RATS all animals will be killed before 12 months of age, and for MICE before 20 weeks of age.

Project	**30. Bacterial infection in airways 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)	Asthma is the world's commonest long-term lung disease, affecting >350 million people. In the UK 5.4 million people receive treatment for asthma: that's 1 in 12 people. Asthma seriously affects quality of life: 1/3 of sufferers find asthma interferes with leisure activities and 1/4 with their ability to work or study. Asthma carries a massive economic burden, with 4.1 million lost working days in UK per year, despite £1 billion of NHS spending. Asthma remains a terrifying and potentially fatal condition, claiming 3 lives each day in UK. These deaths, and most symptoms are caused by 'exacerbations' also called 'asthma	

	attacks'. These are usually caused by viral or bacterial infections which activate immune cells of the airways to cause inflammation. However, the mechanisms underlying this inflammation are poorly understood and are considered the top priority for European asthma researchers to address. Clinical trials have shown that long-term use of an antibiotic (Azithromycin) reduces exacerbations in severe asthma. This is an exciting step forward but raises important questions which must be addressed. How does this drug work? Which asthma sufferers are most likely to benefit? Can we avoid giving it to those who won't benefit but may experience side effects? How long should treatment last? Can alternative, non-antibiotic treatments be equally effective but without risks of antibiotic resistance? How can certain bacteria – like '*Haemophilus'* – persist in inflamed airways in asthma and COPD? Our goal is to address these problems by asking several questions. Firstly, how do bacteria become established as infections in the airways of people with asthma? Secondly, what effects do they have on inflammation in the lungs? Thirdly, do bacteria in the airways make us susceptible to viruses? Fourthly, how does Azithromycin reduce inflammation in airways disease? And fifthly, can we reduce infection by boosting the immune system using vitamin-related molecules?
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)?	If we achieve our goal we will better understand the immune responses which protect us from lung infections. This is essential to defining which patients with asthma should receive Azithromycin and whether there are better alternative therapies. If alternatives can be used we can save this valuable medicine for other uses, reducing antibiotic resistance. If our proposed new technique to boost the immune system is effective, this could lead to development of improved vaccines for a wide range of lung infections, which cause 6% of global human disease.

What species and approximate numbers of animals do you expect to use over what period of time?	Our research group will use 12000 mice over 5 years. Some of these will be genetically modified strains with a particular susceptibility to the types of infections which interest us.
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 infections used are relatively indolent and chronic so – as in humans – they cause low-grade symptoms inflammation of the airways and mild weight loss. Animals will typically display short-lived signs of systemic illness including ruffled fur, altered movement, and body weight loss after administration of the infectious agent. The frequency of this adverse event depends on the infectious agent, the strain and immune status of the mouse. We expect all animals to experience no greater than a moderate severity level, and in reality, we expect a great proportion of animals to only experience transient clinical effects, before returning to normal. Many animals will also experience some additional procedures: blood sampling – withdrawal of a limited amount of blood from an accessible vein – and transfer of cells, by injection of cells into an accessible vein. These procedures can cause transient pain, bleeding or bruising, before returning to normal. Immediately before the end of the study many animals will have their lung function measured which is a procedure carried out under deep anaesthesia, from which the animals will not be awoken again and involves placing a monitoring device into the windpipe. At the end of the study, the animals will be killed and we will remove the relevant organs (lung spleen, lymph nodes) to assess the quality of the immune response throughout the animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The question being asked is a physiological one, which requires analysis of an immune response *in vivo*. Fundamentally the question as to the dominant mechanism of action of a particular antibiotic in this context is one which requires a fully intact immune system and whole respiratory tract. It is therefore not possible to address the importance of specific

	factors *in vitro*, even though we will maximise the use of *in vitro* assays to confirm key findings. Nonetheless we will model certain aspects of the disease *in vitro*, and are funded to do such work in parallel. If we have indications from these cell culture models which could lead to replacement of some of the planned *in vivo* work we would take advantage of this. We are also conducting parallel human studies using bronchoscopies. The experiments planned are only those where we cannot achieve the same objectives by analyses of human subjects. These data, including analysis of human innate T cell responses can be used to plan better experiments in the mouse model, which can then be used to address questions most relevant to the clinical setting.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental plan has been carefully designed with advice from animal research design experts and peer reviewed by internal committees within the establishment and externally at two funding bodies. We are breeding only animal numbers appropriate for the required experiments. Wherever possible multiple outcomes will be measured simultaneously to minimise animal numbers. Numbers are based on power calculations informed by preliminary data from our own previous research and relevant publications from other research 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.	This project requires mammalian species due to the similarities of the immune response and of airway physiology. The adult mouse is the most reliable and well-validated model of immune responses and is considered the mammalian model of lowest sentience. Furthermore, the pathogen being studied normally only infects humans, but the mouse strains we will use are intrinsically susceptible to this bacterium. Mice will be a scientifically more refined species than the previous dominant model system which uses the Chinchilla. Lastly, this work builds on extensive validation and optimisation work

	already performed in mice by our group. Each of the procedures described in this application have been tested and refined and extensively used previously by ourselves and other groups, to use less pathogenic strains of viruses, the lowest effective doses of bacteria and lowest effective doses of immune-modulating substances. Most importantly, by using carefully selected strains of mice which are susceptible to bacteria we will not need to use previously less successful approaches which required the use of powerful chemotherapy drugs to induce susceptibility. Nonetheless, currently there exists no more adequate or refined model of chronic bacterial airways infection and thus this ongoing programme of works has as a key objective development and refinement of this novel model through combining these procedures into validated, refined composite protocols. The choice of infective doses used is based on literature review of studies which have already refined the infection models to optimize endpoints and readouts, whilst minimizing suffering to the animals and ensuring sub-lethal doses. The animals will be closely monitored to detect disease progression and ensure animals are euthanized at the appropriate intervention point. Published guidelines for best practice will be followed. Attention will be paid to animal husbandry, including the provision of environmental enrichment and co-housing animals. Infected mice will be monitored regularly. and killed immediately if they reach clearly defined humane end points.

Project	**31. Behaviour and circuit dysfunction in rodent models of neurodevelopmental disorders**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neurodevelopmental disorders (NDDs) are caused by altered brain maturation due to genetic and environmental factors. Developmental brain disorders can be severely limiting, and there is an increasing diagnosis of NDDs in the UK population. Despite decades of research, current treatments for these disorders focus on managing symptoms and on behavioural therapy rather than on acting on the underlying cause. The unmet need, therefore, is to develop more effective therapeutics to more effectively treat NDDs. Rodent models of NDDs enable studies of the causes underlying these	

	disorders and support their research. However, a major obstacle for NDD drug development is the lack of robust measures in these models that are predictive of clinical efficacy. The overarching aim of this project is to address this challenge by using multiple established rodent models of NDDs that are of interest because of their similarity to corresponding human disorders to identify robust, clinically relevant measures that will allow the rigorous testing of potential therapeutic interventions for NDDs.
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 experiments in this project will yield valuable insight into the underlying causes of the cognitive and behavioural symptoms associated with developmental brain disorders. Furthermore, comparison between different rodent models of NDDs will determine whether different genetic disruptions lead to common disease states. Our experiments will also address the duration and timing of treatment in the appearance and improvement of brain-based deficits and the clinically-relevant behaviours that they lead to. Together, these findings may eventually facilitate classification of NDDs both in terms of their progression as well as their amenability to treatment across the lifespan; this would represent a major advance since current methods focus only on clinical or genetic criteria. Although the principal benefits of the project will be towards understanding NDDs, the methodologies developed in this work also have potential applications and benefits in the study of other developmental disorders and psychiatric diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate concentrating on rats (up to12500) with some work in mice (up to 900) 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?	Adverse effects arising as a consequence of neurological conditions such as seizures or movement problems in certain genetic lines are rare. Many animals will undergo multiple anaesthetics, surgical interventions and imaging procedures which may be stressful; unexpected consequences of these procedures are rare and we undertake careful monitoring so that if and when animals appear distressed or suffering we end the experiment. In

	some cases, we will test animals for performance on learning and memory tasks that involve inducing a memory by delivering a mild footshock, which may cause brief discomfort; on rare occasions, food and water restriction may be used to motivate behaviour. In all testing, attempts will be made to minimise the discomfort to the animals. The animals will always be killed painlessly at the end of study and, where appropriate, their brains taken for histological analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project will investigate the functioning of brain cells within the intact brain, and how brain changes relate to the behavioural changes we are assessing in rodent models of NDDs. Non-animal alternatives cannot provide information on how brain cells in the intact networks of the brain function and communicate to instruct behaviour. We have chosen to use rats and mice rats for these studies as they are the lowest order mammal in which these aspects of brain function and behaviour can be reproducibly assessed while also having the ability to perform genetic and environmental manipulations that cause NDDs in humans. Since these animal models of mimic many human symptoms of the NDDs, they allow us to tease apart processes and factors contributing to the underlying pathology and can be used to develop treatment strategies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is essential that the number of animals we use allows us to collect data that are robust. We have many years of experience in performing experiments using animals and this allows us to make predictions, using specific statistical approaches, about how big an effect we can anticipate and how variable that effect will be. Using this information, we can estimate the numbers of experiments we need to carry out. Good experimental design means that we assess drug effects alongside non-drug treatments or control animals with those obtained from genetically modified animals. Wherever possible, we will take multiple measurements in the same animal over time, across the lifespan, or pre and post treatment, to increase the statistical power of the dataset. We also encourage, where possible, that experimenters share tissue from the same animal which will reduce the overall number of animals required for
	experimentation.

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 the majority of our studies, we will use genetically altered rats as a model system in order to examine an intact mammalian nervous system because they have a rich behavioural repertoire and have extensive social interactions as well as adopt flexible approaches to novel situations, two domains specifically affected in autism and related NDDs. Animals will be housed in groups and cages will be enriched with tubes and objects for exploration to reduce stress and promote healthy social interaction. In all our experiments we are mindful of the need for refinement to reduce suffering, and appropriate modifications to protocols are incorporated where possible. Protocols will be carried out in the most humane way possible. All surgery will be performed under general anaesthesia, using aseptic technique and pain relief will be administered during recovery to minimise distress. Where possible, protocols to monitor brain activity in freely moving animals will make use of wireless transmitters to reduce distress that may occur from cables limiting movement. The animals in these studies will be cared for by trained staff within a well-resourced and well-equipped modern animal facility that maintains specific pathogen-free status/health; they will be carefully monitored to ensure that suffering greater than minor and transient in their home environment does not occur.

Project	**32. Biological Effects of Electromagnetic Fields**	
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)	People are increasingly exposed to a broad mixture of electromagnetic fields in the environment, both at home and work. Some of these are generated by power lines and electrical appliances, and others by mobile telephones and base stations. While there are exposure guidelines to limit exposure both at home and at work, there are persistent concerns that adverse effects may occur at ***low level*** exposures well below these guideline values a***nd further studies are required to address these possibilities. These adverse effects may, for example, affect different types of cancer induction, or the reproductive system affecting reproduction or fertility, or the nervous system, resulting in altered behaviour or brain function*** These include increased risks of some types of cancer, and changes in brain function leading to effects on memory and even neurodegeneration and dementia. There are also suggested effects ***of exposure*** on sleep/wake patterns	

	(circadian rhythms) ***which we will investigate as part of this project***. REDACTED previous work is reassuring in that it does not suggest exposure causes health effects, but these new studies will address **the possibility *that there are biological effects of electromagnetic field exposure, particularly from new and emerging technologies on potentially sensitive groups such as the young or elderly.*** further, and so help to provide more definitive answers. ***Many studies in the literature have tended to use high level exposures to electromagnetic fields to examine health effects, but in this project we plan to*** This project will use a range of electromagnetic field types ***and biological*** effects and mechanisms including cancer risk, cognitive and behavioural development, sleep/wake patterns, ageing and Alzheimer's disease, and reproductive effects in the foetus, children and adults. The project will use a wide variety of techniques to assess changes in cell death and damage to DNA, alterations in gene expression, and behavioural tasks that could reveal changes the structure or function of the brain.
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 information gained in this project will help to characterise more fully the potential of these fields to cause biological effects and will contribute towards an improved assessment of the health risks associated with exposure. The results will be published in peer-reviewed scientific journals and will contribute to filling gaps in knowledge about the health consequences of exposure. ***This project will use a range of frequencies and signal types that are commonly found in the environment and will address a spectrum of possible biological effects, health effects and potential mechanisms including cancer risk, cognitive and behavioural development, ageing and Alzheimer's disease in children and adults. The project will employ a variety of cellular and molecular techniques to study changes in DNA damage, gene expression, and sensitive behavioural tasks could reveal functional changes. It is not fully accepted that EMF exposure may have adverse effects. If certain EMFs are shown to be protective, this could impact on how neurodegenerative diseases could be treated. EMF treatment could be non-invasive may be preferential by some patients.*** ***We are particularly interested in the effects on EMF exposure on the brain and the effects on cognitive function. Data from different biological endpoints will be used together to understand if electromagnetic fields can cause biological effects. Endpoints to be***

	studied include learning, memory, and anxiety, and also changes in brain cells. It is intended that experimental outcomes will be presented at national or international conferences and it that results will be published and used by other scientific researchers. ***Results from this research may feed into wider research programmes, or Government departments to inform policy makers by informing risk assessment and advice given. Where funding is received from charitable organisations or other funders, it may link to patients when they may be a plausible link. Any relevant findings may eventually be used for policy discussions, and relevant data can be used to improve public health.*** ***Some work focusses on studies of circadian rhythm disruptions following magnetic field exposures and the possibility of sleep alteration. In these studies, behavioural data is usually generated as well as gene and protein expression data.*** ***In other work, different animal models of human disease, e.g., Alzheimer's disease (AD) might be used. These models take time to be developed and characterised. These models provide benefits to other researchers in the field of neurodegeneration and may ultimately provide benefits to AD patients due to knowledge of AD pathology and progression.*** ***Results obtained from these studies will contribute to filling gaps in knowledge about public exposures including the potential of low frequency fields to cause cancer and the behavioural effects of new technologies using high frequency fields. Together with information from similar experiments performed in other laboratories, these studies will help to improve the evidence on which to base limits for human exposure as well as advice for policy makers nationally and internationally. Furthermore, if there are no adverse health effects of exposure, this work will be valuable to provide reassurance to the public and workers.***
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 3500-4000 mice in total over the 5-year period. This is based on our experience of similar experiments, ***and this includes a combination of normal and genetically altered mice.***
In the context of what you	With the exposures and behavioural testing procedures we

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?	will use, we do not expect to see any major adverse effects, only very minor or subtle changes, such as alterations in learning or anxiety. Nevertheless, all experimental animals will be carefully observed for signs of stress or illness. The level of severity is moderate to encompass possible adverse effects, particularly those associated with ageing in mice. At the end of these studies, the mice will be humanely killed to collect tissue for studies to determine structural or inflammatory changes. ***The genetically altered animals will have a moderate phenotype, for example a model of Alzheimer's disease, will consist of an impaired performance in some behavioural tests, such as reduced mobility. Some animals have spontaneous seizure leading to sudden death which can occur without warning, but animals are closely monitored for signs of seizure, and any animal that is having a severe seizure will be humanely killed to prevent suffering.*** ***A few animals may be restrained for the moderate severity electromagnetic field exposure. They will be trained to the procedure to avoid stress.*** ***Most of the animals undergo non-stressful moderate severity behavioural testing, where the behavioural effects of exposure are assessed. Testing is not harmful to the mice, and a moderate level of behavioural testing is beneficial for the animals. Some mice will be housed individually, for example to measure their activity rhythms and eating/drinking behaviour. Where possible, these mice are returned to their home groups at the end of testing. Single housing does not appear to be harmful to the mice. Some mice will have their circadian rhythms altered, but changes will be carried out slowly to allow the mice to acclimatise with minimal stress.*** ***Surgical implantation of telemetry devices will be carried out under general anaesthesia and in aseptic conditions as a moderate severity procedure.*** ***At the end of all the experimental procedures, the animals will be humanely killed and tissues removed for analysis.***
Application of the 3Rs	
1. Replacement State why you need to use	It is not feasible to use cells or invertebrates in these experiments because they do not have a brain similar to

animals and why you cannot use non-animal alternatives	humans. Mice brains have anatomy and physiology similar to those of humans. We need to use alert, behaving mice that show a complete range of bodily functions, such as thinking, learning, sleeping, moving, eating and drinking. Overall, the data obtained with such models are more valid for health risk assessment than that provided by any alternative models and are more relevant to potential human risks. It is anticipated that other complementary ***cell based*** *in vitro* experiments will begin in the near future to investigate the effects of environmental agents on specific neuronal cell properties, rather than the whole animal *in vivo*, but behavioural studies will require alert animals with a complete physiological system. ***Less sentient animal models such as flies, worms or fish, do not have the same learning and memory processes as mice or humans. In addition, the structure of their biological tissues is different to that of mice and humans so they are not a good model to assess the effects of electromagnetic field energy absorption through different tissues.*** ***It is not possible to perform all of the procedures on, or collect many of the tissue samples required from human experimental studies.***
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals used will be reduced as far as possible following advice from statisticians to maximise the amount of information obtained during an experiment and to minimise the numbers of animals used. ***Experiments will be designed so that they can be published in accordance with guidelines on publishing work using animals. This includes randomly allocating mice to exposure groups, and blinding of samples prior to analysis which prevents bias.*** We follow a system of written operating procedures to ensure experiments are always carried out the same way, which should to maintain consistency and reproducibility in the results and help to reduce variability. In turn these should help to reduce the numbers of animals needed. The use of healthy animals, obtained from a registered supplier, should also help to avoid loss of experimental animals through disease. We will use specialised video recording to monitor and record the responses of the animals and this will help to significantly increase the potential data return for each animal used. Where possible, mice will be used

	for both behavioural testing and analysis of the brain after death, enabling two biological endpoints to be obtained per animal. By performing multiple analyses on tissue from one animal, we can make direct correlations between the findings. In studies using genetically altered animals, all littermates regardless of transgenic status will be included in experimental studies, ***using an inbred mouse strain helps to reduce variability between animals.***
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 have been chosen for these studies because they provide a very good model system to study the brain and cancer. There is a wealth of biological data concerning mice, and we have extensive experience of using this species in similar studies for many years. Furthermore, using mice maximises the potential for use of genetic markers and the potential for interpretation of results using genetic databases. We will only breed sufficient animals to meet our planned experimental needs, and the mice will be housed in enriched conditions with bedding material. We have optimised and refined the experimental protocols intended for use in this project, which will ensure that the number of animals used and the potential for any animal suffering is kept to a absolute minimum. ***However, we will continually review the scientific literature to assess whether alternatives are available and we will continuously review and amend our existing protocols accordingly where potential refinements may be identified.*** REDACTED Our laboratories are well equipped with state-of-the-art exposure and analytical instrumentation. We also work closely with the animal technologists REDACTED regarding the comfort and welfare of the animals. ***Where possible, electromagnetic field exposures will be undertaken without restraint of the mice.*** ***Many of the behavioural studies are undertaken using video capture and animals can be monitored remotely using this equipment.*** Behavioural tests will be performed and timed in a manner that is the least distressing to the animals. ***Mice will be limited to no more than three behavioural tests. In home cage monitoring studies, we have found from experience that altering the bedding material to a more absorbent material helps to reduce disturbance (and thus stress) to the animals.*** and also by minimizing the number of tests

	carried out per animal. ***Any surgical procedures will be undertaken using sterile conditions to avoid the risk of infection to the animals. This not only limits the harm to the animal but may confound our scientific results.*** None of the procedures proposed in these studies is anticipated to cause any ***lasting*** animal suffering or harm but if any deviation from normal is noted following any treatment or assessment, the animal will be monitored closely, and receive additional care, f***or example, extra feeding or analgesia*** as necessary, with the Named Veterinary Surgeon consulted if appropriate. ***If any animal fails to recover and exhibit signs of pain, distress or of significant ill health, animals will be humanely killed.***

Project	**33. Biological Testing of Potentially Biodegradable Biomaterials**	
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 aim is to identify polymer-based biomaterials which could be safely used inside the body for a range of purposes such as drug delivery or protection of implanted devices like pace-makers. These biomaterials will need to be biodegradable and not cause any damage to surrounding tissues. They are likely to come from bio-renewable sources, or be materials already approved for use in the UK, EU and USA. Following this project, materials showing potential would then be further developed and tested	

	before progressing to use in humans. Materials will first be selected using computer software (in silico) and cell culture (in vitro) testing before they are able to progress to work involving animals (in vivo).
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 development of biocompatible biodegradable biomaterials is field changing fundamental research that potentially revolutionises healthcare technologies by enabling the manufacture of advanced bioelectronic biomaterials well suited for short term applications (e.g. drug delivery and tissue engineering). The technology is at an early stage, needing further development (particularly with regards to biocompatibility) prior to being tested in patients in the clinic. In the short term, the outputs from this programme of work will be the identification of new biocompatible biomaterials that will then form the basis of further development either for clinical or research applications depending on the nature of the biomaterial. This could be in the area of drug delivery, tissue engineering, or biosensors. Thus the short to long-term benefits of identifying biocompatible biomaterials in this programme are worthwhile in the opportunities they represent to improve clinical practice in a broad range of areas. What we hope to ensure through good practice in this licence is that the numbers of animals and severity of procedures used is kept to a minimum through good experimental design.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice (up to 500) 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	Mice will have the biomaterial under investigation implanted under the skin. Following the in vitro work carried out before this step, adverse effects are not expected to be seen. However, if any implantation site becomes clearly sore, inflamed, infected, ulcerated, or if other unexpected side

the end?	effects are observed which indicate a lack of biocompatability, then the animal will be humanely killed by a competent person. Once a procedure has finished then the animal will be euthanised by a Schedule 1 procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The majority of the work carried for biocompatability testing of biomaterials is done *in silico* or *in vitro*, which can give useful information on how a material is going to interact with specific cell types in isolation. However these assays do not take account of how the material behaves in a complex system, i.e. are there interactions with surrounding tissue, circulating immune system cells and systemic toxicity away from the implantation site due to material degradation. Thus it is necessary to evaluate the material in a living organism since the ultimate goal of this work will lead to the development of materials which will eventually proceed to the clinic. Ethically it does not warrant proceeding directly from formulating a biomaterial in an *in vitro* laboratory to evaluation in clinical trials in human patients, and throughout the world there are regulations controlling the screening of a treatment *in vivo*. Therefore some *in vivo* work on experimental animals has to be carried out before progress to the clinic, although through adherence to the 3Rs philosophy and good experimental design, the numbers of animals used is kept to the absolute minimum with the minimal amount of suffering to obtain statistically significant results which will aid progress of an agent into the clinic. Replacement strategies which are adopted include the *in silico* and *in vitro* tests as outlined above, and extensive evaluation in these assays will be carried out prior to consideration for *in vivo* screening.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have previously demonstrated that statistically robust data can be obtained for histological examination of tissues using 3 animals per data point, and we would maintain

	this low number in the experimentation here In assessing for signs of an immune response we will take repeat tail bleeds from long term animals, rather than sacrificing animals specifically for this purpose. The small blood sample taken at each time (no more than 20µl) will be sufficient to prepare smears to check for irregularities in the differential white cell counts.
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 least sentient and most frequently used mammal for *in vivo* biocompatability testing. Although biocompatibility evaluation techniques are well established in lower species, such as the zebrafish, since these materials will ultimately be used in mammals, it is essential that these models are established in the same class. Analgesics and anaesthesia will be used where required, and we will take advice relating to care and welfare via the NVS, the AWERB, and other appropriate sources. Animal suffering will be minimised by always using aseptic technique and we will commit to the LASA Guidelines as set out in 'Guiding Principles for Preparing for and Undertaking Aseptic Surgery' (2nd edition, April 2017).

Project	**34. Biology of Normal and Malignant Blood Cells**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our project aims to model key components of the infection-based mechanism by which childhood leukaemia arises. Our focus for the next several years is to finish prior studies of assay of human leukaemia stem cells in mice and to focus on a model in mice the prevention of leukaemia that is triggered by common infections. Over the past three decades, we have generated a body of data indicating that the most common form of paediatric cancer – childhood acute lymphoblastic leukaemia (ALL) is triggered by an abnormal immune response to common infections (e.g. respiratory infections such as ‘flu). But this only happens in children who are	

	susceptible by virtue of (i) have acquired, as a developmental accident, a leukaemia-initiating mutation in utero; and (ii) have, paradoxically, a deficit of microbial exposures in the first year of life. The latter, principally in the form of benign or beneficial microbes in the gut, are required to prime the new born immune system for proper networked function. We now have a mouse model for this scenario. Mice transgenic for the common leukaemia-initiating gene in patients (ETV6-RUNX1) born in an SPF facility develop leukaemia if moved at 5-8 weeks to a conventional housing facility with endemic pathogens. This gives us a model and opportunity to test the hypothesis that deliberate exposure of our transgenic mice under SPF conditions to the gut microbiome of more 'dirty' mice protects them from leukaemia. Gut microbiome transplants are already in clinical use for some adult illnesses and our mouse modelling could provide a strategy for preventing childhood leukaemia.
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)?	If successful, these modelling experiments with mice will endorse our longstanding infection-based model for the causation of childhood leukaemia. This would, in turn, encourage a search for a prophylactic intervention (microbiome-boosting) that would prevent childhood leukaemia.
What species and approximate numbers of animals do you expect to use over what period of time?	we plan to use 1,530 mice 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?	Procedures involve injections of small volumes of cells into mice, either in the tail vein or directly into a leg bone (tibia or femur) under anaesthetic. No adverse effects will arise as a direct result of the injections. Some of the cellular injections will give rise to leukaemia which, if left untreated, would cause significant morbidity and eventual death. However, we serially monitor the blood of mice and check health daily. If leukaemia

	develops and/or the mice show any signs of distress, they are immediately culled. Some mice may be whole body irradiated prior to cellular injection. At the sub-lethal dose used, we anticipate no harmful effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	90% of our research on childhood leukaemia has been conducted directly on clinical samples. However to validate our model for the causation of leukaemia and the role of infections, we require an appropriate in vivo model. There is no in vitro system that can mimic the process of leukaemia formation, driven by infection.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use mice strains that have been genetically engineered to be profoundly immune-deficient, mice transgenic for a human leukaemia gene and their wild type equivalents, as controls. For both ethical and financial reasons, we plan to use the minimum number of mice necessary. For all mouse work combined, we have estimated a total maximum usage of 1530 animals. It may be significantly less than this. We use three mice in each replicate group, the minimum acceptable for biological consistency.
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.	No adverse effects are anticipated (or seen to date) via the initial irradiation of mice or injection of cells. Daily surveillance of the health status of mice and weekly assessment (via tail bleed) of the development of leukaemia ensures culling before the onset of any severe morbidity.

Project	**35. Bio-synthetic corneal endothelial grafts for transplantation**	
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)	The cornea is the clear window at the front of the eye. The innermost layer of the cornea is a single layer of cells called the endothelium whose main function is to pump fluid out of the cornea preventing it from swelling and losing transparency. In some diseases, the corneal endothelium is abnormal or damaged and does not function properly leading to corneal swelling and loss of vision. Currently the only treatment for this problem is removal of the damaged layer and replacement with a new layer from a corneal donor; one donor cornea treats one patient. There is a global shortage of corneas for	

	transplantation, so cultured endothelial therapies are the future for this field. The expansion of corneal endothelial cells in the laboratory onto a suitable material will enable creation of bio-synthetic endothelial grafts. Several bio-synthetic endothelial grafts will be produced from each human donor cornea so patient waiting times for this sight saving operation will be reduced. We would like to test our novel hydrogel to see if it would be a suitable carrier for endothelial cell transplantation. The aims of this study are to determine to what extent the cultured endothelial cells on our hydrogel are functional and whether they are able to restore transparency in a model of endothelial damage where the cornea has become cloudy due to swelling. We need to do this in an animal model to test the safety of the therapy before moving on to studies in humans. We will do a series of experiments in the laboratory first so that we can minimise the number of animals we will use.
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)?	If the results of this study are favourable then it suggests that it would be feasible to pursue the development of our technology as a new therapy for corneal endothelial disease. This would produce a major advancement of the technology towards the clinic and could mean that a first in human trial would be possible within 5-7 years. Shortly afterwards Fuchs' endothelial corneal dystrophy and pseudophakic bullous keratopathy patients would be the first to benefit from decreased waiting times for sight saving operations.
What species and approximate numbers of animals do you expect to use over what period of time?	In this study we will use 15 rabbits and they will be housed for a period of approximately 6 weeks in total with a maximum experimental time of 4 weeks. We may need to undertake other similar studies over the life of the project licence but the maximum number of animals used will be 45.
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 rabbits will be undergoing eye surgery in a procedure that is very similar to that in human patients performed under general anaesthesia. Analgesics will be given to minimise post-operative discomfort, which should resolve within

the end?	approximately 72 hours. The welfare of the animals will be closely monitored. We expect a moderate severity. Where pain relief does not relieve the animal's symptoms this animal will be humanely killed. After 4 weeks rabbits will be humanely killed and their eyes analysed.
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 completely replace the use of animals in this study as we need to test our bio-synthetic graft in a relevant complex model before moving to clinical trials in humans, the next step in the translational pipeline. The rabbit is a suitable model due to its large eye size relative to its body and its anatomical similarity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Initial experiments will use porcine/rabbit tissue taken from abattoirs or surplus tissue from other experiments to refine our transplantation and cell culture techniques. This will allow us to limit the number of experimental groups, and so animal numbers, that we require for the live animal experiments. Statistical design has been conducted to determine the lowest number of animals required to produce meaningful results.
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 rabbit as our model due to its similarity to the human cornea in terms of corneal layers and dimensions. Eyes of laboratory rodents are too small for the highly- specialised surgical instrumentation required for this procedure. Consequently use of rodents would limit the ability to achieve the scientific aims of the project. Rabbits are the lowest species able to provide the globe size and structure for this work. All surgical procedures will be optimized using rabbit eyes (surplus from other animal experiments) and cadaveric rabbits prior to animal surgery, which will reduce the risk of complications occurring in the in vivo animals. All surgery will be performed by a consultant ophthalmologist who is highly trained in the transplantation procedure, performing similar procedures on a weekly basis in patients. This means that the rabbits will receive similar care and attention to that which a patient would
	expect.

Project	**36. Bispecific antibodies for use in cancer therapies**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This programme of work aims to support the discovery and development of therapeutic antibodies that are engineered to bind to two different targets simultaneously as new medicines to treat cancer patients and aims to: 1. Develop and establish animal models of cancer to allow testing of bispecific antibodies 2. Select the most promising bispecific antibodies to progress into further development	

	3. Develop a detailed understanding of the biology of bispecific antibodies to support clinical trials in cancer patients 4. Perform experiments to identify how best to treat patients with the bispecific antibody. This may include determining the right dose of the drug, selecting drugs to use in combination therapies and identifying factors that can be measured to monitor activity of the drug
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 the development of bispecific antibodies and aims to advance a number of potential new medicines into clinical trials for patients with advanced cancer, who otherwise have limited treatment options. Specifically, the work performed under this project will develop animal models of cancer and perform testing of potential new drugs to increase the chances of bringing effective new medicines to patients. This is a proven methodology as similar lines of experimentation have been successful in recent Progress with immunotherapy in the clinic. This project will also advance our understanding of the basic science behind these drug discovery programmes. Work from this project will be presented at conferences and published in peer-reviewed journals wherever possible. This will help increase knowledge around bispecific antibody engineering, cancer research and immunology for the benefit of the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the next 5 years of the Project Licence we estimate to use about 18,200 animals described in this licence. This estimate is based on the development of 4-5 new medicines being delivered into the clinic and about 5 new molecules being developed for future translation into the clinic.
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	To test the anti-cancer efficacy and potency of antibodies or to predict how the antibody will behave later in patients, in some cases mice will be injected with cancer cells under the skin that, when grown, will be treated with novel bispecific antibodies. In other cases, mice with or without

the end?	a tumour will be injected with the antibody to investigate how long the antibody is in the body or what the antibody does to the body or tumour. The maximum severity experienced by animals under this project will be moderate. Suffering will be minimised wherever possible and it is anticipated that the majority of animals will experience mild or moderate severity. Expected adverse effects from cancer models can include ulceration of externally implanted tumours. At the end mice will be humanely killed by a trained and competent person working under the authority of this project licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal experiments are required to recapitulate the complex process that occurs as cells of the immune system circulate throughout the body and interact with a tumour. Experiments assessing the effect of the body's metabolism on a drug (pharmacokinetics) cannot be performed in non-animal systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Small pilot studies will be performed to ensure that animal tumour models are robust prior to performing larger experiments. Statistical analysis will be used to ensure that only the minimum number of animals required to address the aims of the study are used. For studies using blood sampling, techniques have been established to ensure that very small 'microsamples' can be analysed to allow fewer animals to be used to address scientific questions.
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 will be used as their immune system closely resembles that of humans and has been studied extensively for cancer research. Mice are small and can be genetically manipulated. Studies in mice have been shown to yield reproducible results in our laboratory and other labs with respect to the control of tumour growth by the immune system. Harm will be minimised by performing the least invasive and minimum number of procedures over the shortest time-frame required to achieve

	scientific objectives. The experiments are performed by experienced staff that are well trained to spot adverse events and to reduce stress for the mice. To further improve animal welfare, mice are housed in groups and environmental enrichment in form of nesting material and seeds for foraging is used.

37. Brain development and function

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Mouse, Development, Cerebral cortex, Neuron, Brain function

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

The cerebral cortex is the nerve tissue that covers most of our brain. It consists of several billion neurons, and it is folded to fit inside the skull. The cerebral cortex enables us to perceive the world around us, to think and make decisions. Unfortunately, we have a very vague idea of how it works. Our project aims to understand the general principles governing the formation of the cerebral cortex. Over the next five years we will concentrate our efforts on the molecular and cellular mechanisms controlling the precise allocation and function of different classes of neurons in the cerebral cortex. We would like to understand how much of the information needed in this process is genetically encoded, and what is the influence of the environment in the formation of this complex brain structure.

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?

Understanding the development of the cerebral cortex will help us to decode its function, in health and disease. By studying how the brain develops we ultimately aim to understand human disease, because disruption of the normal development of the cerebral cortex causes disorders such as autism, epilepsy and schizophrenia.

Species and numbers of animals expected to be used

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

The cerebral cortex is a brain structure that has changed very rapidly during evolution, but its general organization is basically the same in all mammals, from rodents to primates, including humans. For example, the cerebral cortex in all mammals consists of hierarchical networks of excitatory and inhibitory neurons. Mouse genetics (i.e., the production of genetically modified strains of mice) allow us to manipulate the function of specific genes that control the development of the cerebral cortex, many of which have been linked to disease in humans. We plan to breed approximately 25,000 mice over the next five years, of which we will use about 60% of them for experiments because the generation of specific genotypes of genetically altered mice is accompanied by the generation of mice with undesired genotypes.

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?

To reach our goals, we will breed and maintain genetically altered mice, which we will subsequently use to perform experiments both in vitro (neuronal cultures) and in vivo. This later set of experiments will include post-mortem analyses (e.g., immunohistochemistry, biochemistry), in utero and neonatal manipulations of mouse embryos, behavioural analyses and electrophysiological recordings in adult animals under terminal anaesthesia. These experiments are expected to have mild or moderate levels of severity. In addition, we will carry out experiments that will involve electrophysiology or imaging methods in mice following recovery from surgery and, in some cases, we will test the susceptibility of mice to develop epilepsy. These experiments are expected to have a severe effect on the animals. Animals will be killed at the end of our experiments.

Replacement

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

It is currently inconceivable that we could generate computer models to advance our understanding of brain development and the disorders that affect this process.

Reduction

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

My research project is designed to minimise the number of animals used to obtain statistically significant results when testing our hypotheses, including the use of factorial experimental designs. We use a highly developed database that aids us in obtaining the precise number of transgenic mice required for our experiments. In addition, we collect the brains of all the mice that we used in our experiments (including many from mice that are

only used as breeders) and we store all the relevant information in a database. Because the mouse brain is relatively large and can be sectioned in hundreds of slices, in many circumstances we can use some of this tissue for pilot experiments in which we test novel reagents such as antibodies. This reduces sensibly the final number of animals that we need to use.

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 genetically modified mice to study the function of genes in specific neural circuits during development. For this purpose the mouse is the best possible animal model because their genome is relatively easy to manipulate, and their brain develops in a similar manner to humans. The use of conditional mouse mutants, in which only a specific population of neurons lacks the gene of interest, provides the most accurate results and hence refinement.

To improve the welfare of the animals, anaesthesia, analgesia and general protection will be provided to the mice to avoid any suffering prior to manipulation or sacrifice for the experimental procedures, using approved methods.

38. Brain development and function

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Mouse, Development, Cerebral cortex, Interneuron, Brain function

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

The cerebral cortex is the nerve tissue that covers most of our brain. It consists of several billion neurons, and it is folded to fit inside the skull. The cerebral cortex enables us to perceive the world around us, to think and make decisions. Unfortunately, we have a very vague idea of how it works. Our project aims to understand the general principles governing the formation of the cerebral cortex. In particular, over the next five years we will concentrate our efforts to elucidate the molecular mechanisms controlling the precise allocation and function of different classes of neurons in the cerebral cortex. We would like to understand how much of the information needed in this process is genetically encoded, and what is the influence of the environment in the formation of this complex brain structure.

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?

Understanding the development of the cerebral cortex will help us to decode its function. By studying this process we also aim to understand human disease, because disruption of the normal development of the cerebral cortex causes disorders such as autism and epilepsy.

Species and numbers of animals expected to be used

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

The cerebral cortex is a brain structure that has changed very rapidly during evolution, but its general organization is basically the same in all mammals, from rodents to primates, including humans. For example, the cerebral cortex in all mammals consists of hierarchical networks of excitatory and inhibitory neurons. For this reason, we use mice as experimental model. Mouse genetics (i.e. the production of genetically modified strains of mice) allow us to manipulate the function of specific genes that control the development of the cerebral cortex, many of which have been linked to disease in humans. We plan to breed approximately 20.000 mice over the next five years, of which we will only use ~60% for experiments because the generation of specific genotypes of genetically altered mice is normally accompanied by the generation of mice with undesired genotypes.

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?

To reach our goals, we will breed and maintain genetically altered mice, which we will subsequently use to perform experiments both in vitro (neuronal cultures) and in vivo. This later set of experiments will include post-mortem analyses (e.g. immunohistochemistry, biochemistry), in utero and neonatal manipulations of mouse embryos, behavioural analyses and electrophysiological recordings in adult animals under terminal anaesthesia. These experiments are expected to have mild or moderate levels of severity. In addition, we will carry out experiments that will involve electrophysiology or imaging methods in adult animals following recovery from surgery, and in some cases we will test the susceptibility of animals to develop epilepsy. These experiments are expected to have a severe effect on the animals. Animals will be killed at the end of our experiments.

Replacement

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

It is currently inconceivable that we could generate computer models to advance our understanding of brain development and the disorders that affect this process.

Reduction

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

My research project is designed to minimise the number of animals used to obtain statistically significant results when testing our hypotheses, including the use of factorial experimental designs. We use a highly developed database that aids us in obtaining the precise number of transgenic mice required for our experiments. In addition, we collect the brains of all the mice that we used in our experiments (including many from mice that are only used as breeders) and we store all the relevant information in a database. Because

the mouse brain is relatively large and can be sectioned in hundreds of slices, in many circumstances we can use some of this tissue for pilot experiments in which we test novel reagents such as antibodies. This reduces sensibly the final number of animals that we need to use.

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 genetically modified mice to study the function of genes in specific neural circuits during development. For this purpose the mouse is the best possible animal model because their genome is relatively easy to manipulate and their brain develops in a similar manner to humans. The use of conditional mouse mutants, in which only a specific population of neurons lacks the gene of interest, provides the most accurate results and hence refinement.

To improve the welfare of the animals, anaesthesia, analgesia and general protection will be provided to the mice to avoid any suffering prior to manipulation or sacrifice for the experimental procedures, using approved methods.

Project	**39. Brain networks for memory and executive function 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)	Our ability to deal with environmental changes depends upon regions of the human brain known to guide behavioural choices. These choices often result in better outcomes when we can recall memories of experiences associated with past success or failure under similar circumstances. The principal aim of the current project is to discover how brain regions that store memories and those that act to produce these choices co-operate to guide behaviour. We will do this by studying the brains of mice and rats and we will also investigate how damage to these brain regions may lead to behavioural deficits and	

	whether current and new drug treatments might halt or reverse this damage. To do this we will use animal models of different human mental disorders where damage to these areas of the brain is well established (e.g., Alzheimer's disease and schizophrenia). We will record brain activity and use various behavioural tasks in our animals to understand how brain regions interact to produce complex behaviour and how this interaction breaks down in human diseases.
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)?	We will add considerable knowledge to our understanding of the brain circuits responsible for many of the most important behaviours in man. We will also gain considerable insight into how these circuits deteriorate in common mental disorders and how current or new drugs can help to halt or cure these devastating human conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use normal and disease-model rats and mice. We will require approximately 5000 animals 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 studies will involve recording brain activity while the animals are under anaesthesia from which they will not recover and so cause minimal harms. Other animals will be trained in various behavioural tests and some will have recording devices implanted into their brains under anaesthesia so that following recovery we can record brain activity using these devices as the animals repeat the tests. If long term dosing of drugs under test is required, animals may have drug delivery devices implanted. Some animals will also have genetic changes bred into them that produce many of the symptoms seen in human patients that suffer from Alzheimer’s disease. These models are well established and cause minimal harm to the animals apart from the Alzheimer’s-like changes in memory and behaviour that we wish to understand and help treat. Harms will be kept to a minimum through the use of pain relief and a defined monitoring regime for any clinical signs of distress, which, if present, will lead to the animals being humanely killed. At the end of
	the study animals will be humanely killed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is impossible to mimic brain and behaviour interactions in cell systems, so studies using live animals are vital to obtain a greater understanding of normal and abnormal mental states and to test the effectiveness of new drugs. This work must use whole animals, as understanding behaviour and the required brain activity to produce that behaviour is a central feature of the project. This cannot be studied effectively by using reduced in vitro preparations, and computational approaches lack the required complexity due to insufficient biological data. To date, there is no suitable alternative to the use of rodents for behavioural studies that do not involve human subjects and we are still extremely limited in our ability to measure neural activity directly from the human brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that we use the minimum number of animals through careful design of studies, minimal animal handling by researchers to reduce stress, making sure that animals are accustomed to any testing arena before a study begins and providing good researcher training. We will monitor the reliability of our studies closely and alter group sizes as appropriate and in consultation with statistical experts. We are working closely with colleagues to develop behavioural tests that improve data yield to reduce animal numbers further by minimising the potential negative effect of animal handling in our 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.	This project aims to reveal the complex interaction between brain regions vital for behaviours such as making decisions and recalling memories of life events. We will use rodents, as these are lower species yet show remarkably similar behaviour and brain organisation to humans. Our rat and mouse models of human diseases show symptoms

	such as memory loss and brain damage that are very similar to human patients. Knowledge gained through our previous studies means we already know when to expect these changes to start in these models, so we can target specific animal ages in order to refine our studies. None of the models that we will use are expected to experience severe side effects; however, if seen in any animal it will be humanely killed. We will use small implanted pumps for drug delivery. Although this requires a surgical step it has less overall negative impact on the animal. This is because pumps remove the need for repeated daily dosing and allow drug levels to remain stable over the entire dosing period.

Project	**40. Brain Regions In Learning, Memory, and Motivation**	
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 will determine how the cells of our brain's internal spatial map communicate with each other to allow us to navigate and to remember places and what occurred in them. Importantly, we will study the relationship between the hippocampus (the area of the brain in which these cells are located) and other brain regions. The hippocampal formation is one of the first areas impaired in Alzheimer's dementia and we will use our knowledge of the functions of these brain regions to study the disease processes in mouse models of this disease.	

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)?	An understanding of how our brain's GPS operates will provide fundamental insights into how this part of the brain operates in normal animals and humans. The hippocampal formation is one of the first areas impaired in Alzheimer's dementia and we will use our knowledge of the functions of these brain regions to study the disease processes in mouse models of Alzheimer's Disease. We will also use our knowledge of how animals and humans solve spatial memory and navigation problems to construct sensitive spatial tests designed to identify the earliest stages of Alzheimer's dementia in people at risk of the disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use both mice and rats. Most of what we know about the functions of the hippocampal formation comes from work on rats. Mice offer the advantage of allowing genetic manipulations to identify cells and modify their activity. We estimate to use 6300 mice (of which 5000 will be for the breeding and maintenance of genetically altered mice) and 900 rats (of which 200 will be for the breeding and maintenance of genetically altered 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?	Rodents will be trained on behavioural tasks, in the vast majority of which they will be rewarded with food for correct performance. Some animals will have tiny electrodes implanted into their brains or brackets for holding measuring devices affixed to their skulls. In some cases, drugs will be injected into their brains or damage made to small areas of the brain in order to assess the role of these brain regions in spatial and other types of learning and memory. All operations are carried out under deep anaesthesia and animals are given painkillers to minimise pain and discomfort during recovery. Rats and mice usually recover within a day, as assessed by normal consumption of food, running on wheels and building a nest in their home cages. It can take 5 days for the rats to recover from surgery when a brain region is damaged, during which time they mostly rest and sleep. In all cases animals will be given sufficient time to recover. Recordings of brain activity are taken from animals as they perform in spatial and non-

	spatial learning tasks. The brain has no sensory receptors and thus the animals are unaware of the brain recordings. Stimulation of brain areas is not intended to cause pain or discomfort but to elicit natural behaviours or to modify such behaviours. Some experiments require mice to be head-fixed and running on an air-suspended ball while performing a task in virtual reality. This procedure is preceded by 2-3 days of habituation to minimise animals' stress. Some animals show transient dislike of head fixation via a brief increase in urination and/or defecation which disappears several minutes after the first exposure. Food reward is then used to encourage navigation reaching desirable performance after 3 to 5 days of experience. Once familiar with head- fixation, mice willingly explore virtual environments, similar to what they do on running wheels in their home cages. A small percentage of animals may not acclimatize to restraint within 5 days and these will be removed from the task. At the end of the experiments or if the animals show signs of ill health, distress or suffering, they will be deeply anesthetized and perfused so that we can examine their brains or be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use rodents because it is not possible to study the role of the hippocampus and related brain structures in real world navigation without using behaving animals. We will use cadavers when living tissue is not required and unconscious anesthetized animals to study brain conductivity and other aspects of hippocampal function which do not require behaviour. It is possible to genetically modify mice to replicate some of the pathological processes underlying Alzheimer's dementia and to use these animals to study the biological mechanisms of Alzheimer's. By studying the pathological anatomical and physiological changes in the brains of these animals and the effect of these on the animal's ability to use its spatial mapping system and remember places we hope to understand some of the mechanisms of

	Alzheimer's. Where possible, we use computational modelling to direct our experiments and to minimize animal numbers.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers are minimised through continual technical developments which enable us to monitor the activities of larger numbers of brain cells in each animal, through the development of more sophisticated behavioural tests requiring fewer animals, and through the use of computational models that enable us to make highly specific testable predictions about the role of hippocampus and other structures in spatial memory and navigation minimizing the number of experiments required to reach a conclusion. Most procedures involve long term experimentation with the same animals which significantly reduces the number of animals needed to reach statistically significant conclusions.
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 and mice are used in these studies since they are very good at navigating in familiar environments and remembering what has happened to them there. We know a lot about the anatomy and physiology of their brains and in particular of the parts of the brain to be studied in this project. They are the most widely used experimental animals for combined behavioural and electrophysiological studies; furthermore, most of the detailed anatomy of the hippocampus has been elucidated in these species. Four major classes of spatial cell (place cells, head direction cells, boundary and grid cells) were discovered in these animals. While rats are docile and display high levels of spatial memory and navigational abilities, using mice offers unmatched access to genetic tools, allowing us to induce specific gene mutations relevant to diseases such as Alzheimer's disease, as well as using genetic techniques to record from and manipulate functionally/genetically/anatomically defined ensembles of cells. It is possible to genetically modify mice to replicate some of the pathological processes underlying Alzheimer's dementia and to use these animals to study the biological mechanisms of

	Alzheimer's. By studying the pathological anatomical and physiological changes in the brains of these animals and the effect of these on the animal's ability to use its spatial mapping system and remember places we hope to understand some of the mechanisms of Alzheimer's. We will use unconsciousness anesthetized animals to study brain conductivity and other aspects of hippocampal function which do not require behaviour. We will use cadavers instead of live animals wherever possible. Furthermore, we use computational modelling to direct our experiments and to minimize animal numbers. Optimal results in behavioural experiments require that the animals are healthy, in good spirits, and motivated to perform well. For this reason, the majority of our behavioural tests involve food and liquids as rewards. We also use minimum levels of food restriction to motivate the animals to work for these rewards. Professional operative techniques and post-operative care ensure a minimum of adverse effects and the minimum level of suffering from any surgical or other procedure. Animals under active testing usually live in enriched environments with ample space and several toys which are chosen to be compatible with the experimental procedures. Because both rats and mice are highly sociable animals, the animals are housed in groups in large home cages where possible.

Project	**41. Brainstem circuits controlling gut-brain communication**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	2 Years 10 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 objectives of this Project Licence are centred on the regulation of the gut-brain interaction, and how visceral information are received and computed by discrete groups of nerve cells to maintain health. To achieve this, we take advantage of genetic engineering techniques to precisely interrogate the function and the chemical content of discrete brain cells and to correlate these specific cell manipulations to changes in brain or gut health.	
What are the potential benefits likely to derive from this project (how science could be advanced	The primary benefit of the work will be the advancement of scientific knowledge on the logic by which the brain compute and store messages	

or humans or animals could benefit from the project)?	sent by the gut. These knowledge will be directly relevant for human and animal health and welfare. For example, in humans there is high co-morbidity of obesity, gastrointestinal and psychiatric diseases (i.e. anxiety, obsessive compulsive disorders, and depressive states). Understanding how the communication between the gut and the brain is regulated may have implications for the health and wellness of animals (including humans), in that it will provide an evidence base for the development of novel pharmaceutical, behavioral or nutritional intervention to treat or diagnose brain and gut diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	This program of work may use 4000 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?	Mice may be injected with physiological doses of hormone or pharmaceutical agents. This is a mild procedure that very rarely promotes adverse effects. In some cases, treatments may lead to reduced body weight. Some mice may experience surgery. Possible pain is minimised by good surgical and aseptic techniques, suitable anaesthesia, good perioperative care and adequate provision of pain relief. This is a moderate procedure. Likewise, some mice may experience gastrointestinal discomfort when subjected to experiments mimicking the human intestinal bowel disease. All other procedures involve the monitoring of natural behaviour, including eating habits; procedures that never promote adverse effects. Any animal showing any signs of ill health will be closely monitored, receive veterinary treatment or will be humanely euthanized. At the end of testing, mice will be humanely euthanized for tissue and data collection.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are the only model for the proposed licence because it is not possible to study gut-brain communication using cultured cells or computer modelling. At present, to understand how these two distinct, yet tightly connected, body

	districts interact requires the use of live animals. However, the research programme seeks to identify means for replacement by incorporating mathematical modelling when available, and basic knowledge that will be acquired with this program of work also have the potential to facilitate replacement.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Sample sizes will be based on statistical power analysis from several prior experiments and power calculations conducted with a statistician. Sample sizes will be reduced to include the minimum number of mice necessary. Built into the experimental design and dissemination of the results are the ARRIVE guidelines established by the NC3Rs. Measures taken to avoid unjustified duplication of procedures will include close monitoring of literature; conference attendance and discussing current procedures with colleagues and veterinary staff.
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 species of choice. There is a large scientific literature on this species and other alternative models do not allow for precise genetic manipulations of brain cells during enacted behaviour. Refinements for injections include the implementation of scoring sheets and humane endpoints. Pain and suffering is minimised by good surgical and aseptic techniques, suitable anaesthesia, good peri-operative care and adequate provision of pain relief. To prevent duplication of experimentation, scientific conferences are attended and discussion held with colleagues. The scientific literature is continually reviewed and veterinarians consulted for alternative surgical treatments and novel means to alleviate adverse effects.

Project	**42. Breeding of Transgenic rodents for supply of tissues to the client**	
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
What's the aim of this project?	To provide a service to clients by breeding Genetically Altered animals and maintenance of the colony for research purpose for supplying the tissues.	
Why is it important to undertake this work?	Applicant has vast experience in breeding Genetically Altered Animals and thus providing a centralised service, managed with technical expertise to the client/s who do not have the facility to breed the animals required for the research purpose and/or are new to the breeding of genetically altered animals. This will help to generate income and to attain financial independence and direct benefit to animal welfare and science, reduction in transport time.	

What outputs do you think you will see at the end of this project?	The output for the PPL holder is to ensure meet the demands of the client for supply of animals/tissue and share the expertise of breeding rodents with the clients and generate the revenue for the facility. The offspring produced as a result of this project will be used by researchers/client using the facility to achieve their personal scientific goal. The long-term objective of the project is to use the appropriate method for genetic modification as a part of the multi-client facility to advance understanding of pathophysiology processes and to contribute to our in-vestment in scientific research by providing relevant models for human diseases.
Who or what will benefit from these outputs, and how?	By providing this service PPL holder will be able to generate the revenue for REDACTED. Clients are acquiring mice rederived by the REDACTED in which a gene of interest has been deleted. This gene is specifically expressed in a subpopulation of brain cells and we are trying to understand whether this gene is relevant in neurodegeneration.
Will this work be offered as a service to others?	Yes
How will you look to maximise the outputs of this work?	The colony will be managed by the experience member of the team and will ensure the breeding is carried out efficiently to meet the demand. Their will be monthly discussion on the breeding performance of the strains and any issue e.g. pre-weaning mortality will be discussed in depth.
Explain why you are using these types of animals and your choice of life stages.	We are using all life stages of the animals under this project. Genetically altered animals, particularly rodents, are in widespread use in biomedical and veterinary science and invaluable for elucidating the function of genes and the pathways in a wide variety of biological, physiological and pathological processes. The primary aim of the REDACTED facility is to supply tissues by breeding and maintaining rodents with genetic alteration for our research client at our modern building.

Typically, what will be done to an animal used in your project?	The rodents will be bred and maintained in IVC cages at a barrier level as a service to client and tissue will be used to compare the pathways between wild type and genetically altered animals.
What are the expected impacts and/or adverse effects for the animals during your project?	Animals are not expected to exhibit any harmful phenotype or abnormalities as we are breeding the animals in well established background strains. Some animals may develop a harmful phenotype e.g. tumours, neurological signs, after certain age but in all cases will be killed .
What are the expected severities and the proportion of animals in each category (per animal type)?	Animals in this project will be produced by natural breeding techniques and are likely to only experience no more than transient mild visible effects which are not expected to impact significantly on the animal welfare or wellbeing. Those animals used for breeding will be killed by a recognised appropriate method at the end of reproductive life or as required for tissue collection.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Need to use the animals as aiming to provide service to a client by breeding animals for scientific research.
Which non- animal alternatives did you consider for use in this	Non-animal alternatives are not considered as client needs animal tissues for scientific research purpose. Clients have tested cultures of cell lines that gave reasonably positive data encouraging the to move to a more relevant model first in-vitro and

project?	then in-vivo.
Why were they not suitable?	This gene is specifically expressed in a sub-population of brain cells and we are trying to understand whether this gene is relevant in neurodegeneration. This project is still in an early developmental stage where we need to clearly validate the pathway of interest. For this reason the initial experiment will compare the phenotype of this subpopulation of brain cells between knock out and wild type animals.
Enter the estimated number of animals of each type used in this project.	mice: 1000
How have you estimated the numbers of animals you will use?	The colony is being re-derived by commercial supplier and colony will be expanded by conventional mating in either pairs or trios. The numbers are estimated on basis of progeny with required genotype at 25% of litter size. Colony will be maintained by experience staff member and will be bred on receipt of breeding form and the study form which justifies the purpose proposed on the project licence [IMAGE RENDER FAILED] Total numbers • Typically animals on C57 background anticipate 8 animals/litter. Smaller litters maybe be pooled to maintain number • Pilot studies will be completed to determine experimental conditions show differences between WT and Tg derived tissue (Typically n=16/condition) • If pilot study show potential difference study will be expanded to test different compounds (Typically n=112 animals per genotype)

What steps did you take during the experiment al design phase to reduce the number of animals being used in this project?	Consideration will be given, wherever possible, to producing only the genotype required for experiments, e.g. Homozygous breeding pairs. Code of practice for the breeding of animals for use in scientific purpose and GA animals framework, will be adhere to which is published on the Home Office website and NC3Rs website. The efficient breeding of GA animals framework published on HO website https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf
What measures, apart from good experiment al design, will you use to optimise the number of animals you plan to use in your project?	Cohort Management Generation of stocks for GAA, including breeding programs will be controlled centrally by experienced members of staff with the assistance of a dedicated animal tracking system. This will enable animals to be easily tracked for many things, including how many breeders in any given line has, stock levels, cohort organisation. Breeding performance of the strain in the unit will be discussed at the local AWERB. We will only maintain the colony and breed on demand to minimise wastage. The clients are going to use both sexes of the progeny. We intend to use REDACTED service with 72 hrs result time prior to weaning, thus unwanted genotype animals are killed by schedule 1 method as soon as possible. NVS at the facility is actively involved in breeding of GA and share guidance for good practices at all the time. Also facility has experienced member of staff who will lead the maintenance of the GA colony. Clients will fill the form which will help us to determine the cohort size and planning breeding. [IMAGE RENDER FAILED] [IMAGE RENDER FAILED]

Which animal models and methods will you use during this project?	GA mice will be used to expand the colony by conventional breeding methods either in trios or pair. None of animals are expected to suffer any pain from this method of breeding mice. GA animals bred and maintained under this project are not expected to experience any harmful phenotype.
Why can't you use animals that are less sentient?	Project is aimed at providing service to client. The client currently needs juvenile and adult animal tissue for the scientific use to prove the hypothesis.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We will refer to the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes by home office for the welfare and ensure best working practice. https://www.gov.uk/government/publications/code-of-practice-for-the- housing-and-care-of-animals-bred-supplied-or-used-for-scientific- purposes We will consult the NC3Rs guidelines and monitor refinement where such practices are published (NC3Rs/LASA websites) for best practice. https://www.nc3rs.org.uk/generation-and-breeding-genetically-altered- mice We will ensure that the user is aware of the breeding of the line via weekly report, ensure the animals bred are used within the initial agreed timeframe and any deviation from the agreed timeframe is communicated immediately, so that the breeding pair could be reduced. Only breed on demand to minimise wastage. technicians looking after the colony are empowered to question the colony manager if they suspect any unusual request on the colony.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	The only protocol currently does not exceeds a mild severity level. To minimise suffering all mice, they will be monitored and humanely killed if they show any signs of distress. Our animal unit is proactive with enrichment e.g. fun tunnels and nesting material or similar products. Tunnel handling or cup handling practice is followed in the unit rather than lifting by tail to reduce stress to the animals. For genotyping the least invasive method will be used. Animal will be grouped house where ever possible with exception of animals separated only for welfare reasons e.g Males fighting

What published best practice guidance will you follow to ensure experiment s are conducted in the most refined way?	Wherever possible, animals will be group housed to minimise stress in social species. It might be necessary to separate animals due to fighting which can result in a single housing for welfare reasons. However, stud males, in particular, separated from active mating mab be have to be single housed as re-grouping is not practical. We will refer to the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes by home office for the welfare and ensure best working practice.

Project	**43. Cancer metastasis by basal extrusion using zebrafish as a model for simple epithelia**	
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)	Most cancer patients die from metastasis, when cancer cells spread throughout the body and colonise different organs, yet we do not fully understand how tumour cells invade the body to form masses at distant sites. We plan to use transparent zebrafish embryos to visualize how cancer metastasis initiates and spreads throughout the body to take over normal tissue functions. 90% of the tumours originate in the skin that coats our organs; therefore, it is important to understand how cancer cells invade from these tissues and become new types of cells that migrate inside the body and become resistant to our current	

	treatments. We have developed a method to follow these events live in transparent zebrafish embryos, which could help us develop ways to target these rogue tumour cells with drugs. Because we are using similar mutations in the fish that cause very deadly cancers in humans, information we find in zebrafish could translate to humans. This approach will allow us to identify parts of the tumour that we may have missed previously and the transparency also lets us develop drugs that will kill these metastatic tumours until every cell of them is gone.
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 current inability to treat (and potentially diagnose effectively) metastasis is why it kills most cancer patients. The results of this project will help us understand how cancer cells disseminate and overtake different organs. This could help us learn why current treatments have been ineffective and provide new strategies to treat metastatic disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use zebrafish in this study. We expect to raise around 7300 genetically altered zebrafish over the course of this five-year 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 analyse the embryos produced from genetically modified zebrafish. These adult zebrafish may carry a gene for a fluorescent protein or carry a genetic mutation. Since the fluorescent genes do not affect zebrafish adult behaviour or breeding, we do not expect them to cause adverse effects. While most of the genetic mutations are only incurred transiently before the embryos reach 5 days old, they should not cause harm. We will use one line for screening for drugs after cells have metastasized that causes high incidences of cancer in humans at mid-life, therefore, we will only raise these zebrafish adults to half their normal life expectancy, when they start to form tumours. For this reason, the highest severity we expect will be moderate, with all other treatments being mild. When the zebrafish reach the end of their breeding life they are killed humanely in accordance with Schedule 1 methods.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have already developed much of our work in cells in culture and these were very important for suggesting that the mechanism we discovered could play a role in initiating metastasis. To test this, however, we needed to test in a well-developed animal system where we could follow live cancer cells invading without causing undue harm to the animal, normally associated with similar mouse studies. Using zebrafish is excellent because we can do most of our studies using unprotected animals (zebrafish embryos) which robustly tests our models with thousands, rather than 5-10 typically used in mammalian models. This restricts our work with protected animals to breeding them to produce the embryos needed in our study.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The latest technologies for introducing genetic changes in zebrafish cells will be used to ensure the procedure is as efficient as possible, thereby reducing the number of animals that need to be used. We will also make transient genetically altered fish that we do not need to raise to adulthood, radically reducing the number of fish needed. Finally, zebrafish that are not currently used will be euthanized but can be made readily using frozen sperm and eggs.
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.	Zebrafish are the ideal model organism for this project for several reasons. Most cancer studies that study metastasis rely on mouse models, however in that system researchers can only observe the behaviours of a few cells adjacent to a tumour that they introduce artificially. Moreover, studies in mice require putting them under anaesthesia for long periods to film with a camera placed in a wound in the animals. This is not only stressful and painful for the mouse but it does not tell us how metastasis naturally occurs and with high numbers to feel confident in one’s findings. We use the zebrafish larva, which is transparent.

	This means that by simply putting them under a microscope, we can track the movements of cancer cells as they disseminate into the body of zebrafish embryos, which are not considered sentient beings. Although zebrafish are not likely to feel anything at this point, we still film them under anaesthetic to reduce their movement and any chance of pain.

Project	**44. CAR T cell therapy for human cancer: addressing regulatory issues**	
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)		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 test whether a potential new therapy for cancer could be safely applied to cancer patients who also have arthritis. We have developed a means to engineer the body's immune cells so that they might attack the blood vessels in tumours, thereby inhibiting the growth of the tumour. However, there is the possibility that these engineered cells might also target vessels in arthritic joints. Since many cancer patients might also suffer from arthritis, before we can test our potential cancer therapy in patients, we must first check in mouse models whether this treatment will	

	cause any further damage to the arthritic joint. Therefore this project will explore this using a mouse model of arthritis.
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 allow us to determine whether cancer patients with arthritis should be included or excluded from treatment with this potential cancer therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will only use mice bred for research purposes. Mice are the most suitable animal in which to study immune responses to cancer because we understand the immune response in this species more than any other animal and this response is sufficiently similar to that in man to justify use in this way. Furthermore, specialised strains of mice are available that enable us to perform well controlled experiments that will yield high quality information. We estimate that we will use 100 mice in total over the course of 2 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?	In this project mice will be injected with antibodies and immune cells. Occasional blood sampling using a needle will also be required. These procedures will induce some stress due to restraint and transient discomfort from needle insertion. Mice will also be exposed to limited doses of radiation to partially deplete their own immune cells and thereby allow us to efficiently introduce the engineered “tumour-targeted” immune cells. High doses of radiation can cause sickness in mice therefore we will use doses that are well tolerated. However, any animal showing signs of distress/pain reaching a moderate severity limit will be culled to avoid further suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All potential T-cell therapies are tested in vitro to prove efficacy before use in vivo. However, in vitro assays cannot adequately reproduce T-cell responses *in vivo*. These obstacles include the 3-dimensional architecture of the target tissue (ie the arthritic joint and its associated vasculature), through which T-cells must

	spread. They also fail to reproduce conditions for circulating T-cells to migrate from the blood vessel into the target tissue. When using T-cells to target the vasculature, in vivo models are again required to reproduce the defective nature of such vessels in disease settings, including their unusual blood flow properties. Finally, the complex interactions that occur between components of the immune response cannot be adequately modelled by *in vitro* systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing the experiments we will perform statistical analysis to ensure we use the minimum number of mice per group that will be informative. In designing our experiments we will ensure randomisation of animals to different treatment groups across the different cages. Researchers performing the analysis will always be are blinded to animal treatment groups to prevent confirmation bias and ensure the accuracy of results. This ensures confidence on the part of the researcher that the experimental result is valid and prevents unnecessary repetition. Better reporting of research should result in better science and more effective use of animals in experiments. Therefore our findings will be reported (using the ARRIVE guidelines) in the scientific literature and at conferences, thereby minimising risk for future unnecessary animal experiments conducted by others. Literature will be continually reviewed to ensure that we are not repeating published work and that our hypotheses are based on the most up to date knowledge.
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 appropriate species for the work proposed here because inbred strains are available that permit studies of immune therapies for cancer. Mouse models are also available that develop arthritis. Furthermore, the murine immune system is very similar to humans. Mice are considered to have the lowest level of neurophysiological sensitivity among potentially suitable species.

	The methods used are designed to involve the least suffering by limiting the number of procedures involved to that required for generating a reliable answer. Furthermore, needle sizes used will be kept to a minimum. Finally, mice will be monitored daily. The advice of the named veterinary surgeon and named animal care and welfare officers will be taken to ensure animal suffering is minimised.

Project	**45. Cardiac conduction 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)	The *cardiac conduction system* is the electrical wiring system of the heart and it initiates the heart beat and controls the heart's rhythm. The *cardiac conduction system* consists of three parts, (i) the sinus node, the pacemaker of the heart, which initiates the electrical impulse that causes the heart to beat, (ii) the atrioventricular node, which transmits the impulse from the top to the bottom of the heart and (iii) the His-Purkinje system, which transmits the impulse to the two powerful pumping chambers of the heart. It can be life threatening when the system goes wrong, because the heart does not beat or the heart	

	beat is irregular. At the moment, the only treatment is the implantation of an artificial pacemaker in the chest and these are not without problems (for example, the battery runs down). We aim to study why the *cardiac conduction system* goes wrong. If we can understand why it goes wrong, we can design new treatment strategies. For example, our work over the last 10 or more years has shown that the system goes wrong because it loses 'ion channels'. Ion channels are the molecules responsible for the electrical impulse. In this project, we want to discover why ion channels are being lost and our work is showing that it is regulatory molecules called 'micro-RNAs' and 'transcription factors' that are responsible. We have also shown that, in two cases, if we knock out the regulatory molecule responsible (we did it using an 'anti-micro-RNA'), we can reverse the *cardiac conduction system* disease. Using mice as models, we will investigate *cardiac conduction system* disease in veteran athletes, heart failure patients, the elderly, and obese patients. Strangely, veteran athletes who have been exercising at a high level for decades are more likely to need an artificial pacemaker. Heart failure patients have *cardiac conduction system* disease and it has been shown to increase the likelihood of them dying. The elderly frequently have *cardiac conduction system* disease and artificial pacemakers are mainly fitted to the elderly. Obese patients frequently have abnormal heart rhythms and some can be life threatening. In addition, we aim to study why heart rhythm problems occur at particular times of the day or night and why shift work, jetlag and insomnia etc. (which all affect the body's 'circadian rhythm') disturb the *cardiac conduction system*.
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. Advancement of current understanding of the mechanisms that control the cardiac conduction system in health and disease 2. Identification of 'druggable' small molecule therapies for cardiac conduction system disease that could ultimately lead to improvement in human health.
What species and approximate numbers of animals do you expect	We anticipate using approximately 4690 mice over a period of five years.
to use over what period of time?	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	When studying disease, adverse effects are possible, but every effort is made to avoid them. With exercise training (severity categorised as mild), mice are swim trained for up to 60 minutes twice a day for 1 month. The mice are constantly watched to ensure that no mouse cannot cope (in the worst case, drown). Mice are natural swimmers and our (extensive) experience is that the mice cope very well and become fitter and sleeker. For heart failure, under anaesthesia, the mice undergo surgery to tie a band around the major artery coming from the heart, the aorta. The heart, therefore, has to pump blood against a higher resistance and this leads to heart failure over six to eight weeks (severity categorised as moderate). Following surgery, the mice are given an analgesic (pain killer). After surgery, the mice are carefully monitored and up to 5% of the mice will display signs of suffering and have to be humanely killed immediately. After six to eight weeks, the mice are carefully monitored for signs of heart failure (for example, loss of body weight, inactivity) and at this point the mice will be humanely killed. For studying the day-night ('circadian') rhythm in the heart, mice are humanely killed at different times of day and night to take heart tissue. Mice are nocturnal and are active at night. To avoid working at night, the mice can be made to be active during our day and sleep during our night by altering the lighting of their cages. Experience shows that this has no discernible effect on the mice (severity categorised as moderate). For studying shift work or jet lag etc. when the body's 'circadian rhythm' is disturbed, the mice will alternate between three days at UK time, three days at Chinese time, back to three days at UK time etc. for 30 days. Experience shows that this 'jet lag' has no discernible effect on the mice apart from small irregularities in the heart's rhythm (severity categorised as mild). To study ageing, mice up to the age of 27 months will be obtained from a breeder. On arrival, the mice will be humanely killed. At the age of 27 months (equivalent to 80 years for a human), half of all mice will have died of natural causes. The severity is categorised as moderate. To study obesity, mice will be raised on a diet high in fat for up to five months.

	Generally, the mice are not expected to have health and welfare problems, although there is a risk of skin abrasions, which can be treated (severity categorised as moderate). In some cases, animals will be fitted with a telemetry system to record the ECG or a mini-pump to deliver a drug. In both cases, this will involve a small operation under anaesthesia. Post-surgery the animals will receive an analgesic. There is a small risk (1% or less) of wound infection or wound breakdown. During the course of the work, animals will be anaesthetised to allow the recording of an echocardiogram or ECG. Other than unconsciousness, this is not expected to cause adverse effects. At the end, all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This study aims to model human cardiac conduction dysfunction (that involves the integrated response of cells and organs) and identify the key underlying genetic changes. There is no viable alternative to using a small mammal as: 1. It is virtually impossible to procure human cardiac conduction system tissue 2. Cells isolated from the cardiac conduction system do not proliferate and are not amenable to culture for more than 48 hour Therefore, the use of animal models alongside *in vitro* and *in silico* work is required. In this study, mouse models will be utilized. Mice are highly used in physiological research as they provide a comparable model of human cardiac physiology and cell function. The murine genome has been well characterised, and genetic manipulation can be performed with relative ease. This allows for the investigation of gene function within the context of the whole organ. While non-mammalian models are available, such as zebrafish, there are key differences in their cardiovascular physiology compared to humans (including incomplete separation of ventricles and circulatory physiology).

2. Reduction Explain how you will assure the use of minimum numbers of animals	We always take measures to ensure that the minimum number of animals will be used. We have carried out these types of studies many times before and are highly experienced understanding the variability typically encountered and the number of animals needed to observe a statistically significant difference. Furthermore, power calculations are routinely carried out to ensure that we are using the minimum number of animals necessary. Where it is possible, we will use non-animal alternatives such as cell lines or computer models to test the validity of hypotheses before working with animals.
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 physiology of the mouse is similar to that of the human. It is a popular laboratory species and because it is commonly studied by others, we know a great deal about it (for example, where the *cardiac conduction system* is in the heart). This means that many techniques are readily available and are refined to be as minimally invasive as possible for the mouse. We know what drugs to use, how to administer them, at what concentrations and what are possible adverse effects. **General measures to minimise welfare costs to the animals** Animals will be group housed wherever possible. We shall use full analgesia following surgery. We will conduct daily monitoring of animals at non-critical times. We will monitor animals more frequently following surgery and if an animal deteriorates. We shall regularly record body weight, respiratory rate, grooming, coat condition, ocular and nasal discharge, piloerection, hunched appearance, activity and other factors to allow us to accurately detect suffering and determine when an animal will need to be humanely killed. All animals will be humanely killed at the end of the study.
Project	**46. Cardiac ultrasound and MRI in Xenopus (aquatic frog)**

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 general aim is to evaluate an animal model (frog) where the heart has only one pumping chamber, not two as in normal human hearts. This is the closest possible model to the heart of children born with congenital heart malformations with only one pumping chamber. The specific object is to perform imaging studies with cardiac ultrasound and MRI of the heart of the frog. The information obtained will help us to understand the mechanisms used by the cardiovascular system of the frog to function well for 25-30 years, with normal life style, despite the presence of only one	
	pumping chamber.	

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 derived by this research project have the potential for a positive impact on a very large number of children born with the heart characterized by the presence of only one pumping chamber instead of two. Currently these children, despite the progress of medicine and surgery, require multiple hospital admissions for repeated investigations and surgical procedures. In any case they are having a short life expectancy with a very high incidence of complications and extremely poor quality of life.
What species and approximate numbers of animals do you expect to use over what period of time?	Twenty (20) frogs within the 5 years 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 investigations will be done in general anesthesia, with all non-invasive monitoring (leads attached on the skin, without any injection or puncture). Therefore the expected adverse effects should occur in maximum 1% of the cases, and be limited to mild discomfort. At the end of the investigations the animals will be allow to have full recovery.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mathematical models have already been used to study the blood flow distribution in an artificially created mathematical model with only one pumping chamber. Unfortunately the limits intrinsic to all the theoretical models do not allow the simultaneous introduction of all the biological variables present in the animal model. Frogs are animals with only one pumping chamber, as are 15% of children born with congenital heart malformations. Differently from the children who have a very short and miserable life expectancy, frogs live many years of unrestricted quality of life.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of 20 subjects will allow reliable statistical calculations of all the measured parameters related to the cardiac function. This number has also been motivated by the absence of any previous study on this matter.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The choice of the frog is based on its characteristics of having a heart with a single pumping chamber. In addition, this model has a great deal of existing data on heart development and function, enabling the comparison of our advanced techniques with the currently available knowledge. The proposed investigations (cardiac ultrasound and MRI) will be performed in animals with general anesthesia, with only non-invasive monitoring methods, proven to be very well tolerated, with no or mild discomfort. To minimize the discomfort the frogs will be kept at their optimum temperature (18-21°C) and their skin dampened throughout the procedure.

Project	**47. Cardioprotective therapeutic interventions for cardiopulmonary bypass surgery**	
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)	One baby in every hundred is born with a defective heart. Many of these babies will undergo at least one operation to correct the defect whilst they are still very young. During surgery the heart is often stopped temporarily to enable the surgeon to repair the defect. During the time whilst the heart is stopped, and in the period immediately after it is restarted, the heart can sustain further damage that adversely affect its function. The techniques used to limit this damage are based on those developed for adult patients however, there is evidence that these techniques are less effective in	

	children. The aim of the studies covered by this licence is to determine the optimal way of limiting the damage to the hearts of children undergo cardiac surgery.
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 study aims to identify interventions that will minimise the damage caused to the heart in the period whilst it is stopped and then restarted during cardiac surgery. In so doing the study will benefit the thousands of babies and children per year who have to undergo cardiac surgery to correct life threatening heart defects. In addition, this study will advance understanding of the factors that damage the immature heart in the period whilst it is stopped and in so doing facilitate the development of improved drugs to protect the heart during heart surgery.
What species and approximate numbers of animals do you expect to use over what period of time?	The study may use up to 380 pigs, during the 5-year duration of the licence. The number of pigs is necessary because we are aiming to answer a lot of questions. Firstly, we need to investigate how heart injury differs at different ages. Secondly, we need to test several different possible interventions which might reduce injury. And thirdly, we need to test any promising interventions in animals which have diseased hearts and therefore are more representative of the human hearts which are operated on.
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 around 70% of the pigs, no adverse effects will be experienced other than the induction of terminal anaesthesia. The remaining 30% will undergo a minor surgical procedure under recovery anaesthesia. This procedure involves inserting a balloon through a very small incision in the neck into a blood vessel. It will then be fed into a vessel in the heart, where it will be inflated for a set period of time to occlude the vessel. Then it will be deflated and removed. The procedure therefore only involves one very small incision. These pigs will be given pain relief in accordance with normal veterinary practice and are expected to return to normal behaviour within 24 hours and show no signs of suffering thereafter. The recovery pigs (30%) will later undergo the same surgery as the first cohort of pigs (70%) which will end with them being killed whilst still anaesthetised and therefore won’t involve any adverse effects except for the

	induction of terminal anaesthesia. We will kill the pigs under anaesthesia because all of the data will have been collected by then and therefore waking them up following major surgery would only cause unnecessary pain and suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The primary aim of the study is to identify interventions that will improve the clinical outcome of babies who have to undergo corrective heart surgery during which the heart is stopped and restarted. It is not possible to replicate this situation using either isolated organ, tissues culture or by computer simulation. Furthermore, it will not be possible to translate the findings into the clinical setting without first demonstrating their safety and effectiveness in a representative animal model. Consequently, it is not possible to conduct this study without using animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The studies will be carefully designed to use the smallest number of animals needed to obtain meaningful results (observation of reduced cardiac damage and improved cardiac function in some groups compared to others). If we use too few animals, we will not be able to see the difference between the treatment groups, however we don't want to use any more animals than necessary. Therefore, we have performed mathematical calculations which help estimate how many animals we need. The project is planned in a stepwise manner so that only interventions that are successful in early phases of the study will be progressed into the main study.
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.	Young pigs have been selected for these studies because their anatomy, size, physiology and state of development is very similar to that of babies and young children, thereby enabling the clinical scenario to be closely replicated. All surgical procedures will be conducted under deep general anaesthesia and will be performed by experienced paediatric cardiac surgeons. For the 30% of animals that undergo a minor procedure under recovery anaesthesia (before undergoing the second surgery under terminal anaesthesia), pain relief will be given in accordance with normal

	veterinary practice. The animals will be looked after by staff experienced in the care of pigs following surgery.

Project	**48. Cell competition and the dynamics of tumour development in epithelial tissues**	
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
What's the aim of this project?	To define the mechanisms that regulate stem and progenitor cell fate in epithelial tissues, and how these programmes become subverted during tumour initiation.	
Why is it important to undertake this work?	In cancer, tumours develop through a multi-stage Darwinian-like process, evolving from a phase of neoplasia (i.e. abnormal growth of tissue) to invasive carcinoma (where tumour cells infiltrate into underlying tissue) and metastatic transformation (where cancer spreads to different parts of the body). Entry into this programme is often preceded by phase of “field cancerization” in which the acquisition of mutations in certain cancer genes, known as driver genes, enable cells to outcompete their neighbours, expanding the pool of cells on which further damaging mutations can impact.	

	To resolve the biochemical pathways that lead to cancer formation, emphasis has been placed on resolving the mutational landscape of tumour types – the “cancer genome”. However, while the repertoire of cancer genes is becoming increasingly well-characterized, the impact of mutations on the behaviour of individual cells – notably, how they balance cell proliferation through division and differentiation in functional cell types – remains underexplored. To probe the earliest stages of tumour formation, methods based on the use of transgenic animal models have been developed to trace the lineages of mutant cells and their daughter cell progeny - known as “clones”- following the activation of mutations in specific cancer-associated genes (Sánchez-Danés et al., 2016). However, current cell labelling strategies struggle to resolve how the fate of neighbouring wild type cells are influenced by the activation of mutations in neighbours, and how these changes can drive field cancerization and cancer progression, with consequences for our understanding of early cancer-detection and risk management strategies. To understand the mechanisms that drive tumour development, there is a pressing need to understand the pathways that regulate cell fate decision making in healthy tissues, and how these programmes become subverted during the earliest stages of tumour initiation and progression.
What outputs do you think you will see at the end of this project?	This project will provide insights into the cellular and biomolecular mechanisms that regulate cell fate decision-making in the epithelial lining of the mouse gastrointestinal tract (including the small intestine, colon and stomach), skin epidermis and oesophagus, both under normal healthy conditions and following the activation of mutations in specific cancer-associated genes. Using a genetic cell lineage tracing strategy REDACTED we will quantify at single-cell resolution the fate behaviour of mutant epithelial cells (marked by a red fluorescent reporter gene) as well as wild type cells (marked by a fluorescent reporter of a different colour). This will allow us to study in parallel the clonal evolution of mutant epithelial cells and the effect they have on the clonal dynamics of neighbouring wild type cells as well as the surrounding tissue. By combining this approach with gene expression profiling, this novel experimental design will allow us to: ● resolve both the cellular and biomolecular basis of epithelial cell fate decision-making during the development, maintenance and repair of healthy epithelial tissues; ● establish a new standard in the study of the mechanisms of pre- neoplastic transformation and field cancerization in columnar and epithelial

	tissues, which can inform new strategies of early cancer-detection and risk management; provide an exemplar for how quantitative modelling-based approaches can impact on cancer research. • REDACTED mouse models will provide a valuable and versatile resource that can be readily generalised to the study of other tissue types and cancer-associated mutations, and used by other groups for the identification of novel biological markers, testing immuno-detection methods, intravital imaging as well as investigating other cancer-related biological process, such as metabolism and inflammation.
Who or what will benefit from these outputs, and how?	The outputs of the proposed research will have immediate impact on the research community through publications, resources and conference presentations. In the medium term, all transgenic mouse lines generated through this project will be made available to the research community, where they can be used to study mechanisms of epithelial cell fate in healthy and diseased conditions, and readily adapted and applied to other tissue types not considered in this programme. In the longer term, this research is expected to provide potential actionable targets for use in regenerative medicine, as well as early cancer diagnostics and therapeutics.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	As a collaborative group, we will remain alert to opportunities to extend our work by sharing our material and intellectual resources. In particular, we will be ready to share our transgenic mouse models, building collaborative partnerships with researchers whose interests extend to other tissue types, or with technological skills (such as intravital imaging) not yet available to our laboratory. Even within the scope of the current project, lineage tracing studies of transgenic mouse models may sometimes provide data associated with other tissue types. In this case, we will readily share tissue samples and data with researchers who have expertise and interest in these areas.

Explain why you are using these types of animals and your choice of life stages.	The activation of cancer-associated DNA mutations can have a dramatic effect on the fate behaviour of epithelial cells, allowing mutant cells to outcompete their non-mutant (wild type) neighbours during tissue turnover, creating pre-neoplastic lesions that can undergo subsequent transformation to more aggressive phases of disease. These changes in cell fate may be driven by direct crosstalk between neighbouring mutant and wild type cells, or they may be mediated by mutation-driven changes to the local tissue environment. To develop a comprehensive knowledge and understanding of the factors that drive epithelial cell competition during the earliest phases of tumour development, it is necessary to study cell fate behaviour in their native tissue environment, making animal studies unavoidable. While some insights can be obtained from in vitro culture systems, the knowledge accumulated from the investigation of the mouse is incomparable. The mouse is the most appropriate animal model for the proposed studies since: (i) it is a mammal; (ii) its physiology is more extensively characterised than that of other mammalian model species; (iii) mice are amenable to transgenic manipulation allowing oncogenic mutations to be conditionally induced; and (iv) a large number of relevant transgenic and gene knock-out lines are already available. The experiments detailed here will involve creating and analysing transgenic mice, and are classified as mild to moderate with respect to potential discomfort, stress or suffering. To contrast changes in cell competition during phases of development, we will study the action of oncogenic mutations in epithelial tissues in embryonic, postnatal (0 to 3 months) and adult (3 to 15 months) mouse tissues. As injury-induced cellular plasticity is considered as a key driver of pre-neoplasia, we will also study the effect of oncogenic mutations on cell fate behaviour during mild tissue regeneration models.
Typically, what will be done to an animal used in your project?	The majority of animal experiments involve the activation of DNA mutations in cancer-associated genes targeted on specific epithelial cell populations using genetically-engineered mouse models. To activate such oncogenic mutations coupled to fluorescent reporter genes, we will use drug-inducing agents (such as Tamoxifen) administered orally or delivered by injection. Animals will then be allowed to age before being killed and tissues collected at various time points following cell labelling, allowing the size and cell composition of mutant cells and their progeny –clones – to be quantified and compared to those of neighbouring non-mutant (normal) clones. To study cellular dynamics in the embryo, pregnant females will be treated with the same drug-

	inducing agent, administered orally or delivered by injection, and pups recovered and killed before delivery. In studies of postnatal development and adult, mice will be treated with the same drug-inducing agent, administered orally or delivered by injection, and tissue collected over a minimal range of time points, from days and weeks to months and up to one year post-induction. In some experiments involving adult mice (3 months of age), two drug treatments may be required, one to activate the oncogenic mutation and fluorescent reporter gene, and another to induce mild tissue "damage" in an injury-like model involving the targeted genetic ablation of specific cell types resulting in the rapid regeneration of tissue. The mice will then be aged, killed and tissues collected at various time points. In other injury models involving adult mice (3 months of age), the activation of the oncogenic mutation and fluorescent reporter gene following treatment by a drug-inducing agent will be coupled with the administration of a chemical agent or an endoscopic biopsy to create mild tissue damage resulting in rapid tissue regeneration. The mice will then be aged, killed and tissues collected at various time points.
What are the expected impacts and/or adverse effects for the animals during your project?	To study the fate decisions made by cells during the normal development and maintenance of epithelia, including tissues of the gastrointestinal tract, skin epidermis and oesophagus, we will use the administration of a drug-inducing agent to induce the expression of fluorescent marker genes in cells. Although oral administration of the drug is our favoured route, consistency in the induction frequency of mutant cells across animals might require the use of intraperitoneal injection. In this case, around 50% of the animals might experience mild discomfort due to the injection, which will last <1 day. From previous studies of tissue regeneration using the proposed procedures, we expect adverse effects on the animals to be mild. Once again, if required, we expect that around 50% of the animals may experience mild discomfort due to intraperitoneal injections of the agents that are used to mark cells and/or induce mild tissue damage to elicit a repair response. Since the epithelial tissues repair rapidly and efficiently, any discomfort associated with injury should be mild and should last no longer than 2 days. To induce the earliest stages of tumour formation, we will make use of genetically modified mouse models that allow oncogenic mutations to be activated in individual cells. We will explore the activation of oncogenes both during tissue development, targeting the embryonic and early postnatal phase, and in adult. The early lesions that form through these

	studies will be small, leading to, at most, only slight discomfort even when the induction efficiency is relatively high because the tumours will grow in places where they don't cause pain. We expect that tumours of moderate severity will only develop from lesions in adulthood in around 5% of mice. This might result in more discomfort to the mice because of the tumour size. Animals will be monitored daily, and should tumours develop, the animals will be humanly killed. Based on one or, maximum, two oncogenic mutations, the mouse models used in this study are not expected to reach a stage of metastasis. Surgical procedures will be carried out aseptically under anaesthesia, and animals may receive analgesics during and after surgery, where possible. All animals will be monitored regularly and, if there are any concerns, animals will be examined and weighed. The majority of animals (>95%) are not expected to show adverse clinical signs. However, some weight loss with or without other clinical signs such as piloerection or hunched posture may be seen. In these cases, if the signs do not resolve within 24 hours or the animal deteriorates, they will be humanely killed. In all cases, animals will be humanly killed after the experiments, either after induction of a tissue repair response or after generation of tumours. We will analyse the presence of particular phenotypes (e.g. appearance of tumours or the efficiency of tissue repair) by using the molecular, histological or culture techniques.
What are the expected severities and the proportion of animals in each category (per animal type)?	93.6% of procedures are expected to result in mild severity and the remaining 6.4% are expected to be moderate.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	The goal of the project is to understand how mutations in cancer- associated genes can drive cell competition in epithelial tissues, promoting the earliest stages of tumour growth. These changes in cell fate may be mediated by mutation-driven changes to the surrounding tissue – known as the tumour microenvironment. To study the pathways that drive early tumour development, it is important to study mutant cells in their native tissue environment. In this case, animal studies are unavoidable.

Which non- animal alternatives did you consider for use in this project?	Currently, there are excellent in vitro culture models - knowns as “organoids” - of the target epithelial tissues that have been developed for both mouse and human epithelia. Cultures of intestinal epithelial tissues can be grown and sequentially repassaged that recapitulate the glandular organization and pattern of tissue, while stratified cultures of skin interfollicular epidermis and oesophagus can also be maintained over the long-term.
Why were they not suitable?	Despite the promise of organoids, current technologies do not allow such culture systems to be grown and maintained together with supporting stromal and immune cells. As such, they cannot be used to resolve mutation-driven changes in the tumour microenvironment and how these changes may mediate cell competition. However, where possible, we will make use of organoid culture systems as a preliminary screening platform to identify the potential effects of oncogenic mutations on epithelial cell fate and in follow-up studies to perturb signalling pathways identified by the in vivo analysis of the mouse model. Using this method, we will be able to focus on the targeted effects of candidate signalling pathways that will significantly reduce the number of animals required.
Enter the estimated number of animals of each type used in this project.	mice: 22140
How have you estimated the numbers of animals you will use?	Protocol 1: Superovulation. Superovulation is required for generating new transgenic strains, freezing lines, and importing new lines when rederivation is required. We anticipate needing up to 60 mice per year for cryopreservation (300 over 5 years). We will also need to generate at least 10 lines and/or import at least 15-20 different CreERT and floxed allele strains (translating to 350 mice in total). Total of 650 mice. Protocol 2: Generation of founders, To generate 10 lines for Protocol 1, genetically modified embryos will be implanted into pseudo-pregnant females to produce “founder mice”. We anticipate needing around 60 founders per line, translating to a total of 600 mice. Protocol 3: Embryo recipient. Embryo recipients are required when thawing embryos from frozen stocks, when generating new strains or when rederiving mice from other facilities. Total of 400 mice over 5 years. Protocol 4: Vasectomy. For each allele, we will use around 6 males to mate with the females for use in Protocols 1 and 2. For 15 alleles, this translates to a total of 90 mice over 5

	years. Protocol 5: Breeding and maintenance of genetically altered animals. When designing the experiments, we will perform quantitative statistical analysis to ensure that the number of mice used per group is the minimal to be informative. To achieve sufficient statistical power, we will need n=5 mice per group when analysing lineage tracing models and when inducing tissue regeneration response. When using stem cell isolation by Fluorescence-activated cell sorting, it is crucial to obtain a sufficient number of cells to ensure statistical significance. To achieve this, we will need 10 mice for each group at each time point. We will breed a number of different mouse strains carrying transgenic lines, Cre-dependent reporters or floxed alleles (estimated at around 20 different lines). In addition, we will generate additional mouse lines. Some of the mice will be used for tissue harvesting and in vitro experimentation. Therefore, in planning to maintaining 10 lines at any one time and also perform necessary experiments, we estimate a requirement of around 2,000 mice per year, translating to a total of 10,000 mice over 5 years. Protocol 6: Embryonic epithelial cell behaviour. We require at least 5 mouse embryos per group x2 groups (control and treated condition) x5 different conditions (viz. time of treatment) x4 time points x15 lines = 3,000 mice. For cell isolation experiments, we will need 10 embryos per group x2 groups x4 time points x15 lines = 1,200, translating to a total of 4,200 embryos over 5 years. Considering average number of pups from a pregnant mouse as 5, we require 840 pregnant mice, which also includes spontaneous stillbirth. For titrating the tamoxifen dose within these different mouse lines, we will need 60 more pregnant mice. There may also be circumstances where the status and stage of pregnancy might be not assessed accurately requiring 100 more mice. We will therefore require 1,000 pregnant mice in total. Protocol 7: Post-natal epithelial cell behaviour. We aim to analyse 5 mice per group x2 groups x3 different conditions (viz. time of treatment) x5 time points x10 lines = 1,500 mice. For the cell isolation experiments we will need 10 mice per group x2 groups x2 time points x10 lines = 400 mice, translating to a total of 1,900 mice over 5 years. Protocol 8: Adult epithelial cell behaviour. We aim to have 2 groups x5 mice per group x6 different conditions (viz. time of treatment) x5 time points x10 lines = 3,000 mice. For cell isolation experiments, we will need 10 mice per group x2 groups x5 time points x10 lines = 1,000 mice, translating to a total of 4,000 mice over 5 years. Protocol 9: Adult epithelial cell manipulation by signalling

	pathway specific agent. We aim to have 2 groups x5 mice per group x5 different conditions (viz. different agent) x5 time points x4 lines = 1,000 mice, translating to a total of 1,000 mice over 5 years. Protocol 10: Epithelial regeneration. We aim to have 2 groups x5 mice per group x6 different regenerative conditions x4 time points x5 lines = 1200 mice. For the cell isolation experiments we will need 10 mice per group x2 groups x3 time points x5 lines = 300, translating to a total of 1,500 mice over 5 years. Protocol 11: Endoscopy and biopsy of colonic epithelium. We aim to have 2 groups x5 mice per group x5 different conditions x4 time points x4 lines = 800. For biopsy, we will need 10 mice per group x2 groups x5 time points x2 lines = 200, translating to a total of 1,000 mice over 5 years. When designing the experiments, we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative. A strength of the current approach is its reliance on quantitative modelling-based approaches that allows cell fate behaviour to be inferred from the statistical analysis of clonal ensembles. Within this framework, we use the statistical distribution of clone sizes and compositions, and its evolution over the given time course, to abstract quantitative models of cellular hierarchy and cell fate choice. Statistical approaches based on Bayesian analyses or least-squares measures are used to obtain model parameter estimates with known statistical confidence. Although the cohort size will depend on the complexity of the underlying cellular dynamics in the appropriate condition, with 50-100 clones per tissue sample, statistical significance can be achieved from a minimal number of mice (n=3-5 per group). Depending on the context and the complexity of fate behaviour (viz. the multiplicity/heterogeneity of cell states and repertoire of cell fate decisions), to trace the fate behaviour of cells in normal mice and mice harbouring oncogenic mutations, we anticipate quantifying at least 3-5 time points per mouse model. Indeed, the ability to abstract precise quantitative information from a minimal number of mice based on statistical clonal ensembles is a signature of our approach and major strength of the programme. Although the induction frequency may vary between epithelial tissue types, lineage tracing assays based on ubiquitous or, in some cases, targeted-promoters (such as Keratin14 or Lgr5) will allow clonal data to be recovered from multiple tissues from the same animal significantly reducing the required number of mice. The number of mice needed for isolation of tissue for organoid generation will depend on whether organoids are to

	be formed from dissociated tissue or from Fluorescence activated cell sorted cells. In general, each organoid isolation would require up to 10 animals from a minimal number of time points and conditions, and 3 separate isolations per genotype would be needed to ensure statistical reproducibility.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	When working with different oncogenic Genetically Modified lines, we will conduct a pilot study to assess the phenotype of the oncogenic mutation based on quantitative clonal analysis over a limited number of timepoints. Only those lines that show an interesting phenotype, viz. driving non-neutral cell competition or inducing changes in the tumour microenvironment, will proceed to more in-depth analysis. This quantitative assessment of clonal fate will allow us to reduce considerably the number of mice used (estimated >75% for those lines that do not proceed beyond the pilot study). The licence holder has a background in theoretical and statistical physics, and is conversant with the necessary mathematical and statistical expertise that will inform the experimental design. Nevertheless, the licence holder is ready to receive and respond to advice from local statisticians.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	To reduce the number of breeding pairs, the mice will usually be kept as homozygous, whenever possible; although we are alert to the need to breed from heterozygotes too, to avoid the risk of mutation selection. As appropriate, we will undertake pilot experiments, particularly with cancer models, seeking training from our experienced collaborators, so we can be assured that we can safely monitor tumour progression to stay within the moderate limit. Agonists or inhibitors will be pre-screened in pilot experiments to obtain an indication of the dose that is likely to be effective. As we will only use previously-validated compounds, the starting dose will be the minimum to have an effect according to literature (taken to be at least the IC50). Specifically, we will refer to the resource ‘Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. British Veterinary Association Animal Welfare Foundation/Fund for the Replacement of Animals in Medical Experiments/Royal Society for the Prevention of Cruelty to Animals/Universities Federation for Animal Welfare. Morton DB et al. Lab Anim. 2001 Jan;35(1):1-41. Tissues other than those that named on the licence might be affected by the candidate gene perturbations since promoters

	might be expressed in the stem and/or progenitor cell compartments of other organs. To maximise the information from a single animal, we will collect samples from respective organs and share these with other scientists, mitigating the need for further breeding and experimentation. We will remain alert to any advances that will enable the replacement of animals.
Which animal models and methods will you use during this project?	The mouse is the most appropriate animal model for the proposed studies since: (i) it is a mammal; (ii) its physiology is more extensively characterised than that of other mammalian model species; (iii) mice are amenable to transgenic manipulation; and (iv) a large number of relevant transgenic and knock-out lines are already available. The experiments detailed here will involve creating and analysing transgenic mice, and are classified as mild to moderate with respect to potential discomfort, stress or suffering. Mice will receive tamoxifen, doxycycline or Diptheria toxin administration in order to activate fluorescent reporter genes, oncogenes or to ablate targeted cell populations. To study the dynamics of tissue regeneration in the colon, we will use Dextran Sodium Sulfate treatment at low dose, which induces mild colitis, an inflammation of the inner lining of the colon.
Why can't you use animals that are less sentient?	Currently, the mouse is the least sentient mammal for which transgenic technologies are available.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Our institution remains a major centre in the fields of developmental and cancer biology, with many labs engaged in animal work and the study of transgenic mouse models. Importantly, there is a close and effective integration of expertise both at the level of academic researchers and the animal facilities, supported by the institutional biomedical services team. This network allows for the dissemination of best practice and information on advances in 3Rs. We are ready to receive and act upon advances as soon as we are made aware of changes in best practice.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	The majority of procedures outlined in the project are ones in which the lab has years of experience and expertise. This expertise has allowed us to design experiments that minimise the welfare costs for the animals. Mice will be maintained in a specific-pathogen free environment in individually ventilated cages. Mice will receive tamoxifen, doxycycline and Diptheria toxin administration, which normally do not harm mice. In cases where timing and dosing is not critical, compound administration such as doxycycline will be supplied in the food or drinking water. If timing and dosing is critical, as is typically the case for tamoxifen administration to induce cells at a defined clonal density, the choice of administration will be optimised against the balance of harm and benefit. Our preferred method will be oral gavage, a method of drug delivery which in our experience has sufficient labelling efficiency with our current genetically altered strains. However, in cases of new genetically altered strains, if the recombination efficiency is not optimal and consistent, we will try alternative routes such as intraperitoneal injection or subcutaneous injection, the pain of which is also considered to be mild. The ability to control the clonal induction rate in the experimental animal by precise dosing will reduce any variability in our quantitative results, which in turn will enable us to reduce total usage of mice. Similarly, alternative routes will be also preferred for labelling agents (such as Bromodeoxyuridine / 5-bromo-2'-deoxyuridine) if the labelling efficiency and timing is not optimal with oral gavage. Diptheria toxin-mediated specific cell loss in the stomach and intestine may cause local inflammation. However, the affected tissue will heal rapidly without causing any clinical signs. Cell ablation methods based on the pharmacological agents DMP-777/L-635 and 5-FU-mediated cell ablation methods were shown by our lab and others to have a transient effect on the target cells in stomach, and therefore treated mice can remain healthy, without major adverse effects, when used with an appropriate dose. Moreover, tissue-specific and cell-specific ablation by Diptheria toxin administration will result in even milder effects on mice. Dextran Sodium Sulfate is one of the most well-characterized agents in rodent models of colon epithelial cell regeneration. For each mouse strain, we will refine our model by determining the dose that is sufficient to cause reproducible partial epithelial loss with minimal clinical signs . For example, the general dose of Dextran Sodium Sulfate administration used in the literature is 3% Dextran Sodium Sulfate solution in drinking water. Based on previous studies, we will set this dose as the maximum, while aiming to use a lower dose, if suitable, to address our scientific questions.

	For the gene knock-out studies, since we will use inducible conditional alleles, mice should not display a phenotype before induction. To avoid unexpected pain and suffering, animals will be first bred and analysed as heterozygous animals. We will only use well-established reagents and protocols to induce expression or deletion of candidate gene(s). Taken together, the overall harm to mice that can be caused by performing the experimental plan will be minimal. Additionally, we will use endoscopic biopsy to induce regeneration in the colon, currently the most refined approach available. It represents a significant refinement compared to other techniques, such as carcinogen treatment. All endoscopic biopsy studies will use anaesthesia and peri- and post-operative analgesia as part of the protocol regime, as discussed with the Named Veterinary Surgeon. Surgery will be conducted using the aseptic technique, which meets at the least the standards set out in the Home Office Minimum Standards for Aseptic Surgery.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	For general health monitoring of the animals, we will be guided by the "Working document on a severity assessment framework" prepared by the "National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes". We will assess 'body condition' of animal as defined by *Ullman-Culleré MH & Foltz CJ, Lab Anim Sci. 1999 Jun; 49(3):319-23.* To assess the actual severity of our procedures on the mice, we will refer to the Grimace scale published by the NC3Rs (https://www.nc3rs.org.uk/grimacescales), which is itself based on the study by Langford DJ, et al. 2010. Coding of facial expressions of pain in the laboratory mouse. Nature Methods 7(6): 447-449. For monitoring general health monitoring in the context of tumour burden, we will follow the 'Guidelines for the welfare and use of animals in cancer research. *Workman P. et al. British Journal of Cancer (2010) 102, 1555–1577'.* We will conduct surgery using aseptic technique in accordance with the following guidance: *Guiding Principles for Preparing for and Undertaking Aseptic Surgery 2nd Edition 2017. http://www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf* For the breeding of Genetically Altered mice, we will refer to the

	guidelines provided by the Home Office, detailed in https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf In estimating the correct dose of chemical agonists or inhibitors, we will refer to the resource: *Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. British Veterinary Association Animal Welfare Foundation/Fund for the Replacement of Animals in Medical Experiments/Royal Society for the Prevention of Cruelty to Animals/Universities Federation for Animal Welfare. Morton DB et al.* *Lab Anim. 2001 Jan;35(1):1-41.* We will remain alert to any advances that will enable the replacement of animals.

Project	**49. Cell senescence and life span in mice**	
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)	Recently, it has become increasingly clear that long-term cancer survivors suffer from serious premature ageing, causing 2-10 fold higher risks for developing multiples of chronic diseases, frailty, and mortality. Radio- or chemo-therapy is considered as a significant cause of this, because these symptoms can be seen in healthy animals receiving radiation or chemotherapy drugs. At cellular levels, the cells in aged animals or the cells treated with radio- or chemo-therapies accumulate DNA damage. This in turn dramatically changes the way cells look, function and communicate with their neighbours	

	– they become “senescent” and are called senescent cells. This limits the ability of tissues to recover from damages and to function properly such that individuals become more prone to multiple diseases and death. In recent years, drugs that selectively eliminate senescent cells (called ‘senolytic’ drugs) have been developed and shown to be effective in alleviating a wide range of age-associated diseases, such as cardiovascular disease, atherosclerosis, lung disease, osteoarthritis, osteoporosis and liver disease in mice models. In our previous research, we were able to provide evidence of relieving early development of age-associated diseases in the mice after whole body irradiation (a model of radiotherapy) by using known senolytic drugs. Thus, our vision is to prevent accumulation of senescent cells in long-term cancer survivors to suppress premature ageing and extend lifespan. At present, less than 15 senolytic drugs are known, and are with various side effects. To improve senolytic treatment for humans, the number of the potential senolytic drugs should be significantly increased and should undergo appropriate pre-clinical testing. The proposed project continues from previously successful work with the objective to identify safer and more effective senolytic drugs that can be used for clinical trials.
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)?	Scientifically, this work will increase our knowledge on the roles of senescent cells in age related diseases, frailty and death, which in turn helps to design novel drugs for healthier ageing. Findings will be published in peer-reviewed journals and presented at scientific conferences. This work will provide comprehensive pre-clinical validation for senolytic interventions to prevent premature ageing in long term cancer survivors, as well as to promote healthy ageing in normal population. Additionally, this work also offers significant pharmaceutical commercialisation and clinical translation opportunities by identifying a number of novel

	senolytic drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately total of around 2,400 mice over 5 years will 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?	Some mice will be treated with drugs commonly used in chemotherapy in humans (injections or in food) or with irradiation as a model to human radiotherapy. Then the effects of senolytics drugs (given by orally or in food) on ageing phenotype will be examined. Instead of senolytics drugs, dietary restriction (to a safe level), which also reduces the amount of senescent cells, maybe conducted. In our previous experience, irradiation or oral administrations of senolytics/ control fluid in mice results in a small reduction in their body weights (1-2g) but recover to their original weights in about 20 days. As mice get older, the ageing symptoms such as hunched back, hair loss, eye discharge, worsening fur condition and weight loss may appear. The mice that have been irradiated or chemotherapy-drug treated will start showing the sign of ageing earlier. However these ageing symptoms are usually not expected to cause more than mild suffering or distress or more than moderate impairment of the well-being or general condition of the animals. The mice will undergo various measurements of ageing phenotype as they age, and we leave long-enough intervals between to ensure the recoveries from any stresses they may experience. The measurements include frailty index scoring, neuromuscular tests, cognitive function test, and blood sampling. Whole body imaging under anaesthetization may also be conducted in a limited number of mice. However, the overall severity of any mice should experience in this project will not exceed

	moderate levels of severity. All the mice will be humanely killed after experiments. Blood and tissue samples will be taken and banked. The analysis of these samples will enable us understanding the processes that occur in tissues during premature ageing and how the senolytic drugs change them.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Ageing in humans is an extremely complex process affecting the whole organism. Itdepends upon integration of many organs and systems in the body, and in vitro techniques cannot replicate this interaction. Non-mammalian models fall short of replicating the changes associated with human ageing, as they do not have all the equivalent systems, thus mammalian animal models are necessary However, wherever possible, cell culture models will be used to replace the animal models to address specific questions. For example, the safety and effective doses of all the potential senolytic drugs will be tested in tissue culture models before the experiments on mice. Furthermore, some organ-specific aspects of ageing and the effects of senolytic drugs will be studied in three-dimensional (3-D) tissue culture models which partially replicate structures in whole organs. For example, currently skin 3-D models are being developed to test the effects of senolytic drugs on skin barrier function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The hypothesis and the experimental designs will be constructed to minimise the number of mice used and the duration of experiments without compromising statistical rigors using

	ARRIVE guidelines. The statistical power calculations will be undertaken with expert advice from statisticians to ensure minimum use of mice to test particular research hypotheses. Information obtained from our previous experiments will be used to improve the accuracy of these calculations.
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.	Humans and mice are mammals and share many biological features including those relevant to ageing, and mice are the lowest sentient animal model suitable for the study of ageing in mammals. To minimize welfare costs, the animals will be given extra bedding for warmth and housed in a rat cage for more space, which also aids better monitoring. They will also receive environmental enrichment and extra saw dust so that they can exhibit normal rodent behaviour such as digging. By implementing daily monitoring, the mice showing signs of distress and suffering will be identified promptly, and will be given supportive care including (but not limited to) provision of soaked diet, extra bedding or an incubator. However if the condition cannot be remedied by appropriate care within 24 hours, they will be humanely culled.

Project	**50. Cell surface receptor functions in development and infection**	
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)	This is a basic research grant that aims to develop new treatments for genetic disease and vaccines for infectious diseases. The aim is ultimately to improve the welfare of livestock animals which humans depend on, and also to develop new vaccines for diseases that affect humans directly. We will also investigate the function of genes involved in basic developmental processes, and in particular fertility, with the aim of creating knowledge that can be exploited to improve peoples' lives. The objective of this project is to investigate the	

	function of extracellular protein interactions identified using *in vitro* protein interaction methods. We are interested in cell surface and secreted protein interactions that are important for the pathogenesis of certain infectious diseases and normal developmental processes, particularly fertilization. We will be studying pathogens which affect human health, and for which no vaccines are currently available or licenced. Our work on developmental processes is aimed at obtaining a basic understanding of how fundamental biological processes work; for example, we are interested in understanding more about how sperm and eggs recognise each other.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work we are performing on understanding how certain infectious diseases can interact with human cells may lead to the development of new vaccines for both human and animal use. We work on cell surface and secreted proteins which, because they are exposed to host antibodies, are inherently very attractive vaccine candidates. The work described in this project will provide new approaches in the preclinical development of vaccines for human diseases such as schistosomiasis and malaria, as well as important livestock diseases such as animal African trypanosomiasis. Our research findings will be made available to other scientists through publication in open access, peer-reviewed journals or on open access platforms, and presentations at scientific conferences and meetings.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using small rodents (mice and hamsters) as a model organism, and all procedures will be performed on adults. We anticipate that we will use 2,500 per annum and the project is expected to last for 2 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 regulated procedures that the animals will undergo will, for the most part, be mild, primarily involving administration of substances such as vaccine formulations comprised of pathogen cell surface proteins, hormones and blood draws. Mostly this will involve needle and syringes in the same way that vaccines are delivered and blood is taken from humans. We do not

	anticipate anything other than mild discomfort for these procedures but possible adverse effects include ulceration at vaccination sites, which can be treated with analgesics. In the case of vaccine testing, animals will be infected with defined pathogens; again, this will usually involve intravenous delivery by needle, although Schistosoma spp. infections will be administered by tail dipping into infected water – the natural route of infection. The symptoms for some of the pathogen infections can lead to observable adverse effects on the mice, and so these protocols are classified as “moderate”. In these instances, we monitor the infections of these animals carefully using the most sophisticated in vivo imaging techniques which now enable us to accurately predict the course of an infection and to intervene before the infections symptoms cause harmful suffering. In the case of investigating the roles of cell surface receptors in fertility, some surgery will be required to transplant embryos into donor mothers. The surgery will always be performed under anaesthesia and postoperative monitoring and care will be guided by the latest opinions and developments as recommended by the named veterinary surgeon. At the end of all procedures, animals will be humanely culled by a Schedule 1 procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals to make antibodies which work well in our protocols since non-animal alternatives typically result in antibodies whose properties are not adequate for our work. We need to use animals in our preclinical vaccine screening approaches since there are few or no adequate *in vitro* infection models available for the pathogens that we wish to study. A vaccine candidate that showed efficacy in an animal model would also lend significant weight to its candidature for progression towards a human vaccine.
2. Reduction Explain how you will assure the use	We have developed a method of making more antibodies at one time in fewer animals. We will use the very latest technologies to quantify the number of parasites in infected animals. These

of minimum numbers of animals	approaches mean that we can accurately quantify the number of parasites in individual animals over time without killing any animals consequently leading to a reduction in the number of animals used. We will use superovulation to increase the number of eggs that we can obtain per mouse, which reduces the total number of animals required.
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 the research outlined in this project uses mice or hamsters as an animal model. Mice are a suitable model for these studies because they are a mammal, and almost always contain a recognisable counterpart to any human proteins that we identify in our *in vitro* screens. Consistent with this, many pathogens that infect humans also infect mice with similar pathogenic outcomes (e.g. *Shistosoma mansoni*, the parasitic worm that causes schistosomiasis in humans) and so they can be used as an appropriate model for preclinical work. Mice have a long history of making important contributions to the understanding of human biology and many valuable resources such as gene-deficient mice are available that enable us to make scientific advances with an increased confidence of correctly interpreting the outcomes of experiments designed to discover new treatments and therapeutics to improve human health. We have refined the protocols for the percutaneous infection of mice with *Schistosoma mansoni* by developing an anaesthesia apparatus which has reduced the stress to the animals during these infection protocols.

Project	**51. Cellular and molecular mechanisms involved in the development of pain associated with peripheral pathologies**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	1. To identify molecules and interaction of molecules in various tissues, for example the skin and urinary bladder, which produce pain, and may serve as targets for the development of new drugs. 2. To identify molecules in sensory neurons (cells that detect environmental impacts), which are key for the generation of pain, and may serve as targets for the development of new	

	drugs. 3. To identify cellular and molecular interaction in the spinal cord, which are associated with the development of, and may serve as targets for the development of new drugs.
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 provide academic, clinical and economical as well as societal benefits. Academics will benefit from the generation of new knowledge on the mechanisms involved in the development of pain in various conditions. Identification of new targets for the development of novel drugs will be beneficial first, for the economy by attracting new investments for future studies and drug development by pharmaceutical companies. Second, in the long term, patients suffering from chronic pain will also benefit from the identification of novel targets when new drugs are available for clinical use. Finally, results generated during this project will contribute to reducing the demand on NHS resources and health care and social benefit costs on the whole of society.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats: 1400 and Mice: 4700 approximately 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?	Protocols used in this work are associated with mild or moderate severity levels. While every care will be taken to avoid the development of adverse effect, they may arise with procedures. These include unintended nerve injury following of injecting substances into the sciatic nerve, bleeding and inflammation after any surgical procedure, unexpected effects of drugs administered, general ill health after cyclophosphamide injection and peripheral nerve injury, autotomy after peripheral nerve injury, post-operative wound opening. By careful design and work many of these adverse effects can be avoided (e.g. following all guidelines and standard operating procedures). Inflammation and the development of spontaneous pain will be prevented by the administration of appropriate drugs. Animals after any procedure will be monitored frequently for any sign of distress/ill health. Animals following recovery

	anaesthesia will be frequently checked until the experiment is terminated. If animals showing signs of adverse effects or poor recovery, advice will be sought form senior animal care technicians and/or veterinary surgeons at earliest opportunity. Animals which do not show recovery within a reasonable time, will be humanely killed. All animals used in this project will be humanely killed. Tissues from the overwhelming majority will be used for various investigations.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever it is possible we will use cell lines or primary cultures or human studies on volunteers or patients. In addition, we have introduced bioinformatics in our work. These approaches are fully integrated in order to maximise effectiveness and minimise animal welfare costs. My track record shows that the great majority of our previous work has been done on cell lines and primary cell cultures, and that I have gained huge experience in such studies on mechanisms involved in the generation of pain. In the present project, we are keen to follow this approach. However, it is inevitable to use laboratory animals for obtaining primary cultures. Further, pain is a response of the whole body. Some aspects of this response to mild inflammatory conditions can be studied in human volunteers. However, studying mechanisms in other painful conditions, such as nerve injury or burn injury, in addition to some limited studies on patients, can only be done in laboratory animals. In pharmacological experiments we must use drugs which are not approved for clinical use due to their known or unknown toxicity. Therefore, studies on the effect of some drugs on pain responses must be done again in animals. However, I would emphasise that the great majority of pharmacological studies will be done on cell lines and primary cell cultures on anaesthetised animals in non-recovery studies.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The approaches that we will employ will significantly reduce the number of animals: First, we will use state-of-the-art, highly effective methods for the identification of molecules. Hence, the number of animals used in these studies is significantly less than those used in studies employing more conventional approaches. Second, we are using cell lines and primary cell cultures instead of animals. Cultured cells cells, in contrast to animals, can be used for testing a significant number of drugs with significantly lower variability. Whenever it is possible cell lines will be used instead of primary cultures. Third, wherever it is possible, we will assess pain by measuring the expression of various biomarkers which exhibits less variability then behaviour studies, resulting in using fewer animals. Fourth, using power estimation ensures the use of no more animals than requited to statistically meaningful results. Fifth, we will study samples, and measuring pain in various pain models of human volunteers. Further, we will also study samples, and measure pain in patients. These studies will complement animal experiments and will allow us to compare nociceptive processes in laboratory animals and human. This comparison is crucial for the identification of targets for future drug development.
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 this project we will use rats and mice to elucidate cells and molecules involved in the generation of pain. The great majority of studies in the field of neuroscience including pain uses rats, and during our previous work we also gained experience with rats. Hence, using rats will allow us to compare our data to our previous data and to those published by other laboratories. Due to the enormous advantages in terms of quality of scientific data as well as 3Rs provided by the use of transgenic mouse, some

	experiments will include wild type and transgenic mice. Based on data available in the literature as well as from our previous work, the use of transgenic mice cannot be replaced. This project aims to study how pain is generated. Hence, the development of pain is inevitable in our work. However, the majority of animals used in studying will undergo general non-recovery anaesthesia during the whole course of the experiment and pain will be assessed using biomarkers. Animals used in behavioural studies will be kept for the minimum time necessary following the induction of the painful condition. As part of observing 3Rs, we will continue to follow, Good Laboratory Practice, ARRIVE and LASA guidelines.

Project	**52. Cellular mechanisms of circadian timekeeping**	
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)	There is a biological clock, or circadian rhythm, within every cell of the human body that controls major aspects of our physiology, such as when we sleep and how we breakdown food. We know that this body clock is important for health, since when it goes wrong or gets out of synch with the day/night cycle (as in jet lag or shift work), people are more likely to suffer from conditions such as diabetes, cardiovascular disease and various forms of cancer. We want to understand the cogs and gears that allow this daily biological clock mechanism to function within each of our cells. To do this we also must understand how it communicates with	

	neighbouring cells, and how our physiology is tuned with the external cycle of day and night.
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)?	Elucidation of the fundamental processes of our cellular clockwork will help us to understand the complex interaction between lifestyle, our genes and human health. Furthermore we hope our research will reveal potential targets for therapeutic intervention and management of sleep and other clock-related disorders, such as jet-leg and shift-work.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use no more than 13100 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?	This project involves using mice carrying genetic manipulations which we already know or have good reason to expect will be not be harmful. The experiments we perform will be designed to ensure that the smallest possible number of animals is used, and that they endure the least possible suffering. The majority of adverse effects are expected to be mild, with a small number having the potential to be moderate in severity. Moderate severity would mean that the mice are experiencing some persistent discomfort, and this is something we can monitor by looking for clinical signs of this discomfort such as reduced body weight and not interacting with other mice. The protocols will involve breeding and generation of genetically altered mice so that we can investigate their biological timing at the level of individual cells, tissues and in whole animals. Because we are interested in understanding how our internal clockwork functions in the body, in some cases we will use common, non-toxic drugs to interfere with these processes. Some animals may be subject to the implantation of miniaturised devices that measure things like brain activity, heart rate and body temperature. At the end of such experiments all animals will be killed humanely.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To be able to better prevent and treat human disease, we need to understand the fundamental mechanisms of the daily biological clock in every human cell, and how it functions in our bodies. The vast majority of our research occurs in cultured cells *in vitro* without requiring animals. Ultimately though, living animals and their tissues must be used to test the relevance of our research, as the health-relevant, biological end-points we seek to comprehend (circadian rhythms in human physiology and behaviour, sleep and wakefulness) are properties of the intact brain and body and so only occur in living organisms. Whilst many individual features of our biological clocks can already, or in the future will, be able to be studied in cultured cells and tissues, this needs to be proven on a case-by case basis. To this end we monitor the scientific literature, and explore new alternatives as they come online. Where we are successful in adopting a new alternative for animal tissues, we share our findings with colleagues to encourage replacement in other labs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We already reduce our reliance on animal models by performing the majority of our experiments in cell lines grown in the laboratory. Where animals are necessary, tight control over breeding programs means that we produce very few animals surplus to needs, whilst robust experimental design enables us to generate statistically valid results from the minimum requirement of experimental stock. Moreover, for most of our experiments with mice, we make long-term recordings from the same animal or tissue, meaning that far fewer numbers are required than if tissue were collected from animals at several timepoints over the daily cycle. As part of good laboratory practice we write detailed protocols for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and a statistical analysis that

	explains the number of animals in each experimental group. These protocols are then reviewed within my lab, before being shared with relevant members of our Biological Services Group (including NACWO), for comments and suggestions. The protocol is then revised to enable them to be shared and published according to the ARRIVE guidelines published by NC3Rs.
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.	Many experiments that are required as the ultimate test of a hypothesis cannot be performed in humans for clear ethical reasons, but would be too far from the research question if performed in lower organisms. Mice are therefore the subjects of choice for studying circadian timing as they manifest robust rhythms in behaviour and physiology. Moreover, the genetic details of circadian biology are closely aligned between mice and humans – for example the proteins are interchangeable in cell-based assays. As such, mice are the least sentient animals that can be used for satisfactory tests of mechanisms that facilitate the circadian organisation of behaviour and physiology in mammals. Transgenic mice are the leading vertebrate model to study gene action because of their relative ease of breeding and genetic manipulation. Furthermore, the enormous recent growth in knowledge of the mouse genome and the ability to manipulate it with temporal and spatial refinement are unsurpassed when seeking to examine the role of circadian processes in mammalian biology. Consequently, a wealth of information and tools are available, both locally and more globally, which will greatly benefit this project: the rich diversity of genetically altered mice provides an unrivalled resource for understanding the molecular genetic basis to circadian physiology. Moreover, refinement of procedures is ever increasing due to the continuing development of mouse lines with, for example, tissue-specific and temporally selectively inducible mutations. Inbred strains will ensure consistency of results, and minimise the variations between individuals, thus allowing us to keep the experimental cohorts relatively small, in compliance with the 3Rs (both

	refinement and reduction). In turn, the results emerging from this project will increase this information, and have the potential to influence scientific progress. Inbred strains ensure consistency of results, and minimises the variations between individuals, thus allowing us to keep the experimental cohorts relatively small. Moreover, only in mice is the genomic knowledge and technology sufficiently well advanced to develop and apply conditional inactivation/activation of genes by inducing agents. The work will be carried out in purpose-built, state-of-the-art facilities by highly trained technicians and scientists, all of whom are dedicated to the highest standards of animal welfare. The procedures to produce genetically new types of mouse, to breed them up into viable colonies and to experiment on them by recording cycles of behaviour and physiology have been refined over many years in our facility. Finally, beyond our clear ethical reasons, we also have a very strong scientific motivation to prevent animals from suffering any adverse effects, since any form of distress has the potential to interfere with circadian rhythms – the phenomenon we seek to understand.

Project	**53. Central regulation of appetite and body weight**	
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)	In 2010, 26% of adults and 16% of children in the UK were classed as obese. A further 42% of men and 32% of women were overweight. Co-morbidities related to obesity, such as diabetes, heart disease and kidney disorders, create massive personal and public health problems, and are projected to cost the NHS £9.7 billion per annum by 2050. Although there are uncommon genetic and endocrine origins for obesity in certain individuals, it is a generally accepted view that the current epidemic in human obesity is due to the fact that over-weight people, even though they have adequate energy stored in their bodies, are still driven to further consume because	
	they become resistant to satiety signals.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By separating different aspects of appetitive behaviour, the pathways involved and the factors that regulate them, we hope to be able to develop new treatment options to bring benefit to people suffering with metabolic disease or who are struggling to control their body weight.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use around 4600 experimental mice over a 5-year period. All the mice in this and in our other research projects will be bred under this one project licence. The numbers of mice bred for all of these various projects over the five years will not exceed 40000.
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?	Often, we need to do surgery on our mice in order to manipulate how the brain responds to different signals. Sometimes, mice will be given an injection (either under the skin, into a vein or directly into the brain) with minimal disturbance. We can carry out a range of physiological tests on the mice. Thus, we might put them in a scanner to see how much fat they have, measure their blood pressure, or measure their metabolic rate. Occasionally, we even train our mice to poke their noses into holes to break an infrared beam or to press a little lever, which provides them with a sugar reward. This can tell us about their motivation to eat. Invariably, the parameters we measure are very simple: for example, how much food do they eat or how much sugar is circulating in their bloodstream. For the latter, we shall take pin-prick samples of blood from their tail and measure these in a sugar monitor, rather similar to how a diabetic patient would. These procedures will cause some minor discomfort. In this case, we carry out the surgery with the mice under general anaesthetic, plus we give the mice pain killers and sometimes local anaesthetics, to make sure that they do not feel any pain during recovery. The mice recover very rapidly, so they can be returned to their home cages to carry on living as usual. At the end of the study, all the mice are killed humanely.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is difficult to study appetite and body weight in anything other than a normally-behaving mouse. However, we can still find out a lot about brain cells by studying them isolated from the rest of the body, in brain slices in a dish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The need less animals because all the mice we use over several projects are bred under this one licence. For individual experiments in this project, data provided from similar studies in the past or from pilot studies, allows us to make precise calculations of the minimum number of animals we will need to provide robust results. When a strain is not being used regularly, we reduce the colony to a minimum or we end their breeding, having first cryopreserved sperm or eggs for future regeneration.
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 minimise any adverse effects, such as stress, we like to handle our mice (often daily) in order to get them used to being picked up. We also do a range of physiological tests on the mice, sometimes in their home cages, but often after acclimatising them to other cages. We carry out surgery with the mice under general anaesthetic, and give the mice pain killers and sometimes local anaesthetics, to make sure they do not feel any pain during recovery. The mice recover very rapidly, so they can be returned to their home cages to carry on living as usual. We use remote radiotelemetry, which is where, during surgery, we implant a small radiotransmitter under the skin or in the abdomen of the mouse. Later, these devices allow us to monitor things like body temperature, blood pressure and brain activity without having to disturb the mice. We now use transgenic mice to identify, control or record the activity of individual cell types in the brain. This allows us to determine how different cells respond to stimuli and how

	they communicate with each other without using the very invasive old techniques. Since we can manipulate the mice while they are still in their home cage, we can record their normal behaviour, whether they are secreting hormones, or if their metabolism is changed, with minimal disturbance. To do this, we breed mice that have so-called “designer receptors” expressed in just a single cell type. The designer receptors lay dormant and the mice behave as usual. But, by then giving the mice a “designer drug” or by shining a light through an optic fibre, we can activate or inhibit selective brain cells, while studying changes in behaviour or physiology. It is now even possible to see and record the activity of specific brain cells in freely moving mice, using tiny camera lenses attached to the mouse’s head.

Project	**54. Cerebellar contributions to motor, cognitive and affective behaviours**	
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 cerebellum is a major region of the brain vital for the coordination of movements. When in mammals (including humans) it is damaged e.g. due to stroke, genetic disorders, or tumours – voluntary movements such as reaching to grasp an object become inaccurate and poorly timed; balance is severely disrupted; and the ability to learn new motor skills is impaired. Recent studies have also indicated that the role of the cerebellum extends beyond movement control to include mental processes such as decision making as well as emotion (including fear and anxiety). A fundamental gap in our	

	understanding of emotional behaviours (e.g. defensive behaviours triggered by threatening or fearful events) is how the initiation, adaptation or maintenance of appropriate motor responses are generated. The cerebellum may provide this link given its role in motor co-ordination. The plan or work outlined in this project licence will provide fundamental new insights into how the cerebellum communicates with other brain regions to contribute to motor, cognitive and emotional behaviour.
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)?	An increased understanding of the major role that the cerebellum plays in the regulation of voluntary movements should in the longer term assist with the devising of rehabilitation methods for patients with movement disabilities of cerebellar origin, while our studies of cerebellar interactions with brain structures involved in emotional behaviours will also provide new avenues to explore in the treatment of certain psychiatric disorders such as phobias, generalized anxiety disorder and post traumatic stress. The findings will also assist the interpretation of imaging studies of the human cerebellum, by providing information about where and at what time during carefully controlled behaviours, specific cell types in the cerebellum are active.
What species and approximate numbers of animals do you expect to use over what period of time?	Based on extensive prior experience of experiments of a similar type it is estimated that the total number of animals used over the 5 year duration of the project will be 1500 rats. Rodents are the animals of lowest neurophysiological sensitivity on which studies of this type can be performed to provide reliable and applicable data.
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 severity limit for this licence is moderate. During surgery under carefully controlled conditions to minimise infection, animals will receive a general and local anaesthetic and following surgery will be given pain killers. Except for a few hours immediately following surgery, the animals are expected to look and behave normally and to live a normal life. In order to motivate animals to perform behavioural

	tasks that involve them receiving a food reward some animals may be kept on a calorie-controlled diet, however, all animals are expected to live perfectly normally. Some animals may receive compounds (drugs) which will be delivered directly into specific brain regions to interfere with the activity of brain cells. The general pattern of motor and sensory behaviour is expected to be normal but in some cases the drugs may cause the animals to show temporary difficulties with movement. At the end of the testing period the animals will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We can only understand the neural processes that are essential for behaviour by studying the intact brain in living animals. Hence, the objectives in this project cannot be achieved using cultured neurons or by using computer simulations. In addition, the neural processes we wish to understand cannot be investigated in lower animal species, such as the fruit fly, as they do not possess a sufficiently developed brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals required to generate the transgenic models necessary for the objectives, will be kept to the minimum by carefully monitoring the colony size and breeding, and matching these to the demands of the experiments. To ensure that the minimum number of animals are used in the behavioural experiments but to allow statistical testing, wherever possible each animal will be used as an experimental test and a control. This reduces by half the number of animals needed to meet our objectives. In addition, we will test the same group of animals across a number of behavioural tasks. To maximize the information from a single animal and to minimize suffering, in some cases we will take slices of brain tissue after death and experiments will be conducted to investigate the neural processes involved in learning and memory formation.

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 throughout the project as they are the species with the lowest degree of neurophysiological sensitivity that are able to perform the behavioural tasks upon which the work depends. All surgeries will be conducted under strict aseptic conditions, with the animals under a deep surgical plane of general anaesthetic. Post operatively the animals will receive analgesia to provide pain control. Animals are expected to make a rapid and uneventful recovery following surgery and to continue to live a normal life. The animals, which will be housed in cages with environmental enrichment, will be involved predominantly in spontaneous behavioural tasks that provide additional stimulation and for which they will receive reward treats. They also receive additional attention from the research staff. Throughout the protocols the health of all animals will be monitored daily. Regular examination of the animals by trained staff and experienced technicians will ensure that steps are taken to minimise any distress or discomfort to the animals. Veterinary advice will always be sought where and when necessary.

Project	**55. Characterisation of normal and malignant blood stem cell biology**	
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)		
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 programme will identify molecular and cellular regulators of blood stem cell function (including the process of stem cell expansion, or “self-renewal” which is the key to producing unlimited blood cells outside the body. This programme also attempts to use this knowledge of how blood stem cells make copies of themselves to understand how diseases such as cancer mimic and subvert	

	this process. This programme will use well-established mouse models of early stage blood cancers to understand this process from its origins in single cells and also to test novel therapeutic strategies in animal models. The most promising of these strategies would then be moved forward to test for their ability to reduce disease burden in patients by other groups. Finally this project will also produce a list of molecules that cause primitive cells to develop differently, a process critical to understand if blood cells are to be expanded outside the body for their use in gene therapy and the treatment of diseases such as HIV/AIDS, anaemia, and leukaemia.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 840 mice per annum over 5 years. (i.e. ~4200 animals during this project.)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of mice under this licence will show no signs of adverse effects that impact materially on their general health. It is estimated that ~30% of animals will have no clinical signs at all. A further 60% would not exceed mild effects and ~10% might reach a moderate impact on general health. Such adverse effects may rarely be such that the humane end points are reached (<5%). These animals may develop blood cancers causing abdominal distension, substantial weight gain or loss, severe anaemia or erythrocytosis, laboured respiration, persistent inactivity or inappetence, combined with signs of hunched posture or piloerection. Animals showing these clinical signs will be immediately and humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is currently impossible to study the complex role of the microenvironment of normal and malignant stem and progenitor cells in vitro since the complete set of important factors have not yet been identified. Also, to study stem cell function, the cell must be shown to possess the ability to sustain lifelong blood cell production and this cannot currently be assayed outside the body. REDACTED a single cell human blood cell

	expansion technique REDACTED dramatically reduce our need to determine human stem cell functional output in mouse models and have therefore not included human cell studies in mice in our current five year plan.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing experiments we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative. We will utilise in vitro and in silico approaches extensively prior to determining the need for mouse studies on a particular blood cell regulator. Finally, we will use human cell lines (including patient derived cell lines) of particular mutations (e.g., JAK2 V617F; CALR) to study the biochemistry of the mutations.
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 most appropriate and most widely used animal model for studying blood cells and cancer. The techniques are therefore very well established and findings can easily be integrated with other groups' data. The mouse is also the species in which reliable gene delivery systems are best established. For our studies that involve blood cell transplantation, we have recently introduced a recipient mouse model that permits much lower, sublethal, irradiation doses.

Project	**56. Characterising and inhibiting vascular disturbances**	
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)	To characterise the microcirculatory disturbances associated with different inflammatory disorders in the presence of co-morbidities such as ageing and hyperglycamie in vivo and identify strategies that can confer therapeutic benefit	
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)?	It is widely recognized that unwanted inflammation and thrombus formation are central to the pathological mechanisms underlying many (cardio)vascular diseases such as heart attacks, stroke and acute renal failure. For many of these diseases, which have a higher occurrence and worse outcomes in aged and diabetic individuals, no	

	effective treatment has been identified. Collectively, these diseases consume vast amounts of limited NHS funds and time. Therefore considerable research efforts are directed at understanding both the physiology and pathophysiology of circulating leukocyte and platelet recruitment, with the ultimate view of developing treatments to prevent injury by these cells. The contributory role of these different cells in mediating vascular and tissue damage may be age dependent. Furthermore, their unwanted activation and this subsequent deleteious effects appear enhanced in the setting of high plasma glucose levels. This may explain why current therapeutic interventions are not always effective in the elderly and in patients with diabetes. This study will therefore investigate inflammatory and thrombotic events in both young, aged and hyperglycaemic scenarios. As well as increasing scientific knowledge of these inflammatory and thrombogenic processes, these studies may provide data that would have beneficial implications for a whole host of diseases. Regenerative medicine, particularly the use of stem/progenitor cells, is currently being considered for treating a whole host of inflammatory and degenerative conditions. However, stem cell therapy is limited by the insufficient number of cells that can be isolated. Therefore, identifying factors that can enhance the recruitment of these potentially therapeutic cells to the blood vessels within sites of injury in experimental co-morbidity models of injury will impact positively on this treatment modality and have huge implications for the treatment of a number of inflammatory and ischemic diseases. Experiments are not conducted with any financial profit in mind
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years, we would expect to use no more than 9,000 mice
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	The majority of experiments proposed within the remit of this licence will be conducted on terminally anaesthetised mice. Thromboinflammatory injuries will be induced in anaesthetised mice and subsequent characterisation of vascular perturbations and the therapeutic effects of stem cells will be performed under terminal anaesthesia.

end?	Hence we do not anticipate any adverse effects and so the severity level is non-recovery. In some experiments inflammation will be induced prior to anaesthesia with all subsequent analysis conducted under terminal anaesthesia. Since these inflammatory models are well characterised and routinely used, we do not expect (nor have we experienced) any adverse effects. A mild severity limit is given for the appropriate protocol. In some mice, hyperglycaemia will be induced by feeding of a high fat diet for up to 16 weeks. Thereafter, these mice will undergo terminal anaesthesia. Again, this is a well established model of type 2 diabetes that is routinelt used and we do not expect any adverse effects. A mild severity limit is again given for the appropriate protocol.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No in vitro techniques are currently available that can fully replicate the complex spatial and temporal interactions that take place within blood vessels following inflammation. Nor is it possible to entirely replicate the many additional complications associated with co-morbidities such as ageing and diabetes. Many of the cells we will be investigating rely upon their surrounding environment for cues or signals – something which cannot be mimicked in single or even multiple cell co-cultures. The presence of blood flow, shear stresses and neuronal/hormonal mediators all influence and exert effects on circulating cell recruitment and this cannot be reproduced accurately in vitro for the various different vascular beds we wish to image.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical analysis / power calculations will be conducted to ensure we use the minimum number of mice per group that will be informative. Statistical analyses from previous studies (and our extensive experience) suggest groups sizes of 6-8 are at least required to measure significant differences. To maximise the information gained from a single experiment we aim to acquire images from multiple

	blood vessels and multiple sites in the body and subsequently take tissue / serum samples for further data generation.
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 species of choice as they are the best vertebrate model for use in in vivo imaging studies that look at inflammatory processes. The microcirculatory patterns in mice are very well characterised and are similar to humans. The main components of their thrombo-inflammatory and immune system are shared by humans, which is essential when these same processes are being studied in mice. Many of the responses we plan to study from a scientific viewpoint are best characterised in this species due to a widespread availability of selective murine blocking antibodies and defined transgenic and knockout mice. The models of inflammation to be used are well characterised, highly reproducible and can be induced easily in anaesthetised mice. The histological and biochemical responses obtained models the clinical disease. By using well established protocols to elicit and inflammatory response, we can mimimise the unknown effects on the mice and subsequently minimise or prevent pain, distress and suffering. Training of researchers would be carried out by the principal investigator and competent researchers in the group who are both fully experienced with all procedures required. This will ensure that the learning curve of new personal licensees is reduced and unnecessary animal distress prevented. During the protocols we will monitor the health status of the animal and cull mice that show physical signs of distress.

Project	**57. Characterising microbiome- mediated pressures on bacterial host-adaptation in the nasal niche**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 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)	Healthy cattle, in common with humans, carry communities of bacteria in their nostrils. Within these communities are some bacteria that are regarded as pathogens, and are also thought to influence both the structure of bacterial communities in the nose and susceptibility to disease. Importantly, some of these pathogenic bacteria can jump between animals and humans – a step which can lead to epidemics. The factors	

	dictating whether these 'host jumps' occur are poorly understood. One hypothesis is that bacterial communities in the nose might vary in their resistance to colonisation by pathogens, a resistance perhaps mediated through gene transfer between bacteria, via competition between bacteria, or by bacterial production of small molecules. By directly sampling nasal secretions from cattle we will be able to characterise the bacterial communities and small molecules present in this environment and compare this to findings in the human nose. This will aid in understanding the molecular basis of colonisation resistance and host-jump events.
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)?	Better understanding of how the pathogen Staphylococcus aureus colonises the nose of cattle could help to prevent the transfer of this pathogen between human and cattle hosts. It could also decrease the number of bovine S. aureus infections in circumstances where the nose acts as a reservoir for disease. Bacteria can transfer genes between one another and this phenomenon can lead to outbreaks of S. aureus infections. However, we do not always understand where these genes originate from. Determining the genes present in nasal bacteria could help us to understand the factors that pre-dispose towards the development of epidemic strains. Likewise, understanding the microbial communities and small molecules associated with S. aureus carriage could assist in the development of strategies to prevent this occurring.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using 100 animals over the period of three 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 severity of the procedure, collecting a nasal swab and aspirating nasal secretions, is mild. It will involve short term restraint of the head for less than 5 minutes using routine handling techniques, and carries a low risk of adverse effects. Whilst one potential risk associated with sampling is the development of a nosebleed occurring as a consequence of damage to the

	inside of the nose, this would be expected to self-resolve and is unlikely to cause lasting harm or distress to an animal. However, animals showing persistent clinical signs of nasal damage, nostril abrasions, or distress will be promptly referred to a veterinary surgeon and treated as advised. Animals will be returned to the REDACTED herd after sampling.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Understanding the factors that govern the structure of nasal bacterial communities involves characterisation of these microbes and their metabolic environment. Both elements are undeniably complex. Whilst strides have been made in developing 'synthetic' microbial communities, these systems are difficult to maintain in a laboratory environment and do not mimic the complex communities present in the nose. Therefore, it is necessary to sample live animals to gain a complete picture of the microbial ecology in this niche. Further, whilst 'synthetic nasal medium' for culturing bacterial communities is presently available, its composition is based on data from human subjects – and may not be relevant in the context of cattle. However, where available, alternatives to live animals will be employed. This includes usage of cadavers to optimise sampling. However, cadavers, in lacking an active immune system, are not an appropriate model to study nasal bacterial communities.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Prior data are used to estimate the variability of the phenomena we intend to measure and the number of animals required. As data is collected we will exercise the opportunity to consider its variability in order to further refine our group size estimates. This process will ensure that the minimum number of animals is used in each instance. Where available, cadavers from previously culled animals will be employed for protocol optimisation. This will minimise the number of live animals required. Indeed, every

	study involving live animals under license is subject to rigorous evaluation by experts in statistics, ethics and animal care.
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.	Cattle are of particular interest for this study based on the high prevalence of 'host jump' events observed for a particular bacteria, *Staphylococcus aureus,* between this species and humans. This observation has led to the suggestion that cattle represent the major reservoir for human epidemic clones, and therefore warrants further investigation of the factors that govern host-jump events. Whilst not anticipated, it is possible that swabs may be contaminated with traces of blood during sampling. In this event the sample would be compromised and a second attempt to collect a sample from the other nostril would be made. Animals will be appropriately restrained during sampling to reduce the opportunity for adverse events, and time under restraint will be kept to a minimum (<5 minutes) to reduce any distress. In the event that sample collection does cause undue stress to an individual animal, the option of using sedation to calm the animal can be employed. Typically swabs are ensconced in a flexible plastic guard to minimise the risk of adverse effect during sampling. In addition, we will investigate different ways of sampling the nasal tract in an initial small cohort of ten animals in order to select the least invasive method compatible with the aims of the study.

Project	**58. Characterising the early immune response to systemic fungal infection**	
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)	Systemic fungal infections kill over 1 million people worldwide each year. The vast majority of these individuals have underlying defects in their immune system which predispose them to such infections. Therefore, discovering so-called “immunomodulatory” approaches to stimulate the human immune system hold considerable promise for improving our treatment of such diseases. In order to achieve this, we need a deep molecular understanding of how the immune system recognises and deals with systemic fungal infections, and how these pathogens are sometimes able to evade this recognition and killing. Here we that aim to pursue one promising avenue in this investigation by characterising how phagocytic cells	

	(specialised white blood cells “eat” pathogens) recognise fungi and then “present” them to the adaptive immune system in order to generate lasting resistance to such infections. Specifically, we aim to answer three key questions: 1. How do phagocytic cells identify fungal pathogens and activate specific signalling pathways to engulf and kill them? 2. How do two groups of fungal pathogens, *Candida* species and *Cryptococcus* species, “hijack” this process in order to survive within the host? 3. How does this hijacking impact on the subsequent ability of phagocytic cells to stimulate the adaptive immune system via antigen presentation?
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)?	We aim to answer a series of fundamental biological questions through this project which will contribute to the wider pursuit of new therapeutic approaches for two major human diseases: invasive Candidiasis and systemic Cryptococcosis. Whilst the immediate opportunities for direct medical or veterinary benefits are small, the long-term opportunity to devise new immunomodulatory approaches are very significant. Given the large numbers of humans and non-human animals that suffer from invasive fungal infections, even a minor improvement in therapeutic approach holds the potential to significantly reduce human and/or animal suffering in the long term. Our previous work, over a period of more than ten years, has made major advances in our understanding of fungal/immune interactions using in vitro and, more recently, Galleria/Zebrafish approaches. However, we are now at a stage that we cannot proceed to test how applicable these discoveries (and potential therapies) will be to human patients without using a whole animal mammalian model. Without the work we propose above these recent discoveries regarding inflammatory regulation of antifungal immunity will remain at the level of an “interesting theory” and not be able to be advanced towards the ultimate goal of influencing medicine
What species and approximate numbers of	We expect to use 2,000 mice in total over 5 years. These mice will come from our breeding colonies,

animals do you expect to use over what period of time?	commercial providers, other academic and non-academic research laboratories. (400 mice x 3 experimental protocols) = approximately 1,200 of these mice will be used for regulated procedures 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 work we propose here is of either sub-threshold or mild severity. A small number of experiments may involve moderate severity. None will be severe. During the course of this programme of work, some animals will experience weight loss and other moderate suffering. All mice used under this Project Licence will be humanely culled at the end of each Protocol or prior to the end, should their welfare deteriorate above the thresholds defined.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many labs, including our own, have made significant progress toward understanding, and ultimately treating, systemic fungal diseases. However understanding the implications of these findings in disease pathogenesis necessitates the use of an animal model. We and others have already focused on the key questions by using nonprotected animal alternatives. Now however we need to test these findings in the closest appropriate nonhuman model – in this case the mouse. Over more than a decade, our group has pursued an intense programme of in vitro work to establish interventions that enhance killing of fungal pathogens by white blood cells in vitro, including both immunomodulatory strategies (e.g. IFNg), genetic approaches (targeting specific phagocyte receptors) and drug therapies (e.g. ERK5 inhibition). We know a huge amount about appropriate drug doses, frequency of administration and individual variability in the immune response to these pathogens. More recently, we have recapitulated key findings using the non-regulated larval zebrafish model, demonstrating that the discoveries we have made have the potential to impact on human and animal therapeutic treatments. However, to demonstrate that these approaches will ultimately be both safe and effective in humans, we now need to move this work into a mammalian (mouse) model. In doing so we aim to achieve two things:

	4. To demonstrate that our in vitro findings can be safely applied in vivo 5. To characterise how the complex immune system of a mammal responds to these interventions which have previously only been able to be tested in relatively simple in vitro models. During this work, review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use. We will use SyRF the free online platform for researchers to perform a systematic review and meta-analysis of animal studies. https://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our proposal is focused around a tightly defined number of procedures which inherently minimises animal use. In addition, however, we will make every effort to maximise the *ex vivo* use of mouse tissues (E.g. To obtain leukocyte populations for *in vitro* experimentation). Typically, such tissues are obtained by culling additional animals, and thus by reusing tissues in this way we aim to reduce the overall number of animals used both by ourselves and other colleagues. Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment. The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines. We have three strategies to minimise animal numbers whilst retaining scientific rigour: 6. All of our protocols commence with a pilot study on six animals (3 control, 3 intervention) to assess the validity of our readouts and ensure there are no unexpected adverse welfare consequences. These pilot studies will nonetheless yield important data that will then contribute to the larger studies, but avoid 'wastage' of animals in the event that initial experimental parameters are suboptimal

	7. In all cases we propose to harvest and store post-mortem tissues (bone marrow, primarily) in order to avoid the need to kill animals purely to obtain tissues for future experiments 8. Wherever possible, we propose to share experimental approaches and data with colleagues in the UK in order to avoid unnecessary duplication and maximise the value of data obtained (e.g. by correlating findings at different timepoints).
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.	Almost all of the experiments we propose here aim to recapitulate the natural route of fungal infection and to map out the early immune response. This means that most of our models use low-dose infections and study immune responses prior to symptoms developing, thus minimising welfare implications to the animal. During our pilot work, as well as the main procedures, we propose to monitor symptoms closely so that we can refine dosing and timing in subsequent experiments. It is important to note that this is not only desirable from an animal welfare perspective but also maximises the value of the data we will obtain – since our work is focused at trying to improve the healthy immune response to these fungi, data from sick animals is less useful than that from animals that have successfully controlled the infection. Lastly, our proposed animal work is closely linked to our ongoing and extensive programme of in vitro work. We will therefore adjust our in vivo protocols (especially in terms of the nature and dosign of PAMP/immunisation reagents) based on in vitro data, so as to avoid triggering unexpected inflammatory responses or adverse side effects.

Project	**59. Circadian clocks in the brain and their dysfunction in neurodegenerative diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The body clock is responsible for the circadian (from the Latin: *circa*= about; *dies*: day- *about a day*) regulation of our daily physiology, metabolism and complex behaviour, such as sleep, memory and cognition. Its importance for general health and well-being has been long underestimated, but it has recently come under intense scrutiny. Up to 75% of the world population is exposed to artificial light at night and low levels of daylight during office hours and 20–40% of the work force is actively engaged in shift work. This long-term perturbation of the circadian system is associated with severe, age-dependent medical conditions, such as diabetes,	

	cardiovascular diseases and brain inflammation. Recent evidence suggests that perturbations of circadian and sleep function are also strongly associated with dementia, including Parkinson and Alzheimer's disease (AD) (the latter accounting for ~70% of dementia cases). These disturbances overlap in time with the earliest pathological landmarks of AD, such as the accumulation of toxic proteins in the brain and precede the onset of clinical symptoms by several years. Importantly, this relationship is also present in several mouse models of AD, although the exact nature of the connection between circadian and sleep disturbances and AD is currently unknown. In this project, we will study how circadian dysfunction affects ageing and neurodegenerative processes and attempt to recruit and exploit circadian biochemical pathways as a novel early intervention to prevent and/or delay neurodegenerative conditions, such as AD
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are currently no available disease-modifying therapies for dementia. The recognition of dementia as a national priority by the British Government in 2015, together with the failure of all clinical trials to-date aimed to find a cure for AD underlie the necessity to broaden the scope of research in unravelling the genetic and environmental triggers of dementia. My research will bring a unique perspective to the dementia field by providing a deeper understanding of the molecular, cellular and circuit mechanisms underlying circadian function and their dysregulation in the early stages of dementia. The benefits, however, will not be limited to the scientific community. The body clock align us to the light-dark cycle, enhancing our sleep, cardiovascular and metabolic health; thus, manipulations aimed at stabilizing/enhancing its underlying biochemical pathways may not only be a highly effective strategy to prevent early disease progression, but also will enhance wellbeing and are therefore likely to be well tolerated. This "gentler" approach in fact will solve a known conundrum of current therapeutic attempts, which by directly targeting molecular pathways presumed to directly responsible for the disease, also interfere with fundamental cellular processes, thus causing unacceptable side effects, especially in the asymptomatic stages of the disease. This greatly restricts their application, by limiting it to the late

	stages of the disease, when pervasive neuronal loss and cognitive deficit have already irreversibly occurred, making such treatment highly ineffective.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use inbred and genetically altered mice, including aged animals and models of neurodegeneration. The maximum number of animals we will use over 5 years is 14,000. Of these mice, a maximum of 5,000 will be used for long-term in vivo experiments.
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 be only experiencing mild severity procedures, as they will be used for breeding, painless killing for tissue collection, or simple low-stress behavioural tests (such as wheel running behaviour, or novel object recognition) to measure circadian and cognitive function, respectively. About 20% of animals may undergo brain surgery of moderate severity. Mice will be given multiple types of pain relief during and after surgery. They will recover for several days after surgery and will be checked daily for any unexpected sign of pain/distress. Prompt advice of veterinary staff will be sought if needed. Infections are unlikely (up to 1%) and will be limited by using good surgery techniques. Any mouse exceeding signs of moderate severity (e.g., weight loss more than 20%) will be promptly and humanely culled, in consultation with the veterinary staff.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Sleep and circadian rhythms of physiology, behaviour and cognition are hard to study in cellular surrogate models, because they cannot be fully replicated outside the body. Furthermore, currently such models do not recapitulate faithfully the ageing process, which is a preponderant risk factor for both dementia and impaired body clock function. Nevertheless, to replace the usage of live animals or their primary tissues, we will utilise surrogate cell cultures for the initial efficacy screening of novel DNA and viral constructs and drugs. We share our data with the wider neuroscience community to predict accurately specific manipulations of circadian pathways. This may replace/reduce the number of animals undergoing experimentation for
	drug efficacy screening in future.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will reduce the number of animals undergoing extensive behavioural experimentation by using specialized tissue cultures of mouse brain. When more extensive in vivo experiments are required, we will reduce the number of animals used by utilising experimental designs that relies on comparing behaviour and other experimental measurements within the same experimental subject before and after treatment, thus allowing for reduced experimental variability and animals undergoing extensive experimentation. Finally, we will co-detect several outputs from a single mouse, including daily patterns of locomotor activity, sleep quality and gene expression, with further reduction of animal usage, thanks to improved data quality. Allocation of animals to the respective experimental groups will be randomized and we will blind experimenters to group assignment (i.e. control vs treatment) when possible.
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 genetically modified mouse models of dementia to detect long-term consequences of altered circadian and sleep behaviour. Our surgical protocols for repeated measurements of locomotor activity, sleep and cellular activity tipically requires only one major surgical procedure, after which the animal can undergo measurements for weeks and months without any pain and/or without the need of further invasive procedures. After the initial surgery, the animal will be given pain relief and allowed to recover from the surgery for at least one week before any further procedures to record various experimental outputs are undertaken. These precautions will minimize any suffering the animals might have otherwise experienced. We will preferentially use wireless recording devices to detect sleep in freely behaving mice to minimise potential risks associated with long-term tethering of the mice. When sleep restriction is needed, it will be reduced to a length which does not elicit significant stress responses (≤6 hours). We will also implement innovative methods for the detection of gene expression in freely moving mice by using bioluminescent probes (derived from fireflies) which can be detected by sensors placed outside the mouse. This system is minimally invasive and only requires the administration of luminescent

	compounds that we aim to deliver orally in the drinking water, or by implanted minipump, thus minimising the need for repeated injections. For fluorescent imaging by head-mounted cameras or wireless sleep recorders, state-of-the-art miniaturised devices will be used to reduce discomfort, while improving the quality and the amount of collected data

Project	**60. Circuit mechanisms of adaptable motor control**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our aim is to try and understand how the brain initiates and controls complex body movements. Since the early 1900s scientists have tried to understand how the brain communicates with the spinal cord and ultimately muscles to coordinate motor behaviours such as climbing the stairs or catching a thrown object. Although significant progress has been made in understanding how different brain areas coordinate their activity to produce movement, we still don't know how neurons (electrically excitable cells in the brain and spinal cord) produce the patterns of motor commands necessary to initiate and control complex and adaptable movement. In this programme of	

	work, we will address 3 main aims: Aim 1: Develop our understanding of how single neurons and connected networks of neurons in the brain generate patterns of activity necessary to generate movement. Aim 2: Determine how different motor areas in the brain communicate with each other to generate adaptable motor control. Aim 3: Investigate how this communication is disrupted in rodent models of neurodevelopmental disorders and investigate the potential for therapeutic interventions that can reverse the behavioural abnormalities.
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)?	Motor control is a basic but fundamental important feature of mammalian behaviour. Almost everything we do, every thought we have ultimately results in a movement of some kind, whether navigating to find food or interacting with others in a social environment. We will build on existing data derived from animal models to develop an in-depth understanding of how neurons in the brain generate patterns of activity that are necessary for initiating and controlling body movement. Our aim is to generate a 'functional blueprint' of the motor-related areas of the brain in health, as this will undoubtedly uncover potential points of entry for therapeutic interventions aimed at alleviating the debilitating symptoms associated with disease and loss of motor control (e.g. stroke, Parkinson's disease, Rett's syndrome). Direct beneficiaries of our work will be local and international science communities who can access our findings via publishing in international peer-reviewed journals and through participation in national and international neuroscience conferences. Although our work is not directly translatable, we will work with relevant agencies to ensure dissemination to Health Sector partners and pharmaceutical companies.

What species and approximate numbers of animals do you expect to use over what period of time?	This programme of research will use rats and mice. To achieve the outlined aims we will require: ~0.6 rats / project / week / year = (0.6 x 2 x 40 x 5) = 0.6 x 2 projects x 40 weeks x 5 years = 240 – 250 rats. *For each experiment data will be acquired from a single rat for 2 weeks. Two projects will run concurrently throughout each year for 5 years. Experiments involving mice require the generation of small colonies of wild type and transgenic breeding mice to ensure sufficient offspring for use in experiments. Cohorts of 3 mice per week will be used for behavioural training and recording, with ~9 projects running concurrently. The number of breeding and experimental mice is estimated to be 5400 – 5800 across the 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	A small proportion of mice will be used for breeding purposes to ensure a stable supply of experimental animals and no adverse effects are expected. Animals that require invasive surgical procedures will be continually monitored and pain will be controlled during surgery by general anaesthesia by administering post-operative analgesics. Stress will be minimised by

	habituating animals to the experimental setup prior to each experiment. To facilitate learning food or water restriction may be used where the health of each animal will be monitored daily. Adverse effects associated with this type of experimentation are expected to be minimal. The application of drugs will be focal and restricted to particular brain regions so there should be minimal behavioural side effects. Deaths resulting from anaesthesia or surgical complications are uncommon (<1%) and will be minimised by correct dosing of anaesthetics, by accurate weighing and maintenance of body temperature during and post-surgery e.g. use of heat pads. Risk of infection will be minimised by good surgical and aseptic techniques. Overall, for the majority of experiments the severity rating will be mild to moderate. The highest severity rating of this programme of work will be associated with the use of genetically altered mouse lines that display progressive loss of motor control with endpoint respiratory failure. We will however conduct the majority of our experiments either when mice are pre-symptomatic or at a stage where motor symptoms are relatively mild. Strict health surveillance will be conducted at all times and humane endpoints for euthanasia will be strictly adhered to. At the end of each protocol, animals will be killed by using approved humane methods and tissues from these animals may be used for post hoc histology.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To understand how brain activity relates to behaviour requires the use of animal models and experimentation. Although computer modelling is becoming more and more useful in terms of simulating aspects of brain activity, computer models are as yet not sufficiently complexity to accurately recapitulate normal brain activity. To complement our animal-based experiments we have developed strong collaborations with UK computational

	neuroscientists to further develop in silico models of brain function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise the use of experimental animals we have estimated group sizes from statistical power calculations based on known effect sizes and previous experience. Wherever possible we will maximise the data yield from any individual animal, thus minimising the total number of animals used across the study.
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-modified (GM) mouse lines provide an invaluable set of tools to interrogate brain activity underpinning motor behaviour. GM mice provide a robust model system with which one can apply advanced recording and manipulation techniques to investigate how neurons in the brain communicate with each other, and to what extent output from the brain is necessary for coordinating movement. To minimise stress during experiments, mice will be handled extensively before and after behavioural training and we will ensure adequate recovery periods between surgeries. It has been well documented that stress reduces the ability of mice to learn simple and complex behavioural tasks so it is within the experimenters' interest to ensure a 'stress free' environment in which to train. The majority of experiments in this programme of work will have a mild/moderate severity rating due to moderately invasive surgical procedures. For these experiments, pain will be controlled by general anaesthesia and post-operative analgesia. Principles for good surgical practice will be followed throughout.

Project	**61. Climshift: growth, size-at-maturity, and metabolic rate of marine sticklebacks**	
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)	Climate change is predicted to cause ectothermic animals (fish, invertebrates, etc) to “shrink”, that is, their maximum body size will be reduced. This has major implications for ecology, and also to economies in how it affects fishing and aquaculture yields. However, the extent of this shrinking is still much debated. We aim to determine experimentally how strong this effect will be.	
What are the potential benefits likely to derive from this project (how science could be advanced or	This project will provide fundamental scientific knowledge of how animal physiology (growth, metabolism, size-at-maturity) is affected by	

humans or animals could benefit from the project)?	temperature. In addition, it will provide essential information on how yields of fish stocks and aquaculture industries will be affected by climate change and inform their management.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use the marine fifteen-spined stickleback, Spinachia spinachia, a common shallow water fish species in the North Atlantic. Over the 1 to 1.5 years of the project we expect to use approximately 100 individuals in the regulated procedure: 30 in pilot experiment for RMR, 10 in pilot experiment for MMR, 60 in regular experiments.
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 any adverse effects on the animals from any of our procedures. Our procedures will generate at most moderate stress (severity level Moderate). This species is robust to careful handling, and respirometry is a ubiquitous experimental practice on fish, with accepted practices to minimise stress. This species is not particularly social (in fact they are often antagonistic to each other so beyond the larval stage will be kept separate) so being separated from conspecifics is not an issue. Being confined in a limited space for short periods (i.e. in a respirometer) should not be stressful; they frequently hide under rocks and in crevices. To remove the positive effects of digestion upon metabolic rate, the specimens used in respirometry will have food withheld for 24h. Ectotherms such as fish do not have the same nutritional demands as endotherms, and can have food withdrawn for substantial periods without lasting harm. Since quantifying body mass changes is the main driver to the project, all animals will be humanely euthanised for drying and weighing at the end of their use period.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since we are examining growth, metabolism and maturity stage in controlled conditions, there are no alternatives to using live animals kept in the laboratory. There are several criteria under which we chose this protected species to work on. It is abundant, easily collected, but

	most importantly relatively large and fast growing for a temperate ectotherm, reaching maturity in under a year. No non-protected organisms meet these criteria. In addition, a major focus of the project is how climate warming may affect yields from fishing and aquaculture industries, so providing experimental evidence from a fish species is essential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will be using a relatively small number of initial animals (ca. 40) in the project to produce a new generation. In the regulated procedure (respirometry experiments) we plan to only use enough specimens to achieve good statistically relevant results. Pilot experiments will be conducted to determine the repeatability of metabolic rate measurements. We plan to use 30 individuals in the pilot experiment for RMR, and 10 in the pilot experiment for MMR. The data from these experiments will inform a power analysis to determine ultimate numbers in the regular experiments, but we conservatively estimate this will be around 60 individuals.
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 identified this species as the most suitable to meet the project objectives under several criteria, including ease of collection adjacent to the establishment, relatively large size, fast growth rate, fast time to maturity, ease of maintenance in simulated natural conditions. No non-protected species meet all of these criteria as well as this species does. The animals will be maintained in simulated natural conditions (i.e. artificial substrate and plants), and fed ad libitum at all times. They will be held separately after the larval stage, since this species is known to be aggressive towards conspecifics. None of our procedures will cause anything greater than transient stress. When we know the repeatability and variance of the respirometry data (i.e. metabolic rates) in this species from the pilot experiment, we may be able to refine the procedure further by using fewer replicates and overall fewer individuals.

62. Coccidia control methods in poultry and game birds

Project duration

2 years 9 months

Project purpose

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

Key words

Coccidia, Parasites, Control

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

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?

Coccidia are protozoan parasites of which damage the gut wall. In the poultry there are a number of species of coccidiosis and their effects vary from harmless right through to life threatening. Coccidia infect every poultry house worldwide, and the infectious oocysts are highly resistant to heat, cold and disinfectants. Because of this and the fact they are very prolific and capable of developing resistance in the gut phase of their lifecycle to chemotherapeutic treatments eradication is nearly impossible.

Due to this coccidiosis is a one of the most important poultry diseases as it thrives in high population/ density farmed poultry which the poultry industry is based on. Around $90 million dollars in the US and $3 billion worldwide are spent each year on coccidiosis defence alone. Coccidiosis has a major effect the poultry industry due to decreased performance, morbidity and mortality and still costing an estimated $300 million US dollars in losses, worldwide.

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?

This is a service licence that aims to provide an ongoing service to customers, pharmaceutical and disinfectant companies. It aims to with the provision of supportive data

for regulatory purposes on the safety, efficacy and quality of vaccines and anti-parasitic drugs and disinfectants against coccidia. It also protocols test and confirm drug sensitivity and resistance to existing chemotherapeutic anti coccidials as well as evaluation of other, non-chemotherapeutic control systems that could be used commercially. As such this will have direct impact on the welfare of commercial poultry and game birds. Coccidia have complex life cycles and different sexual and asexual stages which cannot be totally replicated/ maintained in-vitro. Research into such parasites therefore has to be undertaken in their natural hosts. The strains can be also host-specific; therefore choice of species is dictated by the coccidian strain under study/therapeutic agent to prevent this strain.

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?

To ensure minimal use of experimental birds, coccidia are only passaged to maintain stocks of important strains of coccidia, such as fully sensitive strains, or reference strains.

Most coccidia remain viable in a refrigerator for up to 3 months depending on species, or are able to be stored in liquid nitrogen, so passaging is only done when necessary thus reducing the number of birds used. This work is demand lead by the licencing need, it is estimated that 16,000 chickens, 2,500 turkeys 600 gamebirds and 20 ducks will be used.

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The vaccines and anti-coccidial drugs have been around for a long time and new vaccines and drugs would be using similar methods so risk of adverse effects are minimal. Our challenge models have been used successfully for decades and have been refined over these years. The challenge levels we use are very accurate and can give the determined severity limit required. The expected severity limit would be mild to moderate

Replacement

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

Some replication of coccidia has been achieved in cell culture, but the levels are extremely low and it would be impossible to demonstrate the efficacy of products or grow suitable quantities of parasites. Coccidia like Eimeria are host specific, so the natural host animals have to be used.

Reduction

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

To ensure minimal use of experimental birds, coccidia are only passaged to maintain stocks ofimportant strains of coccidia, such as fully sensitive strains, or reference strains. Most coccidia remain in a refrigerator for up to 3 months depending on species, or are able to be stored in liquid nitrogen, so passaging is only done when necessary thus reducing the numbers of birds used.

The numbers of birds used varies, and in some cases is guided by the minimum requirements of licencing authorities such as the European Pharmacopoeia and statisticians are consulted before new studies are initiated if further guidance is required.

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.

Coccidia like Eimeria are host specific, so the natural host animals have to be used. With over 80 years of experience and knowledge of coccidia we have a vast understanding of the interactions and effects of the parasites. This enables us to determine accurate challenge levels so we are able to; use the minimal number of animals, cause the least harm/severity to the animals whilst still achieving a sample effect and achieve statistically accurate results.

Project	**63. Collection of body fluids and/or tissues**	
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 overall objective is to provide scientists with animal body fluids and/or tissues (typically blood samples) of appropriate quality to support the research and development of new medicines.	
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 of work performed under this licence include; • development and validation of in vitro tests to replace using live animals • calibration/validation of equipment that ensures high quality medicines are made to exacting standards • reducing the total number of animals required by coordinating the supply of body fluids and tissues across the company and, where possible, use of 'banks' to store samples for later use	

	• development of new medicines to treat people that are unwell and improve their quality of life and in some cases to save their lives
What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of this 5-year project we expect to use: 1000 mice and 1000 rats (normal and genetically altered); 100 rabbits; 250 dogs and 250 mini pigs. The actual numbers of animals used may be less than the figures stated above as some may be used more than once. This can occur when the sampling causes only minimal distress and the vet has ensured that the animal has fully recovered.
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 samples collected under this licence will be blood and organ samples such as liver or kidney. Other samples, such as urine will be collected more rarely. The species used will be determined by the scientific requirements of the medicines discovery and development programmes at the company. Samples from rodents will be the first choice to fulfil the needs of these projects and are expected to form about 80% of the samples. Samples from non-rodents (dogs, pigs and rabbits) are used where necessary to ensure successful development of a medicine. Blood samples from rodents will mostly be taken from anaesthetised animals meaning that the animals will be unconscious and therefore feel no pain. Usually the animals will then be euthanised (humanely killed) without waking up from the anaesthesia. Samples from dogs, pigs and rabbits (e.g. blood or urine) will often be taken whilst the animal is conscious. Blood sampling procedures on conscious animals will take small samples (less than 10% of the circulating blood volume) and cause no more than minimal and short-lived pain or distress. For example, blood samples will be taken by inserting a needle into a blood vessel in the same way a blood sample is taken in humans. This may cause a mild painful sensation that is transient and stops once the needle is removed. The amount of blood taken will not cause animals any harm. Urine samples will be collected by placing the animals in specially designed cages that allows urine to be collected as it is excreted. During urine collection food is usually withdrawn from the animals to prevent contamination of the urine sample, this will not cause the animal any harm. Animals are acclimatised to these cages to minimise distress. If signs of distress are observed the animal will be removed from the cage. Alternatively, urine can be collected from dogs, by catheter, directly from the bladder. This may cause a mild painful sensation that is transient and stops once the catheter is removed. In some cases, urine can be

	collected from rodents by housing them in a standard cage on hydrophobic sand which allows urine to form into drops which can then be collected. For all species larger blood volumes (greater than 10% of circulating blood volume) and organ sampling will only be taken under non-recovery anaesthesia meaning that animals do not experience any pain or distress and, at the end of such procedures animals will be euthanised while under anaesthesia. Sometimes samples will be taken from animals that have been sampled before. Animals will not be used more than once unless their general health and well-being has been assessed by a veterinary surgeon and they are deemed fit to be sampled again. Some of the rats and mice (approximately 10%) will be genetically altered i.e. they have had specific genes removed or added to support the research and development of new medicines. These genetic alterations are expected to have minimal effects e.g. hair loss, reduced body weight gain or they have changes made to their immune system. These animals will receive the care and husbandry they require to ensure that their genetic alteration does not compromise their health and wellbeing.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Samples will only be taken from animals after a set of questions have been answered by the requestor and assessed as satisfactory by the Project Licence Holder or his delegate. Questions will include checks that the work requested fits the purpose of the licence, alternatives to animals have been considered and judged to be unsuitable, and animal use is justified as the only way to produce the information needed for the research and development of a new medicine.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In vitro studies will be designed to ensure the minimum number of tissues/volume of body fluids are required and therefore the minimum number of animals will be used to achieve the required objectives of studies. To reduce the number of in vivo procedures performed on animals and the number of animals used, larger samples than those required to meet the immediate objective of the work may be taken where there is no increased impact on the welfare of the animal. The extra sample may be utilised immediately for project work or retained for future use as part of a tissue 'bank'. Wherever possible multiple samples will be taken from

	animals during a non-recovery anaesthesia procedure to provide tissues for multiple projects. The company has tissue request/distribution lists that facilitate efficient use of samples. Where appropriate, we will retain remaining tissues/body fluids as part of a 'bank' for future use.
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 experimental procedures will be reviewed to ensure they comply with the project licence and that the use of animals is fully justified. All study work is conducted by highly trained and competent staff. Environmental enrichment e.g. nesting and bedding material and shelters are provided for all rodents in their cages. Socialisation e.g. daily supervised playtime for dogs is provided. All animals are housed in groups wherever possible unless there is a welfare or scientific justification for single housing, e.g. to prevent animals from fighting. The sampling methods chosen will cause the animals the least pain or distress.

Project	**64. Combination Therapies to Improve Treatment Outcomes in Cancer**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project seeks to develop new treatment options for patients with cancer by combining standard-of-care treatments, such as radiotherapy and surgery, with novel, experimental drugs. In particular, this work will focus on using these new treatments to modify the activity of the immune system either to make it better able to attack tumours or less well able to cause damage to normal tissues. The research will be directed towards diseases that represent significant unmet needs in the clinic (eg melanoma, head and neck, thyroid and breast cancer and sarcoma). The work will be	

	based on a number of key themes that use: (i) gene and virus therapies as a means of killing cancer cells and alerting the immune system to the disease's presence; (ii) drugs that make tumour cells more susceptible to being killed by radiation therapy in a way that also makes them more visible to the immune system; (iii) selective delivery of high doses of potentially toxic therapies to a limb (usually the leg) as a way of maximising the ability to kill cancer cells, but also to trigger anti-cancer immune effects throughout the body; and (iv) gene therapy and drug treatments to reduce the risk of damage to normal tissues after radiotherapy.
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)?	We already have a very strong track record of taking new therapeutic approaches from laboratory studies in to clinical trials. For all of the approaches detailed in this application, we will use the data generated to inform the design and conduct of translational clinical trials that will be performed in the UK and internationally. The goal of such studies is to improve outcomes for patients with cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 15500 mice and 3000 rats will be used over a 5-year time period. The majority of the animals to be used are wild-type mice and rats, but we will also use genetically altered animals.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All animals will be looked after by trained, experienced personnel and scientists. Animals will be housed and experiments will be conducted in a designated research facility. Mice will be housed in cages with sterile bedding, food and water. Animals will develop tumours and will be treated with a variety of standard-of-care and experimental anti-cancer agents. Maximum allowed tumour sizes will be strictly regulated by set guidelines to ensure that animals experience minimal discomfort and distress. Tumour-bearing animals will be closely monitored for signs of pain and distress (abnormal behaviour, loss of appetite) caused by tumour growth or as adverse effects of the therapies being tested. However, the majority of anti-cancer agents used are not expected to result in adverse effects that impact on the general well-being of the animal. For

	example, virus treatments result in mild flu-like symptoms. Animals might lose weight but they will be monitored for weight loss at least twice weekly and they will be sacrificed if weight loss exceeds 20%. Some animals will undergo multiple treatments on the same day but care will be taken to design the experiments to use the minimum number of injections possible per day without compromising the results of the study. Some animals will undergo radiation treatments which might result in skin inflammation. Special skin toxicity regulations are in place to make sure no animals experience suffering. Some animals will undergo surgery but anaesthesia and pre- and post-operative analgesia will be used to minimise suffering and distress. All procedures are characterised as mild or moderate by the Home Office. At the end of an experiment, animals will be humanely killed and samples, such as tumour, lung, spleen and blood, will be taken for processing and detailed analysis to help improve future treatments for cancer patients.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible, detailed laboratory studies will be carried out on cancer cells growing in plastic dishes/flasks or as clusters of cells called 3D tumour spheroids. Such *in vitro* studies will enable us to replace many experiments that would otherwise require animal testing. However, no *in vitro* system is able to recapitulate all of the effects of an intact (or partially defective) immune system. Therefore, it is unavoidable that some experiments will need to be performed in tumour-bearing animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will reduce the number of animals required for experiments by optimising experimental protocols through detailed preliminary *in vitro* studies. In addition, we will ensure excellence in the conduct of animal procedures by adherence to standard protocols and by the involvement of experienced animal technicians/consultant surgeons, to reduce the need for unnecessary repetition of studies. We have optimised group sizes to ensure that study objectives are met, without including unnecessarily large numbers of
	animals.

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 smallest animal species (mice and rats) possible for all studies. Rat experiments will be restricted exclusively to those in which the vascular anatomy precludes the use of mice. Animal welfare will be ensured by selecting non-toxic combination therapies and by monitoring closely for the occurrence of toxicity. For surgical procedures we will adhere to policies on the use of anaesthesia and peri-operative pain relief. We will minimise the number of injections an animal receives every day to limit the suffering caused. We perform pilot experiments to determine humane endpoints and thereby reduce the adverse effects that an animal may suffer. This information is useful not only for current studies but potentially also future and external studies.

Project	**65. Control of arousal and autonomic output from the brain**	
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)	In 2010, 26% of adults and 16% of children in the UK were classed as obese. A further 42% of men and 32% of women were overweight. Co-morbidities related to obesity, such as diabetes, high blood pressure and kidney disorders, create massive personal and public health problems, and are projected to cost the NHS £9.7 billion per annum by 2050. In order to find new ways to treat metabolic diseases we need to understand the balance between energy intake (the food that we eat) and the energy that we expend (for example, through exercise and adaptive thermogenesis). We aim to understand how dieting, exercise, sleep,	

	blood glucose and blood pressure link to obesity, and how they might be manipulated separately and safely.
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)?	New approaches or interventions developed as a result of our studies, could potentially benefit those suffering with metabolic diseases such as obesity, diabetes and high blood pressure.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use around 6000 experimental mice over a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Often, we need to do surgery on our mice in order to manipulate how the brain responds to different signals. Sometimes, mice will be given an injection (either under the skin, into a vein or directly into the brain) with minimal disturbance. We can carry out a range of physiological tests on the mice. Thus, we might put them in a scanner to see how much fat they have, measure their blood pressure, or measure their metabolic rate. Occasionally, we even train our mice to poke their noses into holes to break an infrared beam or to press a little lever, which provides them with a sugar reward. This can tell us about their motivation to eat. Invariably, the parameters we measure are very simple: for example, how much food do they eat or how much sugar is circulating in their bloodstream. For the latter, we shall take pin-prick samples of blood from their tail and measure these in a sugar monitor, rather similar to how a diabetic patient would. These procedures will cause some minor discomfort. In this case, we carry out the surgery with the mice under general anaesthetic, plus we give the mice pain killers and sometimes local anaesthetics, to make sure that they do not feel any pain during recovery. The mice recover very rapidly, so they can be returned to their home cages to carry on living as usual. At the end of the study, all the mice are killed humanely.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is difficult to study appetite and body weight in anything other than a normally-behaving mouse. However, we can still find out a lot about brain cells by studying them isolated from the rest of the body, in brain slices in a dish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For individual experiments in this project, data provided from similar studies in the past or from pilot studies, allows us to make precise calculations of the minimum number of animals we will need to provide robust results. When a strain is not being used regularly, we reduce the colony to a minimum or we end their breeding, having first cryopreserved sperm or eggs for future regeneration.
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 minimise any adverse effects, such as stress, we like to handle our mice (often daily) in order to get them used to being picked up. We also do a range of physiological tests on the mice, sometimes in their home cages, but often after acclimatising them to other cages. We carry out surgery with the mice under general anaesthetic, and give the mice pain killers and sometimes local anaesthetics, to make sure they do not feel any pain during recovery. The mice recover very rapidly, so they can be returned to their home cages to carry on living as usual. We use remote radiotelemetry, which is where, during surgery, we implant a small radiotransmitter under the skin or in the abdomen of the mouse. Later, these devices allow us to monitor things like body temperature, blood pressure and brain activity without having to disturb the mice. We now use transgenic mice to identify, control or record the activity of individual cell types in the brain. This allows us to determine how different cells respond to stimuli and how they communicate with each other without using the very invasive old techniques. Since we can manipulate the mice while they are still in their home cage, we can record their normal behaviour, whether they are secreting

	hormones, or if their metabolism is changed, with minimal disturbance. To do this, we breed mice that have so-called “designer receptors” expressed in just a single cell type. The designer receptors lay dormant and the mice behave as usual. But, by then giving the mice a “designer drug” or by shining a light through an optic fibre, we can activate or inhibit selective brain cells, while studying changes in behaviour or physiology. It is now even possible to see and record the activity of specific brain cells in freely moving mice, using tiny camera lenses attached to the mouse’s head.

Project	**66. Control of breast tissue morphogenesis and function**	
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
What's the aim of this project?	The overall objective of this proposal is to determine mechanisms by which adhesion to the extracellular matrix (ECM) regulates tissue organisation and cell behaviour in the mammary gland.	
Why is it important to undertake this work?	Understanding mammary developmental mechanisms is essential to explain the nature of the defects that occur in breast cancer, both at its earliest stages, for example in ductal carcinoma in situ, and at later stages of the disease, including the formation of invasive breast cancer. Our work will provide new avenues for developing novel biomarkers of cancer progression, as well as novel targets that can eventually be exploited in the clinical care and treatment of breast cancer patients. Our work also has important implications for stem and	
	regenerative medicine.	

What outputs do you think you will see at the end of this project?	Our findings will be published in peer-reviewed journals and we will present our work to the scientific community at workshops and conferences. Tools and knowledge will be shared with colleagues and sperm and/or embryos from novel genetically altered mouse models will be made available to those that request them. These may be stored frozen in national sperm and or embryo banks.
Who or what will benefit from these outputs, and how?	Breast cancer is the most common type of cancer within the western hemisphere, affecting approximately 48,000 women in the UK alone and causing significant suffering to individuals and their families. Our work aims to identify how the mammary gland functions during all stages of life, puberty, pregnancy, lactation and reorganisation after milk production has finished. In our earlier work we have shown that genes involved in this process of mammary gland function when disrupted show a similar organisation of tissue that has been found in certain types of breast cancer. The work here will expose potential biomarkers that may be altered within precancerous tissue and micropapilliary cancers. This information is necessary for future investigations that confirm and translate our findings in disease models and patient samples. Being able to predict the outcome of an irregular change in the breast will have significant cost and health benefits within the UK because it may avoid unnecessary surgery and emotional trauma in women who are not at risk. By helping to understand the normal functioning of breast cells, the research will provide insights that will help to advance science towards better, more efficient and cost effective diagnosis and treatment of breast cancer.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	The primary route for communication and dissemination of our data with the research community will be through publication of our findings in peer-reviewed scientific journals and presentation of our findings at invited seminars and at international conferences as posters and oral presentations. In compliance with the 2012 report from the Finch Group, and the Research councils preference to open access publication, we will aim to

	publish our work in journals with open access options or publication policies to ensure accessibility to all. Where appropriate we will also relay our findings to patient groups through public and patient involvement days. We will also bring our findings to the attention of the major cancer charities in the UK and internationally such as CRUK, BCC, AACR, AICR so that they can communicate our findings to their members. For work of particular importance that we deem to be of potential interest to the general public we will liaise with the University media offices to coordinate press releases. Collaborative interactions with colleagues who have expertise in mammary gland biology, and adhesion will forge new opportunities for cross-disciplinary working, and the sharing of skills, expertise, tools and reagents. These interactions will achieve more than their primary scientific objectives by i) bringing the early career researchers into direct contact with other laboratories and interests, ii) providing those researchers with a set of highly sought-after laboratory skills that will be useful in future career development and iii), stimulating the transfer of knowledge within the University. As is customary in such collaborations, the resulting work will lead to co-authorship on papers.
Explain why you are using these types of animals and your choice of life stages.	The study of mammary gland function requires the use of mammals. In all protocols, mice are the most appropriate species due to their ability to have their genes altered to study their function. We have developed this model system for over 10 years and therefore to continue adding to our knowledge we need to use mouse for our future studies. To understand the mammary gland and its function we need to study all ages of animals to capture the different stages of development of this gland during puberty, pregnancy, lactation and reorganisation after lactation.
Typically, what will be done to an animal used in your project?	Typically animals will be mated that carry different combinations of altered genes to study how these interact in mammary gland function. We may use dyes, or compounds that will be incorporated into tissues, cells and/or DNA to help us understand how the mammary

	gland develops and functions in the various stages of life.
What are the expected impacts and/or adverse effects for the animals during your project?	Most mice (>90%) maintained under this project are not expected to exhibit any harmful phenotypes. Protocol 1 : Mice expressing reporter gene constructs used are unlikely to show adverse side effects, however pups and adults will be monitored frequently to ensure that appearance (skin/coat colour, body weight, etc.) and behaviour (grooming, movement, feeding, mating, etc.) are normal. Any animal showing signs of pain or distress will be euthanised. Potentially harmful defects may occur in the mammary gland. Any defects arising may cause mild inflammation or prevent the animal from making milk but are unlikely to cause disease. Mothers will be monitored particularly closely in pregnancy, lactation, and after weaning. Pups will be monitored for signs of stress or malnutrition, and those showing signs of distress will be euthanised. Mice may exhibit temporary side effects, for e.g. small amount of weight loss following administration of inducing, deleting and labelling reagents. Protocol 2: Mice maintained under this protocol might exhibit harmful phenotypes. Phenotypes or adverse reactions might occur in the following tissues; mammary gland, salivary gland, upper oesophagus and skin. They will be inspected carefully and frequently (at least once a day) to ensure that appearance (skin/coat colour, body weight, etc.) and behaviour (grooming, movement, feeding, mating, etc.) are normal. Mice with unexpected changes in appearance and/or behaviour will be humanely killed. Protocol 3: The surgical procedure used as part of this project is straightforward, involving a small incision into the skin (but not the body wall muscles), removal of a small portion of fat and injection of cells into the mouse mammary gland. This small skin incision will minimize suffering and accelerate wound healing. As a result, mice are expected to make a rapid and uneventful recovery from the implantation procedure. Pain relief will be administered as required. Mice failing to recover quickly from surgery will be euthanised.

What are the expected severities and the proportion of animals in each category (per animal type)?	Protocol 1: Mild. Maintenance of conditional and inducible lines will exhibit sub-threshold severity. For in vivo studies (1000 mice) ~50% of mice (females with Cre-mediated gene deletions) may show a mild phenotype. This is less than 10% of the total mice used in this project. Protocol 2: Moderate. We aim to experiment on approximately 100 mice each year, but 50% of these will be of a Cre negative genotype and we do not expect any harmful phenotypes to manifest in this group. If we predict that every single floxed allele causes adverse effects then ~50% of mice (females with Cre-mediated gene deletions) may show a moderate phenotype. This is less than 5% of the total mice used in this project. Protocol 3: Moderate. We cannot accurately predict numbers but ~50% of mice may suffer a moderate phenotype as a result of cumulative steps or possible infection. This is less than 5% of the total mice used in this project.
What will happen to animals at the end of this project?	kept-alive, used-in-other-projects
Why do you need to use animals to achieve the aim of your project?	Mammary gland function can only be studied with the use of mammals. Mice are the most appropriate species as tools are available to alter gene expression. The complexity of how the mammary gland develops cannot be studied in isolation, however where applicable we do use tissue culture to understand gene function in greater detail.
Which non-animal alternatives did you consider for use in this project?	We will use immortalized cell lines where possible but these have their limits. Mathematical simulations will also be used to predict the outcome of some experiments.
Why were they not suitable?	Currently there are no alternative immortalized mammary cell lines that can fully re-capitulate mammary tissue

	growth and differentiation. Since our work is focussed on addressing how both mammary cell compartments are organised within tissues, ex-vivo mammary cultures are the only source. Cells for tissue culture will be harvested form mice harbouring conditional-null alleles. These alleles will be deleted by subsequent Cre-mediated recombination.
Enter the estimated number of animals of each type used in this project.	mice: 6360
How have you estimated the numbers of animals you will use?	We have carefully considered all the aspects of mouse colony management and optimized experimental design to minimize the number of mice to use in order for us to: maintain stocks of various mouse strains and provide female mice whose mammary glands will be used for experimental purposes. The estimated number of mice is based on our previous published studies and using statistical power analysis. Calculations are based on the assumption that each breeding pair produces one litter of 6-8 pups per month. i) Mouse stocks including conditional null-alleles for ex vivo analysis ii) Mice for genetic analysis of mammary gland iii) Mice for in vivo implantation into mammary gland
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Our predicted usage of mice is based on our previous published studies and 2-way ANOVA power analysis. We have taken advice from local statisticians to calculate power analysis.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	We have designed a very efficient regime to maximise the number of experiments we extract from every single mouse in both in vivo and ex-vivo studies. For e.g. In vivo: As mice have 5 pairs of mammary glands, we use all 5 pairs to collect tissues for protein, RNA, DNA, wax embedding, OCT cryo-blocks, carmine wholemounts and fluorescent wholemounts. By doing this we significantly reduce the number of animals undergoing experimentation and nothing is wasted. For the ex-vivo

	studies cohorts of at least 5 mice are needed per prep to make good primary cultures. The work is organised such that each prep is shared by several lab members to maximise use of all cells per prep and therefore reduce the number of preps/mice. We will also aim to collaborate with other scientists that may need to use other body parts from animals that we have removed mammary glands from. We are currently collaborating with a mathematician to predict outcome of studies so that we streamline experiments in mice.
Which animal models and methods will you use during this project?	Taking advantage of the inducible CreER system greatly reduces harmful phenotypes as we are able to switch genes off in a specific tissue type, or cell within the body (mammary gland) and at a time of our choosing. Mammary fat pad transplantations confine transgene perturbations to the mammary gland, negating harmful systemic effects. In cases where floxed alleles targeted to basal cells produce harmful systemic effects, we will use this refined technology to reduce severity levels and reach time-frames and endpoint of experiments which will not be permitted otherwise. Use of young mice ~ 3-4 weeks old to clear fat pads containing indigenous mammary tissue permits a more refined surgical procedure involving a very small incision around the nipple and therefore reduced anaesthetic deployment and faster recovery. We do not expect that these mice will show any harmful or abnormal phenotype following implant of cells into mammary fat pad. The use of matched Cre-negative genotype littermates as recipient mice for e.g. where the donor tissue is of a mixed genetic background will reduce the chance of rejection following transplant and reduce the use of immune-compromised mice which might be susceptible to infections following surgery.
Why can't you use animals that are less sentient?	Mammary gland function can only be studied with the use of mammals. Mice are the most appropriate species that have been studied sufficiently for experimental conclusions to be valid. The gland develops in puberty thus in most cases pubertal and adult mice are needed for extraction of the tissue.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We will keep abreast of the latest 3Rs advances by attending relevant conferences including meetings organised by NC3Rs, reading published articles, newsletters from NC3Rs and the 3Rs resources library. If we come across more refined approaches that still permit experimental outcomes we will integrate these into our current methods.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	Protocol 1: We do not expect any harmful phenotypes. Protocol 2: Mice maintained under this protocol might exhibit harmful phenotypes. They will be inspected carefully and frequently (at least once a day) to ensure that appearance (skin/coat colour, body weight, etc.) and behaviour (grooming, movement, feeding, mating, etc.) are normal. Protocol 3: Use of juvenile mice ~ 3-4 weeks old to clear fat pads containing indigenous mammary tissue permits a more refined surgical procedure involving a very small incision around the nipple and therefore reduced anaesthetic deployment and faster recovery. Sterile surgical techniques and good practice will be employed. To limit adverse effects of anaesthetics, mice will undergo pre-op checks before procedure. Only mice with good health and those that are acclimatised with healthy eating habits will be used. As the surgical procedure is quick, ~5 mins, only the shortest duration of anaesthetic will be applied to limit adverse effects and ensure a faster recovery. We will minimise fur clipping and preparation of the skin, the procedure will be conducted in a warm room and heated pads inserted underneath the animal if necessary. Recovering mice will be supplied with wet food and hydrated gel pads if necessary. Mice will be closely monitored over the first 48h following surgery. Post-operative analgesics will be supplied if necessary.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We will use: 1) the 3Rs resources library for information. 2) The home office best practice standard breeding protocols. 3) The LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017).

Project	**67. Creation, Breeding and maintenance of genetically altered mice**	
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 purpose of this licence is to breed rodents with genetic alterations and supply them for work that supports research into understanding and treatments of diseases. Until 1980 mutant models of mice and rats arose mainly from the spontaneous discovery of new variants but with the advent of genetic engineering in the 1980's this allowed the artifical manipulation of genes to create new carefully designed rodent models of a particular disease. This technology has made an enormous contribution to the study of disease and understanding of the function of different genes. Research models are becoming more sophisticated and in the future will be engineered to provide researchers with an even better animal model precisely designed	

	to answer questions that will advance the discovery of medicines and provide cures for serious diseases. Although it is possible to identify genes and targets that may cause susceptibility to a certain disease this can only be investigated in an animal model that has been manipulated to study the gene effect. Without animal models it is impossible to determine what effect these changes have on a whole living organism. Genetically altered rodents rarely look any different from normal animals and the change to their genetics has no effect on their health and welfare.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are many research groups within the establishment that use genetically altered mice as a fundamental part of their scientific studies. Studying in particular areas such as cancer, the immune system and infectious diseases aging and metabolism. These genetically altered mice are esssential top allow advancement f these research projects.,
What species and approximate numbers of animals do you expect to use over what period of time?	155,200 mice will be used over the 5 years of this licence supporting around 350 researchers.
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 will not experience and changes from a normal mouse as a result of the changes to the genes. A small number may develop clinical signs of disease such as the development of tumours. These animals will be carefully monitored and humanely killed before the development of significant disease. Some of the procedures that are used to create new genetic changes will involve surgical methods for example placing embryos into the uterus of foster mothers. Any surgery is undertaken under general anaesthesia and pain relief is provided. Where possible non surgical methods may be used to transfer the embryos into the foster mother.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The continuing development of GAA techniques such as conditional knockouts and inducible gene switch systems will allow the accurate application of animal models to particular research needs. Although it is possible to identify genes and targets that may cause susceptibility to a certain disease this can only be investigated in an animal model that has been manipulated to study the gene effect. Without animal models it is impossible to determine what effect these changes will have on a whole living system. We will continue to review the scientific literature to look for non animal alternatives that may become available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Expertise in breeding programmes is available at this institute and this will be used to ensure that there is no overbreeding of animals. We have an in-house cryopreservation facility to ensure that strains are held as frozen embryos or sperm reducing the need to keep live colonies if there is no scientific requirement. We will ensure that the breeding matches the scientific demand so that there is no wastage of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are a well recognised species for work involving genetic alterations and there are standard protocols, methods and reagents used that have been optomised for this species and there acknowledged benefits for use. Husbandry procedures ensure that all animals are provided with free access to food and water, a mouse house and nesting material to allow as much expression of natural behaviour as possible.

Project	**68. Creation, production, maintenance and supply of genetically altered mice**	
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 main aim of this licence is to provide a service for the creation, production, maintenance and supply genetically altered mice. Genetically altered mice provide complex systems, essential for the study of biological processes. The procedures and protocols within this licence will result in genetically altered mice being made available for use in a range of other approved project licences, to understand fundamental molecular and cellular functions and disease processes in the fields of biological, medical and veterinary science.	

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)?	GA mice have made significant contributions to biomedical research. However the function of many genes is still not known or is not fully understood, either individually or in the ways they interact to produce their intended effects, or how they go wrong in disease processes. The use of animal models is necessary to determine these processes and to find new treatments for human diseases which are too complex to be modelled in lab or computer based systems. In addition, having a centralised license to perform the protocols described, ensures the use of skilled, highly trained staff and facilities for the local dedicated and standardised breeding and supply of animals that are healthy. This reduces the number of animals used and reduces the duplication of lines.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, around 50,000 over the 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 with altered genes will be used as egg donors to generate healthy genetically altered mice that are free from most disease causing organisms. The eggs collected will be transplanted into normal female mice where they develop during gestation. The recipient female will give birth and care for her litter normally. Male mice may undergo a surgical procedure to render them sterile. This is necessary in order to generate pseudo pregnant recipient female mice that can be used as the recipients for the donated eggs. Mice with altered genes bred under this license will be retained on this license to maintain the breeding colony or transferred to specific experimental licenses for further scientific study. Mice bred under this license are not anticipated to have any overt or obvious negative behavioral or other adverse symptoms and are classed as Mild. When and if a mouse is observed with any symptoms greater than mild e.g., unusual behavioral characteristics which might impinge on its ability to feed or groom, it will be closely monitored and reclassified as Moderate severity if appropriate.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This service PPL by its nature requires the use of animals, and will result in GA animals being made available for use in most of the PPLs used across the university, for which the benefits are clearly described within each PPL and will be published via the scientific groups holding these PPLs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The generation, breeding and supply of mice is unquestionably appropriate. Animals will only be bred once a user requirement has been established and agreed. The breeding programme will be subject to regular review to optimally meet anticipated demand. Numbers will be kept to a minimum by training staff to high standards, quality control and database tools to allow the tracking of animals and any adverse effects accurately. Through centralising the supply of animals, over production and duplication can be reduced. The employment of specialist staff skilled in the breeding and maintenance of GA animals and transgenic technologies, yields a high rate of management success.. Cryopreservation is actively encouraged as a method to reduce the number of live animals maintained on PPLs.
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 frequently used laboratory animals and services operate to best practice using only approved tried and tested techniques. We make every effort to refine our procedures wherever possible, and there is a high level of culture of care that is self-evident within the service. Modification of surgical techniques is constantly reviewed and evolves in line with new developments and best practice to improve success rates and welfare. We keep up to date regarding new genetic tools which reduce the severity of phenotypes in animals. Breeding will be kept to within the prescribed severity classification. No procedures or phenotypes on this licence are categorised as severe. Wherever possible and appropriate, tissue removed during ear notching for animal identification purposes will be harvested for
	genotyping. Data base management of the colonies enables accurate and rapid colony trends to be detected and resolved.

Project	**69. CRISPR/Cas9 library screening of gene mutation contributions to lung cancer development**	
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 is to measure and understand the relative cancer-causing properties of different members of a family of genes frequently mutated in cancer and panels of mutations identified in cancer patients. We are initially focusing on Ras genes that are mutated in ~20% of cancer patients before extending the analysis to other genes that are components of the Ras communication system often found to be rewired in cancer cells. Data from patients, from animal models and	

	from cell-based studies have all shown that Ras family members are not equivalent. There is also an increasing appreciation that different mutations of Ras family members might specify different outcomes for patients. However, most data from animal models and patient studies are not comprehensive and it is difficult to compare between model systems. Importantly these studies also generally lacked any insight into the mechanisms underpinning any differences. We will employ novel methodology allowing many different mutations of interest to be simultaneously compared in an individual mouse. The method uses gene editing to replace the normal copy of the gene with a mutant version. We will perform gene editing with a library of mutations so that we create populations of cells in the mouse lung that each have a different mutation. We will then compare their relative tumour promoting properties and characterize the underlying mechanisms that cause the differences in tumour biology that we observe.
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)?	Nearly every major cancer-causing gene is present in the human genome in the form of several closely related family members with poorly understood overlapping functions. Understanding the relative contributions to cancer development of different gene family members or mutation variants is essential for developing appropriate personalized medicine approaches. The specific genes that we are studying are frequently mutated in human cancers and have proved difficult to directly target with therapies. Our data will indicate how the rewiring of their communication systems contributes to their cancer-causing properties and will potentially identify new therapeutic vulnerabilities of our genes of interest. Furthermore, the new approach that we are employing that allows parallel comparison of many mutations in a single individual is easily translatable to any gene of interest - with significant benefits for reducing and refining animal use.
What species and approximate numbers of animals do you expect	Species: mouse Numbers: 375 Time: 5 years
to use over what period of time?	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Severity category: moderate Adverse effects: Gene edited mice will develop lung tumours; however, experiments will be ended before they become life limiting. All mice used in experiments will be killed to enable analysis of mutation frequencies and the different mechanisms leading to cancer development.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying whether panels of mutations are equally effective at promoting cancer. We have extensively researched these cancer-causing genes using cell-based models and shown that different mutations generate different consequences relevant to cancer. However, we also know that cancer cells in a petri dish do not replicate everything seen in a tumour where mixtures of cancer cells and non-cancer cells work together to create the full range of tumour behaviour. To definitively test the relative contributions of the mutations to cancer development we need to see if and how they generate tumours. There are no non-protected animal models that allow us to ask these questions in a lung cancer context.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The new approach that we are using simultaneously compares the potential disease-causing actions of multiple different mutations in the same individual. This is in contrast to traditional methods that require the generation of different mouse strains that each harbour a different mutation. As a result we are getting much more information per mouse and the comparisons are more reliable because all of the mutations are being compared under exactly the same conditions. We have engaged with expert statisticians to design the experiments to use the minimum number of mice necessary to address our scientific questions.
3. Refinement Explain the choice of species and why the animal model(s) you will	The ability to compare multiple mutations in a single individual relies on the mice harbouring a genetic modification that allows CRISPR gene editing to occur in target cells. The mouse model

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.	can be used to study any gene of interest with this approach and therefore represents a refinement on current methods that rely on the generation of different strains of mice to study the biology of different genes. The latest gene sequencing and imaging approaches that we will employ mean that we can use smaller tumours without impacting on the ability to address our scientific questions. Pilot experiments will determine the earliest endpoints that provide sufficient relevant material so that disease burden and suffering are minimized.

Project	**70. Cytoplasmic regulation of mRNAs**	
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)	Genes encode the genetic blueprint of our bodies and must be tightly regulated to ensure that they make the proteins needed by our cells in the right time and place and in the correct amounts. The multi-step process by which genes are decoded into proteins is known as “gene expression. Failure to tightly regulate gene expression leads to numerous diseases including reproductive, neurological and metabolic disorders. Some genes encode proteins that act to control the expression of other genes – these can be considered “master regulators” of gene expression. Our work examines a large class (over 1,500 examples in humans) of such gene	

	regulatory proteins, which likely to impact on many of the different biological processes required to develop and maintain a healthy body. Supporting this idea, human genetic studies which examine families where diseases are passed down the generations, suggest that they are important for many different aspects of human health. However, despite this there is a relative lack of information on their function. This project aims to fill this gap in our knowledge by focusing on specific examples of these types of regulatory proteins. In particular we aim to establish their specific roles in and mechanisms by which they regulate gene expression, and the developmental and/or life-long health consequences of their function being disrupted
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 provide insight into the fundamental mechanisms by which gene expression is regulated and the consequences of this being disrupted. For example, we study how this contributes to a number of important clinical conditions including 1: Infertility: which affects 1 in 6 couples worldwide; 2: Intrauterine growth restriction which is a risk factor for increased perinatal death, childhood neurodevelopmental conditions and metabolic (e.g. diabetes and cardiovascular) disorders in adulthood, 3: Stillbirth which affects 1/200 births in the UK; 4: Diet-induced obesity: Obesity is a growing epidemic affects over 600 million people worldwide and is associated with major health issues including type 2 diabetes, heart disease and stroke. This epidemic is driven in large part by sedentary lifestyles and diets that are high in calories. Thus, information gained from the different strands of our research can be exploited in the longer term to provide specific diagnoses, predict long term health, and/or to open avenues for the development of tools that will allow the identification of those at risk or novel therapies/treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Xenopus laevis (African claw-toed frog) 850 Mouse 24, 600
In the context of what you propose	Frogs are used for the collection of immature

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?	eggs after culling or to lay eggs. These eggs will be manipulated and in some cases fertilised to test the importance of our regulatory proteins in development and future reproduction. The vast majority of mice on this licence will be used for breeding. Breeding both creates animals for experimental use, but also because we are studying reproductive function, allows us to study their reproduction capacity. Some mice born may be used for harvest tissues/cells/bloods for further study after schedule 1 culling, but not subject to any procedures, the data obtained are nonetheless very valuable. In a minority of cases, tissue/bloods collection will involve non-schedule 1 killing where procedures such as cardiac puncture (to obtain large quantities of blood) occur under terminal anaesthesia. These animals will not be allowed to regain consciousness and are killed as part of this procedure. Thus the average lifetime experience of mice on this licence is being born and either being bred and/or culled for tissues/bloods at the appropriate age (circa 90%, including those in which materials are collected during terminal anaesthesia, but undergo no procedure other than breeding). However, some mice (circa 10-15%) will be used in experiments. In most cases (10-12.5%), discomfort associated with these procedures should be transient and long-term health should not be adversely affected. For instance, they may be injected with compounds (e.g. hormones to modulate ovulation), have blood taken, their diet altered (e.g. high fat or protein content), tissues imaged (e.g. ultrasound, MRI, CT), or their behaviour studied (e.g. learning and memory tests). To link different aspects of our data together greatly increasing the level of knowledge that we can obtain, mice may be subject to repeated measures and/or more than one type of procedure as part of the same experiment (e.g. diet may be altered and the consequences may be measured by taking bloods (e.g. to test for signs of diabetes), doing non-invasive spectroscopy to measure fat content and measuring their metabolic rates, food intake and activity etc by housing in special cages). In some cases, such as imaging mice may be anaesthetised, this reduces stress and also allows for static images greatly improving

	resolution. These types of experiments will inform us (over and above what we can learn by observing breeding, natural behaviour and materials collected post-mortem) about the importance of our gene regulatory proteins in different aspects of mammalian biology (e.g. development, reproductive function, and metabolism). However, a small number of mice (circa 2.5-5%) will be subject to procedures that can adversely affect health. For instance, surgical procedures, as in humans can cause pain and there is a risk of infection. However, such procedures are performed in a sterile surgical environment with full anaesthesia and pain will be controlled by analgesics. However, any animal found to be suffering will be culled. Animals that undergo procedures in this category as part of the experiments in which they are involved, are considered to be most adversely impacted. In some cases the surgical procedures here are carried out under terminal anaesthesia where the animal is not allowed to recover consciousness after the procedure will be killed. This work is necessary for particular insights into why reproduction/metabolism is altered, that cannot be achieved in any other way. At the end of the experiments in which they are involved almost all animals are culled. However, due to the very mild nature of some experiments, a small minority may be kept alive for further use under this licence (e.g. frogs that have laid eggs may be kept for further rounds of egg laying). This also applies to a small number of mice where their oestrous cycle was measured by vaginal smear or they were super-ovulated (by a hormone injection) to improve their fertility. This reduces the total number of animals required (e.g. especially important in our mouse strains that have very poor reproduction). However, these animals will be culled after further use and no animals will be released into the environment.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We primarily use animals to study the roles of our regulatory proteins in complex biological processes which involve many cell types and organs and therefore cannot be recapitulated to any great extent outside of a living organism. This enables us to understand their contribution to human health and disease. However, much of our work uses non-animal based approaches e.g. biochemistry, cells in culture, cell-free systems, biophysics, molecular biology, yeast genetics, in silico approaches, structural biology and molecular modelling where applicable. These approaches help to refine the questions that need to be asked in animals, reducing numbers required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals are only used when all the other approaches we use (see above) cannot answer our scientific questions e.g. what is the effect on health when a specific gene is disrupted. Experimental questions using animals are refined based on data obtained by other approaches (e.g. in vitro) and pilot data are analysed to further guide experimental design and to ensure that statistic power is reached using the minimal number of animals. Similarly our design of mouse lines with similar genetic background limits variability meaning that that statistically meaningful data can be gained with fewer animals. Moreover, protocols are continually refined taking advantages of information and best practice from around the world meaning that the same information can be gained with fewer animals.
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.	Where applicable, Xenopus are used to explore the in vivo functions of our gene regulatory proteins. However, differences we have found between the function of some of our gene regulatory proteins in non-mammalian and mammalian species means that, currently, much of our work uses mice. This makes our results more directly relevant to human health as their genetic make-up and physiology are closer to that of man. Good experimental design and appropriate collaborations means that we can utilise the least invasive approach to answering

	our scientific questions. For instance, where new non-surgical alternatives have become available these are utilised. In the small number of cases where surgical procedures are required appropriate use of anaesthetics and post-operative analgesics limits any pain. Any genetically altered animal or animal involved in an experiments are particularly closely monitored. Monitoring regimes are clearly laid out and are tailored for specific alteration/experiment type, to make sure that the monitoring is appropriate for what may happen. Using these regimes, animals are observed for signs of pain, distress or ill health and strict criteria are applied to ensure that animals are culled, when appropriate, and therefore do not undergo unnecessary suffering. Experienced and dedicated staff and continual training and development further minimises the risk of welfare issues.

Project	**71. Defining cellular and molecular mechanisms which control anti-tumour immune-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
		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)	There are over 360,000 new cases of cancer per year in the UK and 165,000 associated deaths. More than 1 in 3 people in the UK will develop some form of cancer during their lifetime. The risk of developing cancer up to the age of 50 years is 1 in 35 for men and 1 in 20 for women, and breast, lung, bowel and prostate cancers together account for over half of all new cancers each year. Although, cancer can develop at any age, it is most common in older people. Greater than 60% of all cancers are diagnosed in people 65 years of age and over, whereas approximately	

	one per cent of cancers occur in children, teenagers and young adults up to the age of 24. Most first line cancer treatments in the UK currently involve surgical removal of tumour tissue; chemotherapy, which targets cancer cell division; and radiotherapy, which aims to kill cancer cells using ionising agents. These treatments are at best only partly successful and incur serious side effects for the patients. Consequently, there is an urgent need to find more effective and less damaging ways of treating cancers. In recent years, the immune system has been shown to have enormous potential to eradicate cancers. This type of approach is referred to as anti-tumour immunotherapy. Many strategies have been tried in an attempt to enhance the immune system's ability to combat tumour growth. However, the majority of these have failed, due to the highly immune suppressive micro-environment that exists within tumours mass. The aim of this project is to find ways to prevent or reverse tumour-mediated immune suppression within the tumour mass to enable the immune system to effectively attack the cancer cells.
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 primary benefit of the studies outlined below will be to advance understanding of the cellular and molecular pathways involved in disrupting the normal function of tumour-specific cytotoxic lymphocytes (CTL) within the microenvironment of a tumour. Using this knowledge, the study will also evaluate interventions that could be used to redirect CTL to kill the cancerous cells. In the longer term the findings of these studies are expected to contribute to the development of more effective treatments for cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	During the 5-years of this project we estimate using 1500 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?	The vast majority of animals used in these studies are not expected to experience more than mild very brief pain associated with the administration of substances via injection, and are expected continue to live normal lives. All animals will be carefully monitored and in the event, that signs of suffering do occur the animal will be promptly

	killed. At the end of the study all animals will be killed to enable tissue to be harvested for scientific analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to replace the use of animals in our studies we have developed a 3 dimensional cell culture tumour model that replicate many elements of tumours in life. This model will be used as the basis for the bulk of the studies undertaken to address the stated aims. Furthermore, through the wide dissemination of our finding we will encourage other groups to adapt this *in* vitro model approach for studying immune-oncology and thereby contribute to the replacement of animals within the wider field of cancer research. Nevertheless, the complexity of both the host immune system and the influence it has upon tumour growth cannot as yet be fully replicated in a cell culture system. Consequently, conclusions drawn using the culture systems will ultimately have to be confirmed in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimize animal usage, as far as possible, we have developed a 3-dimensional cell culture tumour model that replicate many elements of tumour in life. This model will be used as the basis for the bulk of the studies undertaken to address the stated aims. Nevertheless, the complexity of both the host immune system and the influence it has upon tumour growth cannot as yet be fully replicated in cell culture. Where animals have to be used every effort will be taken to ensure that only the minimum number are used. This will be achieved by careful experiment design and the use of power calculations to determine the minimum group size needed to obtain statistically valid data.
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	These studies will be conducted using mice. Mice are the species with the lowest neurophysiological sensitivity suitable for the studies. In addition: i) mice provide an ideal species for studying immune responses relevant to humans as their immune system has been widely studied and shown correlate well with that of humans, ii) the necessary reagents to undertake the study are

(harms) to the animals.	readily available for mice iii) the availability of numerous conventional inbred and genetically altered strains of mice provides a repertoire of relevant immunological research tools that is not available in other species. The procedures used in our studies have been refined over many years to cause the least level of suffering. The injection techniques used have been selected to minimise any suffering (e.g. we will no longer carry out more than one subcutaneous inject of immune-modulatory material in Freund's complete adjuvant per mouse). All tumour growth experiments we will adhere to the guidelines on the use if animals; as set out by the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), [http://www.nc3rs.org.uk], and the guidelines for the welfare and use of animals in cancer research (British Journal of Cancer. 2010 May 25; 102(11) 1555). In our experience, the response of the animals to tumour development varies widely with different strains of mice and with different tumour cell lines. Whenever overt signs of adverse effects occur additional monitored will undertaken and supportive measures taken, including the provision of moistened feed and additional nesting material. The weight of these animals will also be recorded not less than twice weekly. Any animal with weight loss >20% will be killed. Whenever experiments are performed which involve any degree of distress or potential pain, we routinely check the procedures in order to 'Refine' them further for future studies so that these effects may be minimised. This will continue to be our policy in the future.

Project	**72. Defining dermal heterogeneity and interaction in skin development, regeneration 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 skin is our largest organ and most important protective barrier. It is constantly exposed to damage caused by injuries or environmental stress such as UV light induced sunburns. Skin repair requires coordinated function of two tissue layers, the outer epidermis, and the underlying dermis. The objective of this project is to study a special type of dermal cells called fibroblasts, which produce proteins such as collagen that ensure the structural integrity of the skin. Aberrant fibroblast function and behaviour are involved in many skin diseases including	

	abnormal scar formation, fibrosis and cancer. The aim is to investigate how different kinds of fibroblasts contribute to normal skin function and repair of tissue damages induced by injury or environmental stress. We will discover how these functions are altered in disease and thus our research could lead to new ways of preventing or treating these skin conditions by targeting fibroblasts.
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)?	Fibrosis is the final, common pathological outcome of many chronic inflammatory diseases including scleroderma, rheumatoid arthritis, Crohn's disease and ulcerative colitis. While progress has been made in identifying the signalling pathways that contribute to fibrosis, we are still lacking a clear understanding of the cellular interplay and early pathogenic processes in the dermis inducing and maintaining aberrant fibroblast function. By gaining new understanding of the properties of different fibroblast populations we can potentially prevent or treat these and other diseases of the skin and other organs. In addition the development of computational tissue models that recapitulate key aspects of skin biology will help to generate more targeted hypothesis and reduce the use of animals in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 5200 mice over the time 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 are the development of skin wounds, inflammation and cancer. At various time points of our experiment a drug may need to be given to the animals which may cause brief discomfort. Some mice will have a minor surgery (less than 30 minutes) to take a skin biopsy or implant a device in the skin to transplant selected cell populations. A small skin biopsy may be taken from newborn mice to study how the skin healing ability changes with age. All animals are expected to recover quickly and will be given painkillers and post-operative care. To study the effect of UV light on skin, mice will

	be placed in a chamber of a UV light machine for short-term (less than 60 seconds) where only a small skin area is exposed to UV radiation,(similar to people in a sun tanning machine). In some cases we anticipate to inject skin with selected cells or apply a drug to investigate how different cell types communicate in the skin. The imaging of mice by MRI and other modalities may require longer anaesthesia which could lead to hypothermia and dehydration. This will be carefully monitored during the experiments. At the end of each experiment tissues will be subject to analysis after mice have been humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible we replace mice with studies of cells that are grown in culture in the laboratory and our experiments will be supported by computational tissue models. However, there are three situations in which cultured cells cannot be used: (1) when the properties of the tissue need to be studied for over 3 weeks, which is the limit for maintaining skin cells in culture; (2) when communication between multiple different cell types within the tissue, such as epidermal, nerve and blood cells, needs to be analysed; (3) when the development and progression of diseases such as cancer or fibrosis needs to be tested.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To prevent unnecessary breeding of animals for studies we keep stocks of frozen mouse sperm and embryos. So we only produce offspring animals when needed. In planning our experiments we perform statistical analysis of the minimum number of mice required to obtain a statistically meaningful result. Furthermore our experiments will inform and be supported by computational tissue modelling that recapitulates key aspects of skin biology to generate and test hypothesis. We share post-mortem samples with other research groups so that they can obtain data without having to breed their own mice.

	Whenever possible we will measure changes in animal over time using imaging rather than killing individual animals at specific time points. This will reduce the number of animals used in this 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.	Mice are the lowest form of mammal that can be used to study normal and diseased tissue and are the only mammal in which genetic modification works reliably. Genetically altered animals are essential to study individual cell populations enabling us for example to label or modify them at specific developmental or disease time points. Whenever possible, we minimise harm to mice by carrying out procedures for the shortest time periods to achieve scientific outputs. All surgeries for wound healing or cell transplantation experiments will be done in a surgical theatre under aseptical conditions. After surgery animals will be closely monitored for pain or discomfort and treated with analgesics accordingly. In case skin cells need to be treated with a drug which we have not used before, an initial experiment is carried out with a small number of mice and the conditions predicted from the literature to be most effective and minimize the risk of adverse effects.

Project	**73. Defining immunoregulation during parasitic helminth infection**	
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)	Gut dwelling parasitic worms are extraordinarily common and cause ill health in around one quarter of the world's population. The project is important for both humans and animal. This project aims to understand why some individuals are more likely to become infected with worms than others. The ultimate goal is to develop new and better ways to control these sorts of infection. By the end of five years we hope to have discovered new factors important in determining the ability of an individual to clear a worm infection; to have identified up to three new vaccine candidates; and to have trained up to 5 new PhD students.	

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 generate a deeper understanding of how the immune system works following infection by worm parasites. Relatively little is known as to the ways in which the body protects itself following this kind of infection. In the short term we will generate new knowledge which we will share with the scientific audience through conferences and publications. In the long term, understanding better how the immune system responds or does not respond to parasites will be key to the development of new ways to control infections, such as vaccines.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using approximately 30,000 mice over a five-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will be infected with gut dwelling parasitic worms. The course of infection will be monitored and in some cases mice will be treated to see if the specific treatment can alter the course of infection. Mice may also be vaccinated to see if we can protect mice from infection. The majority of treatments will result in general discomfort following infection, or temporary irritation during an injection or vaccination. In this case the animals resume normal activity almost immediately. Occasionally, animals may appear “quiet”, show reduced activity and reduced feeding for a short while (eg overnight). This is usually associated with the immune system controlling the infection and in these cases mice will be carefully observed until they start to behave normally again. All mice will be humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The body’s defence system operates as a co-ordinated response involving multiple cells and molecules at a variety of body sites. Thus, at present, culturing cells outside of the body, or computer modelling cannot accurately model the immune response. Also, no worm parasites can complete their life cycle outside of their host. In order to study the immune response to worm parasites animals are required.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use appropriate statistical expertise to ensure that we design experiments using the minimum numbers of animals required to generate meaningful results. We have received advice on the design of our experiments from experts in statistics. Thus, example data, based on numbers of worms from several different historic experiments were used to determine how many mice per group we would need to use in order to detect a real difference and not one that just occurs by chance. We maximize the information gained from each individual animal through the use of the most advanced methods enabling extensive analysis of cells and molecules. We will also develop new cell tissue culture methods to complement our animal studies wherever possible.
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 species to be studied in conjunction with parasites that naturally infect rodents in the wild. The gut dwelling parasitic whipworm, Trichuris muris in the mouse is a validated model of whipworm infection in man. Importantly the mouse species of Trichuris is remarkably similar to the human species of Trichuris and also triggers similar immune responses in its host. Thus the mouse model will enable us to develop new therapies to treat human disease. Further, in order to study the mammalian immune system we use the mouse as a model system as it is the best understood animal in terms of how the immune system works with remarkable similarity to other mammals including man. All animals are carefully monitored every day by scientific and animal care staff and we use carefully designed criteria to understand if an animal is in pain. If so, we also have clear criteria which tell us if an animal should be humanely killed. It is our aim to ensure that any pain experienced by the animals is short term and kept to a minimum through the use of careful handling and good technique.

Project	**74. Delineating the physiological consequences of G protein activation**	
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)	Opioids, like morphine, are the most commonly used drugs to treat severe pain. Unfortunately, opioids cause adverse effects such as addiction and dependence, tolerance (need of higher doses to obtain an effect), constipation and decrease in breathing rate (the cause of fatality in most overdose cases). Opioid receptors, a protein found on the surface of neurons, control the body's responses to opioids. Twenty years ago, it was suggested that opioid receptors were able to switch on two different proteins inside the neurons whereby one of these proteins (G protein) mediated pain relief (analgesia), while the other (arrestin) mediated	

	adverse effects. However, recent research disputes this finding. To develop a safer opioid drug, we need to understand how opioid receptors activate processes in neurons that cause analgesia or unwanted side-effects. Up until now, this was difficult because we lacked tools that enabled us to switch off G proteins or arrestins at a precise time and place within the brain. We have generated and validated such blockers in model cell systems. We can now prevent G protein or arrestin activation with unprecedented control. The aim of this work is to use these tools to shed light into how opioid receptors mediate the effects of opioid drugs. We will address this aim through different strategies that combine the use of these blockers with genetically altered mice to investigate the contribution of G proteins and arrestins to opioid-induced analgesia, respiratory depression, constipation and locomotion.
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)?	Pain affects 1 in 5 people worldwide. New approaches to develop effective safe analgesics are a health priority. This project will help us determine whether opioid receptors, proteins that mediate the action of these drugs, control distinct cellular signalling processes within neurons that mediate pain relief and adverse effects. This information is key for the generation of new safer pain treatments. While this project focuses on opioid receptors, the new methods we develop can be used to understand the function of other members of this receptor protein family that are targeted for the treatment of cardiovascular, neuropsychiatric and metabolic diseases. Our project, therefore, has the potential to have a large impact on the development of safer drugs that target this family of proteins.
What species and approximate numbers of animals do you expect to use over what period of time?	2,093 adult mice over 5 years; 347 adult rats 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	We aim to specifically block the proteins that are suggested to control the pain relieving or adverse effects of opioid drugs. The first strategy involves the injection of a toxin that inhibits the action of G proteins in defined parts of the brain. We have modified this toxin so that it acts to inhibit G proteins

end?	only when a second agent is administered to the mouse. Using this strategy before opioid administration will allow us to see if these proteins control the analgesic or adverse effects of opioids. To be able to attribute the actions of the toxin and the opioids to a specific brain region the injection of the toxin into the mouse brain is required. This procedure is associated with a moderate severity. The injections of the activating agent and the opioids will be mild in severity. The second strategy relies on the use of genetically altered mice. One group of mice have already been used by other researchers and are normal functioning mice. The second group of genetically altered mice have not been used previously. However, our in vitro work provides evidence that these mice should also be normal. Pilot studies will ensure that this is the case. Pain relief will be measured using a heat stimulus with time and temperature cut offs that prevent any damage to the paw or tail of the mouse. Changes in respiratory rate will be measured in a chamber where mice can move freely and constipation will be assessed by the time required to expel a glass bead inserted in the colon. All these behavioural assays are mild in severity. At the end of each experiment, or when signs of pain or distress are displayed, the animals will be humanely killed by a Schedule 1 method and their tissues harvested for further research to understand molecular changes in response to opioids.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The 3Rs will be implemented where possible to improve the scientific models used in this project and the EDA tool will be used throughout. Although *in vitro* work can replace some aspects of whole animal studies, the aim of the current project is to evaluate the complex physiological effects of opioids in a robust model of integrated, intact systems. Pathway-selective drugs targeting the opioid receptors have recently been developed using cells in culture. Unfortunately, these promising results did not translate to the whole animal, highlighting the relevance of in vivo validation of the pathway-selective hypothesis.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Our group has extensive expertise in opioid pharmacology and behavioural assays. We have collected sufficient data for power analyses that ensures that we use the most appropriate animal numbers and statistical analyses. Behavioural assays for locomotion, constipation and respiration are minimally distressing and allow measurements of multiple parameters from a single mouse. Similarly, antinociceptive experimentation uses an acute stressor with no long-term damage to tissue. The ability to use several, low stressor behavioural assays on the same mouse will significantly reduce the number of mice utilised overall, whilst also minimising any decrease in welfare experienced by an individual mouse. Finally, our rigorous data interpretation will help to inform the design of further experiments, with support of pilot studies and the EDA tool, ensuring robust experimental design and forward planning which contributes to reduction in overall animal use.
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 appropriate species due to their editable genome and reproductive cycles. They also elicit behaviours in response to opioid drugs that are analogous to man. Rats, on the other hand, are more utilised in translational behavioural research and the ability to compare behaviours in response to opioid drugs that are analogous to man in simple autonomic behaviours e.g. respiration and in more complex behavioural paradigms e.g. addiction/dependence/relapse. The behavioural assays employed are known to provide minimal unwanted stress, therefore we will not be indirectly measuring stress-dependent responses which would confound our data. We will continually review and revise our protocols as appropriate (e.g following advice from the NVS or HOI) to ensure that our techniques are the most refined.

Project	**75. Dental development, regeneration and repair**	
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 common theme of the different aspects of this project is to understand the behaviour and function of tissue-resident stem cells. Whereas a lot is known about stem cells in vitro, very little is understood about how stem cells behave in the body to repair and regenerate tissues. Orofacial tissues are subject to a range of diseases, many of which result from the expose to the external environment and dietry intake. A combination of bacteria and sugars for example are the root cause dental caries. We have already begun to develop new approaches to treat dental caries by using drugs to stimulate resident stem cells in the tooth. By studying these cells in other orofacial tissues we aim to develop biologically	

	based treatments for these diseases whereby tissues are induced to repair and regenetate themselves.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The direct aim of this project is to advance our knowledge of key processes in orofacial tissue development and disease. This will lead to new ways of disease treatment that restore lost, damaged or surgically removed tissue with new tissue that is either induced to be made in the animals or from transplanted cells. In diseases such as periodontal disease, xerostomia and dental caries for example, current treatments do not restore healthy tissue.
What species and approximate numbers of animals do you expect to use over what period of time?	Mainly mice with small numbers of rats. The total expected usage is approximately 20,000 animals 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?	All our protocols are mild/moderate. The two mild severity protocols are for breeding of animals to induce genetic recombination in specific genes to either create gene mutations or mark cells. The gene mutations are specifically induced by administration of a drug whose concentrations are controlled such that they have no adverse effects. The gene mutations are selected to result in abnormalities in tooth growth and development. Sometimes gene mutations may affect other tissues and the animals are thus carefully monitored for any adverse effects. The moderate severity protocols are carried out to (i) growth teeth and tooth tissues or (ii) to experimentally induce oral diseases. To grow teeth small clumps of cells are transplanted either into the kidney or the mouth. The kidney is used as a generic site for transplantation that requires a short, simple surgical procedure that has no adverse on the animals other than those that may be caused by the administration of anaesthetic. The teeth that grow in the kidney do not affect kidney function. Transplantation in the mouth is used to show that fully functional teeth can form. The mice are fed a soft diet while the teeth grow and eventually erupt as they would do normally during weaning. The induction of dental disease protocols either involves drilling or experimentally damaging the teeth to create

	caries (tooth acre) or treating the animals in various way to induce periodontal disease (gum disease). When the teeth are drilled they are filled and protected as would a human tooth and no adverse affects are observed other than those that may occur from the anaesthetic. The protocols used to induce periodontal disease in the mouth may also have affects on feeding and digestion and these are carefully monitored. All animals are humanely killed and tissues collected at the end of each Protocol.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use animals in order to study processes that relate to human disease. Orofacial tissues are highly complex and fundamental apsects of cell interactions in these tissues cannot be satisfactorily reproduced in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Many of the types of experiments produce qualitative data in the form of visual images of tissues and these require few replicates. For experiments where quntitative results are produced, statistical analysis is required which necessitates a minimium of 6 replicates, Where possible replicates are provided from the same animal eg. treatment one side of the mouth with the opposing side being used a as control. Where possible tissues are shared amonst different projects in the REDACTED eg. teeth, salivary glands, cranial bones may all be used from the same animal. We aim to develop in vitro systems to mimic our in vivo observations that will ultimiately reduce animal 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 research studies aspects of orofacial development and disease and aims to directly rel ate these to the equivalent human tissues and processes. The mouse is the smallest laboratory mammalian model to study human diease that also offers the advantage of genetic manipulation. For some protocols rats are used as the human model becasue of their increased size.

Project	**76. Design and function of novel antigen-specific immunotherapies**	
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 immune system has evolved to protect us against infection and cancer. However, in about 10% of the population, the immune system attacks our own tissues causing diseases such as type I diabetes, Graves' disease and multiple sclerosis. At present, therapies for these diseases are non-selective treatments that can disable the whole immune system. We have discovered a way to selectively 'switch off' only those cells causing disease. We are now using the most appropriate experimental models to understand how this works and to design new treatments for the many	
	autoimmune and allergies that affect people.	

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)?	We expect 2 major outputs from the work of this project. First, we will better understand how antigen-specific immunotherapy works. Only through understanding this will we be able to improve how it is delivered to patients. Secondly, our recent success with this approach in Graves' disease and multiple sclerosis has spurred us on to develop therapies for many other diseases for which there is a clear unmet medical need i.e. no satisfactory method of treatment
What species and approximate numbers of animals do you expect to use over what period of time?	We aim to continue this work for at least the next 5 years. Our work on the mechanism of antigen-specific immunotherapy uses both conventional inbred mice and mice transgenic for the antigen receptor on T cells. In addition, we use mice expressing human HLA class II molecules: these mice can mimic the immune response in humans. If our therapies can switch off the response in these transgenic mice then it is likely they will work in people suffering from the relevant disease. The number of animals used in the 5 year period will be up to 19,000 the majority of the mice used will be involved in breeding programmes.
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 described in this project have not induced harmful side effect in the many years that we have been applying them to support our research activity. We breed mice that can, however, develop signs of inflammation in the CNS.. For this reason, the current licence has a moderate level of severity. All of the procedures described in this licence require analysis of lymphoid tissues and blood samples in vitro in order to assess the impact of the interventions tests. For this reason, each procedure will ultimately result in mice being humanely killed
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the past five years, we have used in silico approaches for the initial definition of protein targets of interest. Furthermore, we have developed in vitro techniques allowing us to use human immune cells for the initial screening of fragments of these proteins that we can develop as

	antigen-specific immunotherapies. However, we need to use experimental mice for our work because it is not possible to determine the mechanism of antigen-specific immunotherapy in humans or by studying human immune cells in the test tube. Other alternatives are not appropriate because they do not have the same cells or immune tissues that have evolved to protect mammals. Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed transgenic mice that allow us to reduce the number of mice needed for experiments. First, these mice have infinitely more antigen-specific cells in their lymphoid tissues meaning that one mouse can provide the cells that would otherwise require 1000s of mice. Secondly, the number of HLA-DR transgenic mice required to provide statistically significant data depends on the level of expression of the DR protein. We select only the highest expressing lines of mice for breeding. Finally, the number of mice required for an experiment is determined by the level of variance between individuals within a 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 autoimmune diseases that we study are strongly linked to so called immune response genes found within the HLA-DR locus. Different diseases are linked to different HLA-DR types. Furthermore, the therapeutic peptides that we use are fragments of the proteins attacked by the immune system. The peptides bind to the HLA-DR molecules and it is therefore appropriate that we utilise HLA-DR transgenic mice to study their properties. We also use the Tg4 transgenic mouse for defining the mechanism of action of the peptides. Importantly, we can now selectively target individual genes in these mice allowing us to clearly define the role of these genes in antigen-specific immunotherapy. The therapeutic effect that we have develop involves simple injection into the skin of highly

	soluble peptides dispersed in a saline solution. We ensure that this does not cause harm to the mice since, ultimately, the same approach will be applied to humans. We do challenge the mice with strong adjuvant; however, we have discovered that reduction of the volume of material injected reduces the swelling associated with this procedure.

Project	**77. Detecting bladder volume and pressure from sacral nerve signals in sheep**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 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)	Accidents in people can cause damage to the spinal cord. This causes paralysis and incontinence for which there is no cure. In this project, we aim to develop a new treatment for urinary incontinence. To do this, we plan to design an intelligent implant able to monitor the bladder, for use in man. This has never been done. Our objectives are: 1. to place in surgery a biocompatible implant on the nerves controlling the bladder;	

	2. to analyse, during surgery, electrical signals from the nerves controlling the bladder; 3. to continue recording electrical signals with the implant in awake animals during their normal activities. Altogether, the project will deliver a new implant and surgical protocols for bladder control.
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 could benefit people or animals (such as companion dogs) that have sustained damage to the spinal cord. In the United Kingdom, around 50,000 people live with spinal cord injury, with about 1,000 new cases every year. This cost annually approximately £1 billion, which is 1% of the total NHS budget. Worldwide, spinal cord injury affects about 2.5 million people with approximately 130,000 new cases each year. This new treatment will offer affected humans a method to better manage urinary incontinence, instead of using drugs or bladder catheterisation that currently reduce life expectancy. We will also describe a new surgical technique for this implant with less adverse effects for people. For the scientific community, our results will advance knowledge in: (i) the design and surgical implantation of implants for nerves; (ii) nerve signal processing techniques. It will be applicable to other medical conditions and to radar and sonar systems.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use a maximum of 16 sheep over 3 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?	All the surgical procedures are of moderate severity and will be conducted under general anaesthesia. From past experience, pain after the surgeries we propose to do will be mild and transient and can easily be controlled with drugs routinely used for animals. All possible adverse effects we might see are anticipated to be transient and controllable with routine veterinary care and medication. None of the

	procedures done in awake animals will be invasive, for example, none of these involve breaching the skin.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals to test our implant because there is no other alternative that model the live urinary and nervous system. We need to do some recordings from a live animal, which size is comparable to humans. This is to allow future use in human patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of sheep by using one animal at a time and analysing data before going to the next animal. We will also use animals over long periods of time using non-invasive tests to obtain a maximum of 'real-life' data without having to rely on biopsies or post-mortem evaluation only.
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 are using sheep because their nervous system better mimics that of humans (compared to rats for example). This allows more straightforward application of our treatment to humans or other large animals. We will minimise welfare costs by using totally implantable and biocompatible systems and allow sheep regular access to grazing during the study period. Sheep will be able to express their natural behaviour throughout the project and methods of assessing our implant will remain non-invasive on awake animals.

Project	**78. Developing and testing novel treatments of vascular disease in large animal models**	
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 is to develop and test novel surgical vascular grafts and percutaneous (accessed through the skin) stents/devices for the treatment of severe vascular diseases of the blood vessels (vascular disease). This involves generating, maintaining and characterising large animal models (in pig/sheep) of human stable vascular disease in protocol 2, to be then used in protocols 3 and 4 to test novel tissue engineered vascular grafts, or percutaneous stents that are safe and more effective than those currently used in the NHS.	

	Myocardial infarction (heart attacks), stroke, limb ischemia and related coronary-vascular diseases (C-VD) are still the most common causes of death in the United Kingdom and the rest of the world. The immediate consequences of C-VD to patients have been reduced over time with the advent of interventions such as coronary and peripheral vascular bypass surgery using as conduits patient's own vein harvested from the legs or artificial grafts vascular conduits to bypass the blockages or metal stents delivered percutaneously through a needle. However, after 5-10 years these devices may fail leading to late over time the effectiveness of these interventions declines, often leading to a 50% narrowing within 5-10 years or to heart attack, strokes, or limb amputation. Hence, the aim of this project is to use modern technologies to develop more long-lasting effective bypass grafts or stents to be tested in the proposed advanced preclinical models with a view to identify those highly effective to be then tested in the NHS.
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 new bypass grafts or percutaneous stents lasting longer it will be possible to improve the life expectancy of those suffering from severe vascular disease in terms of both quality and duration. The studies undertaken will enable the proposed new treatments to be optimised in a way that they will be safe whilst maximising their beneficial effects. It is to be expected that some of the novel proposed treatments tested under this PPL will be quite effective with potential to be used in the NHS to benefit patients whilst reducing hospital costs.
What species and approximate numbers of animals do you expect to use over what period of time?	These studies will use predominantly pigs and a smaller proportion of sheep. Pigs/sheep are the most relevant species for these studies because their size, anatomy and physiology closely match that of humans. We estimate that we will use up to 320 pig and 100 sheep over the 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	The animals used in this study will be anaesthetised and either undergo vascular surgery or percutaneous deployment of vascular stents in a manner that replicates the procedures conducted routinely in the NHS. For about 25%of the animals there will be no suffering, as the whole procedure will be conducted while the animal is

end?	anaesthetised. For those animals that are allowed to recover following the procedure, pain control, consistent with that given to patients in the NHS, will be given to minimise any suffering. Based on our previous experience we expect most pigs/sheep to return to normal behaviour within 24 hours of the procedure. The vascular injury incurred by the pigs/sheep will be the minimum needed to evaluate treatments and should not compromise the animal's general wellbeing. All surgical procedures will be performed by expert veterinary and NHS doctors with high sensitivity for the animal's wellbeing. All procedures will be undertaken while the animal is sedated or under a general anaesthetic. Recovery will be managed by expert professionals who will optimise pain control after any surgical procedure. Animals will be housed in normal pens and always grouped with companion animals of the same species. On completion of the study protocol all the animals will undergo a final general anaesthesia to facilitate data acquisition and acquisition of scans and then be killed whilst anaesthetised. The overall severity of the procedure for pigs/sheep allowed to recovery post-surgery is moderate but in all cases pigs/sheep are expected to show normal behaviour within 24 hours of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	These studies will evaluate treatments, destined for introduction into the NHS, that are aimed at restoring normal blood supply to the heart, brain, kidney, limbs, etc in patients with severe vascular blockages and requiring following vascular surgery or percutaneous stenting. It is not possible to undertake this work without using large animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Sample size is calculated by expert biomedical statisticians based on very sensitive biochemical markers or in-vivo imaging scans. In addition, we will: 1) Use internal controls for evaluation of end points within the same heart 2) Use live heart scan as in the NHS for mid-term evaluations, instead of using interim histology/

	termination; 3) Refine the sensitivity of our study by combining NHS imaging standards with advanced post mortem histopathology; 4) Store extra tissue and other specimens from the termination procedures in the on-site GLP preclinical bio-bank fitted with modern NHS type of cryo-storage systems. These specimens will be used for future academic questions, reducing markedly the number of animals used by us and by other 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.	All the procedures will be undertaken by Veterinary and NHS experts, using the same level of expertise, sensitivity, and care used for work in companion animals or in the NHS. An expert anesthetist will be available during any procedure and during the post-operative stay in ITU and/or recovery. Painkillers will be used according to clinical standards to minimize post-operative pain. We will use absolutely sterile conditions and modern ventilators as well as state of the art heart-lung machine, Cath Lab for angiography, and surgical instrumentation. Sterilisation of instruments will be in a NHS-standard autoclave. Access to the surgical/Cath Lab theatre will be strictly regulated and will be at NHS standards. Antibiotic, anticoagulation and/or antiplatelet treatments will be appropriate to the procedures being undergone, and the intraoperative and post-procedural recovery and housing will be to clinical standards. We routinely use state-of-the-art MRI, echocardiography and other sensitive scans for serial assessment of endpoints and to monitor any functional decline before the animal shows any clinical signs. In selected experiments telemetry based monitoring of vital signs may be used.

Project	**79. Developing intervention strategies against global infectious diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to help researchers discover and develop new ways to treat or stop globally relevant infectious diseases. For malaria this is focussed on the way the parasite passes between mosquitoes and humans (and back again), the project aims to find potentially new vaccine candidates or parasite drug targets that, when targeted, block the way parasites complete their infection journey, stopping their development and potentially stopping disease. In addition, the project aims to test the initial ability of candidates against other pathogens (such as the SARS-CoV-2 virus responsible for	

	COVID19) to work as vaccines. Finally, the project includes the study of the fundamental biology of the malaria parasite, performed to advance our general understanding of this globally important disease.
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 aims to contribute to the control and elimination of globally relevant infectious diseases which still account for 3 of the top 10 leading causes of death. For malaria, 3.3 billion people are at risk of infection with 216 million people infected with malaria every year and an annual death toll of 445,000. The discovery of novel malaria drugs or vaccines that block its development is clearly a pressing challenge to biomedical research. Furthermore, the recent outbreak of COVID-19 has also shown the need for major developments of vaccine candidates against newly emerging diseases. Our research team as well as having world class expertise in infectious disease biology have crucial links with clinicians, field sites and biotech/pharma and we believe our work can inform the use of current and future interventions at the global level. In addition, malaria is also an invaluable model for analysis of the way pathogens (from viruses, bacteria to parasites) interact with the mammalian host. There is as such inevitable overlap between basic biological studies and our primary aims (vaccine and drug development) and more generalised understanding of human infections. Discoveries in malaria have had implications for immune responses to other pathogens and to immunity in general and have contributed to the study of insect vectors of disease in general including those of both human and veterinary importance.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years: Mice = 8050 Rats = 110
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	We do not expect any adverse effects on animals in any of our protocols as animals are humanely killed before symptoms from malaria would be expected to develop.

the end?	Likely severity levels are therefore Mild/Moderate. Vaccination for other infectious diseases do not involve live infectious agents nor infectious challenges. Animals will be checked daily following infection with malaria. Increased frequency of monitoring may be undertaken for certain procedures and remedial action taken as advised by the NVS. Animals will be humanely killed at the end of the studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Human malaria is caused by several species of Plasmodium most of which are specific for humans and some can also infect other primates. However, due to ethical and safety restrictions, the transmission stages of human malaria are experimentally intractable, and animal models are the only models for such research. The rodent malaria parasite P. berghei is a safe, versatile, biologically relevant and reliable model to study malaria transmission and infection in laboratory mice and rats. In vitro culturing of this parasite is not feasible except from very few stages of its lifecycle. Therefore, replacement of the mouse model is currently not possible or experimentally relevant. Furthermore there are no non-animals models currently available to assess the immune response to general vaccine candidates. In addition, maintenance of mosquito colonies requires regular provision of a bloodmeal for egg production, which is best done through feeding on live animals due to the mosquito physiology and behavioural biology. Therefore, although much of the mosquito maintenance is carried out through artificial blood feeding on human blood products, mosquito feeding on live animals remains an essential component of this research. Furthermore, feeding on human blood products cannot precede feeding on live animals for *P. berghei* infection and testing of antimalarial drugs and vaccines due to immunological interference or feeding on humans for testing the efficacy of transmission blocking interventions due to ethical and safety

	restrictions. For all the above reasons, feeding of mosquitoes on live animals is required for some of the mosquito maintenance procedures.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals proposed to be used under this project licence will be reduced by using well-established, robust systems for propagation and generation of material. The robustness of these refined systems greatly reduces the number animals used, as ~100% of animals become successfully infected upon inoculation. Our long experience of working with these systems enables us to reduce the number of animals used by careful planning, using appropriate controls and replicas to avoid unnecessary repetition. Mosquito colonies are maintained on rats or mice. By using the appropriate rodent species according to colony size we can minimise the number of animals used.
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 almost all our work, the infecting parasite dose is large (to ensure rapid establishment of infection), animals are monitored daily and are used within a few days of infection. Under these conditions, infections are well tolerated, causing little discomfort to mammalian hosts, with animals typically displaying a normal behaviour. The procedures outlined above inhibit establishment of chronic infection. Different species of mosquitoes display different preferences for blood sources. Rats and mice are well tolerated by our colonies as a blood source. This maximises progeny output following feeding, which intrinsically reduces animal usage. The fact that mice and rats are well accepted by our colonies also reduces feeding time, allowing feeding under conditions of gentle anaesthesia. Light anaesthesia, in combination with rehydration following feeds ensures that animals rapidly recover and experience minimal discomfort. During antibody production, resulting immune responses are monitored by taking small (~30ul) blood samples by the least invasive method. Different immunization programs are established to raise robust immune responses,

	ensuring maximum chance of success using the smallest number of animals under minimal duress. The appropriate animal species will be chosen to best serve the procedure whist using the least number of animals. Rats will be favoured over mice for larger scale antibody production, as dictated by naturally available blood quantities at the end-point. Vaccines which do not produce ulcerative effects will be preferentially used. Any animal showing symptoms exceeding a mild severity limit will be killed using a humane method. For the study of potential anti-malarials, animals will be given doses based on data derived from *in vitro* studies, and initial doses used are not anticipated to cause health problems since compounds have been tested for toxicity. Known anti-malarials, which are investigated for their effects on transmission or liver stages, will be used at doses equivalent to those already routinely used for humans and are unlikely to result in toxicity effects. At all times, animals will be housed in groups where possible, with appropriate environmental enrichment and fed according to current institutional 'best practice'.

Project	**80. Developing methods and tools for engineering the mouse genome**	
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)	This project aims to apply and develop new methods for alteration of the mouse genome. The aim is to develop the ability of generating increasingly complex alleles with increasing levels of accuracy and employing a decreasing number of animals. For this we will either trial emerging technologies or develop new approaches, often based on recent advances in cell culture models. It also covers the performance of quality control tasks for current mutant generation processes and products: Although current protocols are able to produce the modified mice required for research, part of	

	the animals produced contain imperfect modification that, if they were taken further for research would lead to misleading results. The extend to which these unwanted events do happen needs to be defined for all new transgenesis protocols and we will develop strategies to tell apart correct from imperfect events alongside evolving new transgenesis techniques.
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 that are expected from this project include: - a more efficient use of (fewer) animals for generation of mouse mutants for research, - the development of methods that will allow making more sophisticated/precise changes in the mouse genes (current methods leave additional alterations in the genome or afford no control over where new genetic material is inserted in the genome), - introduction of methods for faster and cheaper generation of mouse models (as it will become possible to make the mutation in the mouse strains that are relevant to the research project instead of only the strains that are permissible to current methods). - while new transgenesis methods come on line, we will develop in parallel appropriate molecular methods for checking the accuracy of the mutants generated, to ensure the quality and reproducibility of the research that will be derived from these animals. We will disseminate these validation methods alongside protocols for mutant generations. This works underpins the evolution of quality standards as the transgenesis field changes.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 12500 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?	Generation of founders: The generation of Genetically Altered animals involves administration of hormones and implantation of embryos in foster mothers, protocols which are of Mild and Moderate severities, respectively. The welfare of these animals will be closely monitored to ensure that they remain within the level of severity of these protocols. Breeding of Genetically Altered mice: It is difficult to predict

	what adverse effects to expect as the work will include random insertion of genetic materials and there are potential risks of off-target effects with the new methods that will be piloted. This is why the welfare of the animals generated will be closely monitored and the animals will be humanely killed if they show any sign of exceeding the expected severity limit. At the end of the experiment, most animals will be humanely killed. A very small number will be anaesthetised and exsanguinated to allow further experiments with their tissues.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The experiments to be covered by this project can only be conducted in the context of the whole organism. Although it will not be possible to completely replace *in vivo* systems in the studies being performed, alternatives will be considered both prior to and during the experiments. Wherever possible, *in vitro* culture systems will be exploited as a substitute for *in vivo* systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	**Maintenance of GA mice** Mouse lines will only be maintained whilst they are part of ongoing scientific programs of work. The breeding of any mouse strain with no predicted usage will be stopped and sperm from the line frozen. **Minimising mouse numbers** At all times the minimum number of mice will be used to obtain a valid scientific result. This will be achieved by reviewing historical data associated with previous work carried out in the laboratory. For example, a microinjection session usually requires 20 females to generate 100 embryos to inject with a given strain. The number of females used for a microinjection session will be adapted if/when a higher success rate is anticipated from the microinjection and fewer embryos should be needed to produce a number of genetically altered animals.

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 most appropriate animal model for this project because our intended aim is to work out the function of all mammalian genes and proteins. Animal models are important because we are able to manipulate their genes using genetic engineering and investigate the consequences for the whole organism. The mouse occupies a unique position in determining gene function and the genetics of disease for a number of reasons. Firstly, as a mammal it demonstrates a remarkably similar development, physiology and biochemistry to the human. Secondly, mouse geneticists have developed a very extensive genetic toolkit that enables very specific alteration of genes in the mouse. Thirdly, we now know the complete sequence of all the DNA the mouse carries. We will minimise the welfare costs to the animals by using the minimum number of animals at all times and by using anaesthesia and analgesia where necessary. One of the aims of this project is to improve the techniques we use to introduce mutations in mice and therefore reduce the number of animals needed for this type of experiment in the future.

Project	**81. Developing new treatments for vascular retinopathies**	
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 determine whether controlling expression of vascular growth factors can prevent or reverse blood vessel growth and neurodegeneration underlying blindness in animal models of : A Neovascular eye disease, such as wet age related macular degeneration B. Type I diabetic retinopathy C. Type II diabetic retinopathy	
What are the potential benefits likely to derive from this project (how science could be advanced or	The aim of the project is to identify new targets and develop new drugs to treat common causes of blindness, particularly age related macular	

humans or animals could benefit from the project)?	degeneration and diabetic retinopathy. The benefits that will result from this work will include the identification of new molecules that can be assessed for the usability as therapies in humans, and an understanding of new targets that contribute to these blinding diseases in animal models. The benefits that might result include the development and characterisation of drugs that will be used in humans during the lifetime of this project. In the longer term it could also result in ways of preventing blindness in people.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (1350) Mice, (4000), Rabbits (300) 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?	We intend to induce models of disease in the eye by: A) make a tiny hole (<0.1mm) in the membrane at the back of the eye, either with a laser or a ophthalmic surgeons microknife, which stimulates blood vessel growth. B) Using animals with models of either type 1 or type 2 diabetes. The Type 1 diabetes will be induced either by treating animals with a chemical that prevents them making insulin, or by breeding transgenic animals. Type 2 diabetes will either be generated using diet (e.g. feeding high sugar, high fat diets), or breeding animals that are genetically prone to type 2 diabetes. We will measure the function of the eye by imaging the retina with a microscope in anaesthetised animals, non-invasively (similar to a visit to the optician), and in some experiments measure the electrical activity of the eye by placing a small electrode outside the eye. Most of these experiments have a very low likelihood of adverse effects and the animals will recover quickly from the anaesthetic and not experience any adverse effects under normal circumstances. The severity of the models will vary from mild for some animals to moderate for animals undergoing induction of diabetes or anaesthesia/surgery. Most animals will not undergo surgery, but at the end of the experiment the animals will be killed by a humane method and tissues taken for analysis after death.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We do test the approaches in cells in culture first, but this cannot tell us whether blood vessels (which cannot be grown in culture) can alter their behaviours, and their effect on vision (which cannot yet be sufficiently modelled in cultured cells).We also test the ability of molecules to reach the back of the eye in tissues taken from animals killed for other purposes, build mathematical and empirical models to tell us whether the molecules will reach the back of the eye, before undertaking experiments on animals. However, the eye is a complex organ and in live animals has varied blood flow in multiple different beds, which cannot be modelled or measured without undertaking experiments on live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To enable the minimum number of animals to be used we used advanced statistical modelling. The least number of animals necessary to definitively show the properties (or lack of them) of test substances will be used. We also use experimental designs that reduce the number of animals, (e.g. making multiple measurements on single animals over time rather than single measurements on multiple animals).
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 of the extensive characterisation of these models of retinopathy shows that they are similar to humans in their pathology of both neovascularisation and diabetes. We have now shown that rats have physical changes that are similar to humans (loss of capillaries), and they have larger eyes. Mice are the species with the lowest degree of neurophysiological sensitivity in which retinal vasculopathy and neovascularization with similar properties to human disease has been characterised. Rabbits are used because they have a similar structure in their eye to humans, and drugs are thought to penetrate into human eyes more similarly to rabbit eyes. The imaging and recording techniques we use are both non-invasive procedures that do not result in suffering of the animals other than the injection for the

	anaesthetic. Diabetes can be associated with significant morbidity in humans, but the mouse and rat models we use are relatively mild for the first 16 weeks of diabetes, during which these experiments will be undertaken.

Project	**82. Developing vaccines for Neisseria gonorrhoeae 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)	*Neisseria gonorrhoeae* (gonococcus) infection is rising dramatically worldwide (>78 million cases annually) and there are no vaccines to prevent infection. Gonococci infect the human lower genitourinary tract and gonorrhoea is characterised by painful urethritis in men and cervicitis in women. However, infection without symptoms can occuer in up to 25% of women, and the bacteria can travel from the lower genitourinay tract to the womb, Fallopian tubes and ovaries. Here, infection can cause pelvic inflammatory disease, PID, which has serious consequences for women's healthlath, leading to	

	ectopic pregnancy, chronic pelvic pain and infertility. These are issues for women's health worldwide. Although antibiotics have been tremendously successful in treating gonorhoea since the 1940s, today we are faced with bacteria that are resistant to all classes of antibiotics - essentially untreatable infection. Thus, prevention through vaccination has been highlighted as the most effective solution to the problem. To develop vaccines, scientific knowledge is needed about the components of the bacterium that are important for protection against infection. The Overall Aim of the project is to translate information from our laboratory studies of human immune responses to gonococcal infection to develop candidate vaccines. These vaccines will be used to vaccinate animals to generate antibodies that can kill the bacteria. This information will be used to produce gonorrhoea vaccines for phase I human trials in the longer term.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The major benefit from our work in the laboratory and throught the use of animals is the development of new vaccine components for preventing gonorrhoea, an important sexually transmitted disease of humans. There are ~78 million cases of gonorrhoea reported annually worldwide, which is probably an under-estimate due to significant undetected infection. New vaccines will also help to prevent infection by bacteria that are now resistant to many of our antibiotics. In the longer term, our research will contribute to public health intervention strategies for a global infectious disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year term of the project we anticipate using up to 3000 adult mice, 20 adult rabbits and 100 rats for antibody production.
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 our vaccine studies, high animal welfare standards will be met with environmental enrichment, good husbandry and frequent monitoring. We will typically inject animals with vaccines that are made from i) individual components of the bacterium Neisseria gonorrhoeae produced from the organism itself

	or by genetic engineering in the laboratory, and ii) synthetic vaccines that are produced in the laboratory by chermistry. We will administer different adjuvants with these vaccines: adjuvants are chemicals that help the immune system produce a strong response to the vaccine. These adjuvants are unlikely to cause adverse effects because the ones we propose to use are routinely used with human and animal vaccines. We will inject animals with our vaccines principally by the subcutaneous route. Other routes such as intramuscular are preferred for genetic (DNA) vaccines specifically, and the intraperitoneal route of injection will be uncommon. Our schedule for vaccination is generally 3 injected doses over a period of 28 days. At the end of the schedule we will anaesthetize the animals and maintain anaesthesia to make sure they remain asleep and then bleed them by heart puncture. The animals do not suffer during this procedure and are finally dispatched by neck dislocation. The taking of blood samples is critical to the development of these vaccines. We will use the blood samples to test how effective the vaccines are at producing protective responses against the bacteria. Adverse effects from vaccination are expected to be uncommon; the only adverse effect that may occur is with the use of one particular adjuvant, which may cause dry abscesses. Overall, the procedure for producing immune response antibodies to vaccine antigens in experimental animals is mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no alternativelaboratory models for producing antibodies to candidate *Neisseriagonorrhoeae* vaccines. The use of animals is essential for this overall aim, as antibody production requires a fully functional immune system that cannot be reproduced adequately in the laboratory. No gonorrhoea vaccines would be tested in humans without pre-clinical evaluation of efficacy in small animal models.
2. Reduction	All experiments are designed to minimise the animal numbers used for obtaining statistically

Explain how you will assure the use of minimum numbers of animals	valid data and for ensuring a commitment to refinement, replacement and reduction of animal usage. We use statistical power calculations to calculate the minimum number of animals, in particular mice, that we need to test our vaccines. No animals will be re-used.
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 our experiments are designed to minimise the animal numbers used for obtaining statistically valid data and for ensuring a commitment to refinement, replacement and reduction of animal usage. High animal welfare standards will be met with environmental enrichment, good husbandry and frequent daily monitoring for any adverse effects. Mice, rabbits and rats are the appropriate species for producing antibodies to candidate gonorrhoea vaccines and the antibody reponses produced give an indication of vaccine potential for next-step use in humans. We do not expect any overt adverse effects during the testing of our vaccines as the vaccines are safe. In addition, problems arising due to the use of adjuvants to stimulate the animal immune reponses to our vaccines would be uncommon; we would only use the adjuvant that is known to produce adverse effects as a last-resort only in the unlikely event thast the other adjuvants do not work. All animals used for our vaccine studies are monitored daily for any adverse effects. We also reduce the number of times the animals have blood samples taken and use appropriate anaesthesia to limit discomfort.

Project	**83. Development and Adaptability in Central Nervous System Sensory Pathways**	
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 is to investigate pain development in newborn rats and mice and provide the basic scientific knowledge required to effectively treat childhood pain. The objective is to investigate the structural and functional development of spinal cord, brainstem and cortical pain pathways. In particular we wish to understand how pain processing in the spinal cord and brain develops, how pain behaviour emerges in newborn rodents and how these processes are affected by surgery or inflammatory injury in early life. The normal developmental trajectory of key brain areas involved in pain perception	

	will be studied in whole live animals and compared to that in animals exposed to injury in early life. Since pain perception requires an intact central nervous system, we will measure brain and spinal cord activity in anaesthetised or awake whole animals. In addition, neurochemical changes in these regions will be measured. We will compare naive animals with animal models of childhood procedural, surgical and inflammatory pain. Our results will be shared with scientists and clinicians dedicated to the understanding of childrens pain and the alleviation of suffering.
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 lack of knowledge of pain processing and of the actions of pain-relieving drugs in infants and young children is an area of great concern in paediatric medicine. The potential benefit of this project is that the basic biological mechanisms underlying childhood pain will be discovered, thereby providing a foundation for more effective pain relief for this vulnerable sector of society.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use a total of 2600 rats and mice over 5 years, specifically 276 adult and newborn rats and 244 adult and newborn mice per year. All animals will be bred for scientific use.
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?	Since the aim of this project is to understand pain in children, moderate pain is generated in the animals. To model clinical paediatric pain experience, animals undergo localized surgery, inflammation or nerve damage. The majority of these procedures are of moderate severity and some are of mild severity. The animals will be regularly monitored and if their pain is greater than that expected from these models, they will be humanely killed. All surgery is performed under anaesthesia, and animals closely observed in the first 6 hours, then checked twice daily for the first two postoperative days and daily for the following week. Adverse postoperative events are not expected in this project, but in the unlikely event of unexpected suffering, animals will be humanely killed. Some animals will be used for recording brain activity

	under anaesthesia, from which they do not recover. Others are humanely killed for removal of parts of the brain for analysis of cells and small traces of neurochemicals. Other animals will be used for wireless brain recording while awake and free to move around and humanely killed at the end of the recording period.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Pain sensation and pain experience requires an intact nervous system. To understand and prevent pain we need to investigate the changes that occur throughout the intact nervous system following tissue injury. This can only be done in whole live animal models. Detailed analysis of individual cells can be undertaken in isolated slices of spinal cord or brain, artificially maintained in a dish, but the results from such studies cannot always be interpreted in terms of animal or human sensation and experience. The use of whole live animals is justified by the potential reduction in suffering for infants and children. Rats and mice are good models for understanding human pain as their pain pathways are very similar to those of humans and the newborn rat is at an equivalent stage of development to a premature infant.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We do not use more animals than necessary because it is ethically unacceptable. It is also inefficient. Experiments are therefore statistically designed to minimise animal numbers. In most cases we are measuring the difference in brain function in control and experimental animals. Our data base of control data combined with pilot studies provides us with the standard deviations required to calculate the required sample size for a significant effect at the 5% level. In practice, in most of our experiments 6-8 control animals and 6-8 experimental animals are sufficient to produce significant data. Numbers used take into account the fact that studies need to be performed at several ages representing newborn, childhood, adolescence and adulthood. In some cases, such as in models of nerve injury, sham surgery is necessary because it is essential to know

	whether the outcome is due to the nerve injury itself or to the preceding surgical procedure. Without it, the whole experiment would be wasted and the data uninterpretable. Sham surgery is only used where strictly necessary for statistical and scientific reasons.
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 rodent models used here are chosen to model the post-surgical and procedural pain of neonates in intensive care and the inflammatory pain of children with arthritic conditions. Newborn rats and mice are born so immature that they can be used as a model of premature human infants. All surgical procedures are performed under anaesthesia and in cases where animals recover from surgery, care is taken to ensure that there is no discomfort through the appropriate use of analgesics. Following surgery, animals are closely observed in the first 6 hours, then checked twice daily for the first two postoperative days and daily for the following week. Some animals will be used for recording brain activity under anaesthesia, from which they do not recover. Others are humanely killed for removal of parts of the brain for analysis of small traces of neurochemicals. Other animals will be used for wireless brain recording while awake, which involves wearing head caps. They are free to move around and humanely killed at the end of the recording. Since the aim of this project is to understand pain in children, moderate pain is generated in these animals. If the pain is greater than that expected from models of localized surgery, inflammation or nerve damage, then animals will be humanely killed.

Project	**84. Development and evaluation of imaging agents in rodent models of 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)		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)	Brain diseases such as Alzheimer's (AD) and Parkinson's disease (PD) are becoming more common as the population gets older. The symptoms are thought to be caused by the death of brain cells (neurodegeneration) involved in memory (AD) and movement (PD) and start decades before signs appear. In AD, the first signs are forgetfulness but as the disease spreads, patients become increasingly confused and angry because they do not recognise family and friends. In most cases, sufferers will end up in hospital or a care home and will require 24	

	hour care until they pass away. In PD, the first signs are shaking of the limbs. As the disease spreads, patients find it increasingly difficult to move, walk and swallow and usually end up also needing 24 hour care until they pass away. Although these disorders cannot be cured yet, it is very important to identify patients who will develop the disease as soon as possible. This may allow any future drug treatments to be given before the brain damage is too severe for them to work. Fortunately, these types of disorders show the build-up of different toxic substances (proteins) in the brain that are a specific to each disease. If drugs, termed imaging agents, can be developed to bind specifically to these proteins then they could be used diagnose the disease using brain scans. In addition, the effect of drugs designed to reduce the build-up of these toxic proteins could also be measured using these imaging agents In contrast to these specific proteins, there are also common features of these diseases such as brain injury. Imaging these changes could also be used to identify these diseases early and test the effect of potential treatments. The aim of this project is to develop and evaluate imaging agents that can identify the changes in neurodegenerative diseases using rodents.
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)?	Development of new imaging drugs specifically targeted at brain proteins or more general features of brain injury may be critical for early diagnosis and development of new drugs for these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will mainly use rats, but mice will also be used. It is anticipated that no more than 600 animals will be used over the 5 year project duration.
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	Most imaging studies will be done in unconscious, anaesthetised animals and with careful monitoring there should be no side effects to the animals. Animals may be treated with new test agents beforehand, which have been proven in the literature to be safe so only mild effects are

end?	expected. Some animals will be used that have had human genes added to their DNA structure that produce the abnormal toxic proteins seen in human diseases. Some will become increasingly unable to walk over time mimicking the disease progression but will be used before they show any signs of pain, distress or weight loss. These symptoms are described as moderate. Animals undergoing surgery, such as injection of substances into the brain to produce brain injury, will also have a moderate severity limit but with good care following surgery they will be expected to make a full recovery within 12 hours. At the end of the experiment animals will be killed by a humane method such as overdose of anaesthetic and the organs and brain tissues collected for further experiments in this study. At the end of this experiment brains will be frozen and stored for use in future studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since neurodegeneration is a complicated process that occurs in the living brain it is not possible to use non-animal alternatives. Similarly, when testing how an imaging agent behaves in the body it must be done in animals. However, initial studies to decide if an imaging agent is suitable for animal testing, studies will be done on donated human brain slices and/or rodent tissues in the laboratory collected from previous studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be reduced by careful selection of the best species to use, e.g. rat and mouse. Experiments will be designed to get the best and most accurate information out of a single experiment to allow a rapid decision to do further animal testing or not, thereby reducing the number of animals used
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	Studies will be conducted in rats and mice and the models chosen are those that produce the best effects for brain imaging with less side effects Transgenic rats that express toxic proteins will be used and these are directly comparable to the disease in humans. For animals that have surgery to produce brain inflammation, sterile surgery and

minimise welfare costs (harms) to the animals.	post-operative painkillers will be used.

Project	**85. Development and function of blood cells and cells of the immune system**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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 investigate the molecular mechanisms that control the development and function of cells of the immune system and other blood cells. All blood cells including cells of the immune system are produced from stem cells through a process called haematopoiesis. We will investigate the way in which these developing cells interact with their environment during haematopoiesis and the way in which their environment regulates their development, fate and function.	

Why is it important to undertake this work?	It is important to undertake this work because advancing our basic understanding of lymphocyte biology and haematopoiesis will benefit many areas of medical science. An understanding of how blood cells are produced and how they interpret signals from their environment is important for the treatment of haematological and immunological disorders and malignancies (blood cancers, anaemia, immunodeficiency). The part of the project on the basic mechanisms that generate white blood cells and immunity will contribute to an understanding of the immune system that could eventually impact on the treatment of many human and animal diseases (infectious and autoimmune diseases, immunodeficiencies, cancers). An understanding of factors controlling the renewal and differentiation of blood stem cells into white blood cells will also be important in refining stem cell based therapies for diseases such as immunodeficiencies, cancers and for ageing. White blood cells called T-cells are particularly important to enabling us to fight infectious disease. These cells are produced in an organ called the thymus. The part of the project that investigates the way in which the f the thymus influences T-cell development is important for an understanding of the causes of autoimmune disease, and for the development of new therapies for autoimmunity. Experiments to investigate thymus function and thymus transplantation will have specific clinical benefits as they will help to improve treatment for complete DiGeorge syndrome. Complete DiGeorge syndrome is a rare disease in which infants are born without a thymus and so do not have any T-cells and cannot fight off infectious disease. Thymic epithelium transplantation for DiGeorge syndrome has recently been established in the UK, and there is an urgent need to improve this technology. Without treatment complete DiGeorge patients die before two years-of-age.
What outputs do you think you will see at the end of this project?	This project will contribute to our understanding of the molecular mechanisms that regulate blood cell development and immunity. An understanding of how blood cells are produced and how they interpret signals from their environment is important for the treatment of haematological and immunological disorders and malignancies. Knowledge of how immune cells function and of factors that control their function is important to our understanding of how we combat infectious disease

	and cancer, and an understanding of how immune cells can become inappropriately active, is important to designing new treatments for diseases that involve inflammation, allergy and autoimmunity. The project will lead to publications in peer reviewed journals that present our findings. We will also present our findings from the project at scientific meetings and conferances.
Who or what will benefit from these outputs, and how?	In the long term, a detailed understanding of immune and blood cell development and function is important to anyone who suffers from diseases that occur when these processes go wrong (blood cancers, autoimmunity, inflammatory and allergic disease, immunodeficiencies) as this is required to provide new strategies to treat these diseases. Likewise, this is important to design novel strategies to enhance or improve immunity to infectious disease and cancer (vaccine design and immune therapy). In the medium term, the findings may provide economic benefit to society by contributing to industry through drug discovery. In the short-term, the findings will progress the academic field through publication of new findings and the presentation of new findings at scientific meetings and conferances.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We will publish our findings in international peer-reviewed journals and also present our findings at scientific conferances and meetings.
Explain why you are using these types of animals and your choice of life stages.	We have chosen to use mice because relevant genetically modified mice are available and technology to generate genetically altered mice is well-developed. Mice have long been used as a model to investigate immunology and haematopoiesis and our research findings will be interpretable within the context of this wide body of literature. Mice are an excellent model for human immunology and haematopoiesis (production of

	blood cells), and nearly all our understanding of human immunology and haematopoiesis is based on findings from mice studies. The use of genetically altered mice allows us to test directly the impact of the factor or signalling pathway we are investigating on the immune response. We may maintain aged genetically altered animals (>1 year) in order to investigate the influence of time on the impact of their genetic alteration on their immune organs.
Typically, what will be done to an animal used in your project?	Most of the animals used in this project will be bred and euthanized humanely in order to provide genetically modified tissues and cells for our laboratory experiments. In order to investigate the immune response, some animals used in this project will be immunised. Immunisation would involve either an injection, or application of a substance to the skin, or making the mouse inhale a substance placed as a drop on its nose. In order to investigate how red blood cells are made during recovery from anaemia, mild anaemia will be induced in some mice, either by injection or by mild irradiation. This degree of anaemia will not cause suffering to the mice. In some experiments surgery will be performed to transplant a piece of tissue under the skin or a small piece of tissue will be inserted under the skin by injection. This is not expected to cause suffering because anaesthesia and pain management will be used, and mice will be carefully monitored after surgery.
What are the expected impacts and/or adverse effects for the animals during your project?	The breeding, immunisation by injection or inhalation, and induction of anaemia in genetically modified mice are not expected to cause adverse effects or suffering. Immunisation by administration of substances to the skin may cause some inflammation (reness), itching and soreness at the site of administration. Surgery may cause transient soreness at the site of incision, and mice may feel subdued after anaethesia for surgery. Anaesthesia and pain management will prevent suffering. Mice are expected to recover to normal within 24 hours of surgery.

What are the expected severities and the proportion of animals in each category (per animal type)?	>90% of the mice used in this project will be classified as Mild. <10% will be classified as Moderate, because they will undergo surgery.
What will happen to animals at the end of this project?	used-in-other-projects
Why do you need to use animals to achieve the aim of your project?	Use of animals is essential, as we study dynamic physiological systems (haematopoiesis and immunity) that cannot be investigated in isolation, in which there is homeostatic control and feedback on rate of differentiation, and in which multiple cell types and tissues interact with one another through time. Mice are most appropriate species because relevant genetically modified mice are available and technology to produce genetically altered mice is well-developed. Mice have long been used as a model to investigate blood cell production and immunology and our research findings will be interpretable within the context of this wide body of literature. Mice are an excellent model for human immunology and haematopoiesis, and nearly all our understanding of human immunology is based on findings from mice studies. The use of genetically altered mice allows us to test directly the impact of the factor or signalling pathway we are investigating on the immune response.
Which non-animal alternatives did you consider for use in this project?	We have considered using cell or tissue culture systems in which animals are used as a source of tissue only.
Why were they not suitable?	We cannot rely entirely on tissue culture experiments as alternatives to using animals because we investigate dynamic physiological systems in which there is homeostatic control and feedback and in which multiple cell types and tissues interact with one another through time. This cannot be recapitulated in the laboratory.
Enter the estimated number of animals of each type used in this project.	mice: 12,000 mice
How have you estimated	We have estimated the number of animals that will need

the numbers of animals you will use?	to be bred to generate animals of the required genotypes for our experiment groups. Size of experimental groups was estimated using power calculations.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Where possible we try to use as many different tissues as possible from each experimental mouse and we will freeze material from experimental animals where possible for future use. We design breeding programmes in which as many animals as possible from a litter can be used in our experiments.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	We try to use as many different tissues as possible from each experimental mouse. As the project progresses we may carry out pilot studies to carry out power calculations to use the smallest possible experimental groups.
Which animal models and methods will you use during this project?	Where possible we will use genetically modified mice as a source of tissues only and we do not expect the in vivo experiments in which we immunise mice or induce mild anaemia to cause any pain, suffering, distress or lasting harm to the animals. In the experiments in which we transplant tissue, we will minimise pain and suffering by use of appropriate anaesthesia and pain management, and using careful post-procedure care and monitoring.
Why can't you use animals that are less sentient?	In order to investigate the immune response we need to use adult mice in which all tissues of the immune system are fully developed and interact with each other. Mice are the most appropriate species to investigate immunity because they are the most used model for which reagents are widely available and technology for genetic alteration are most developed. Our data will be interpretable within the context of the vast body of literature pertaining to mouse immunity. There is not an appropriate model to investigate mammalian immunity which is less sentient.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We will be informed of advances in the 3Rs by UCl and by referring to the NC3Rs web-site.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	We will consult with our veterinary surgeon to ensure that we use state-of-the-art post-procedure care (and pain-management if appropriate), and regularly review our monitoring procedures.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We will follow advice from the NC3Rs.

86. Development and plasticity of synapses and networks

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants.

Key words

Synapse, circuit, mitochondria, glia, cytoskeleton

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

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?

Nerve cells signal to each other at special sites called synapses by releasing neurotransmitters that act on receptor proteins to activate or inhibit the cell. A key goal is to better understand the currently poorly understood mechanisms that regulate the formation and function of synapses, to regulate nerve cell communication. These mechanisms are also often disrupted in neurological and neuropsychiatric diseases including epilepsy, stroke, Alzheimer's, Parkinson's and Huntington's diseases, anxiety, autism and schizophrenia. Our aim is to better understand how synapse development, function and strength are regulated, which is crucial for understanding how the brain works, and may also lead to the identification of therapeutic interventions in a wide range of diseases.

For neurons to continue to function they also need a constant energy supply, which is generated by the power-houses of the cell called mitochondria. Maintaining the correct function, distribution and turnover of mitochondria in large and complex cells like neurons (for example a motor neuron axon can be up to a metre long) is critical for brain function and has emerged as an important regulator of correct animal and brain development and physiology. A key goal is to determine how brain cells regulate the position and function of the energy producing mitochondria. Defective mitochondrial trafficking and function is also

implicated in many neurological and neurodegenerative diseases (like Alzheimer's disease and Parkinson's disease) and also causes cellular dysfunction in diseases like stroke, cardiac arrest and spinal cord injury. Studying the mechanisms that underlie these regulatory processes will allow us to understand better how the brain works under healthy conditions, and how dysregulation of these processes alters nerve cell function in disease.

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

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

The main aim of this research project is to generate new scientific knowledge about receptor and organellar trafficking and function and the role this plays in brain development, connectivity and function. Understanding how the brain forms connections (between neurons or between neurons and other brain cell types such as the supporting glial cells), maintains these connections and regulates their strength, in the healthy brain are valuable in their own right. This is also essential for understanding neurological disorders, such Parkinson's disease, Alzheimer's disease, schizophrenia and autism, which exhibit disruptions in and/or abnormalities of neuronal development and signalling. The knowledge gained of the membrane trafficking properties of receptors, transporters and organelles in neurons, glia, and other cell types, and of the protein machinery and signalling mechanisms that regulate these processes, will substantially advance our understanding of the fundamental mechanisms by which nerve cells develop and communicate, and by which the brain functions. This knowledge will not only inform us regarding important mechanisms of animal and brain development and function but will also help to provide a basis for the development of therapeutic strategies, when these processes are disrupted in pathology.

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?

Up to 5000 animals will be used over the 5 year research programme, which includes the generation and breeding of multiple genetically modified strains.

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?

In some experiments we will use genetically modified mice carrying a genetic mutation allowing the function of that gene to be examined, while some mice may carry a fluorescent-transgene, allowing a particular protein or cell type to be observed using a specialised microscope. Neither genetic mutation nor fluorescent-transgene expression are expected to cause any significant harm to the animal. Some surgical procedures such

as making a very small window in the skull so that we can image and record activity of neurons or glial cells will be performed under anaesthesia. Potential adverse effects include postoperative pain but appropriate analgesia should ensure that animals do not experience lasting distress of pain. When unexpected clinical signs appear, we will immediately consult our designated welfare officers and vets. At the end of an experiment all mice will be euthanised using a schedule 1 method or humanely killed under terminal anaesthesia and tissue will be utilised for experiments.

Replacement

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

Rodents have been chosen for this work as the lowest species to mimic the human nervous system well enough for our work to be relevant to understanding of human brain function and disease. Live brain tissue and intact animals are essential for studying brain development and the properties of nerve cells and their connections. The basic synaptic, neuronal and network properties are common to all mammals, and the large body of data available for rodents makes them a good model system for understanding human brain function. Mice are also essential because the experiments require the use of the latest mouse genome engineering technologies to identify cell subtypes and to allow the knockin, knock-out and mutation of genes and reporter proteins. Moreover because some of the work to be carried out will study interactions between different brain cell types (such as neurons and their supporting glial cells), it can only be done on tissue from intact animals.

Reduction

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

Each experiment is designed, as far as possible, to include its own control, reducing variability, and using methods which enable us to estimate the minimum number of animals required to detect an effect, thus minimizing the number of animals required to ensure reliable and reproducible results. Variability, will be further minimized, by using well-defined strains of mice will little genetic variation. By using transgenic technology to make cells of a particular type and / or proteins within the cells fluoresce a particular colour, we can also reduce the number of animals used. Experimental techniques and methods are used that maximise data collection from a minimal number of animals and wherever possible, tissue will be shared amongst researchers. This together with careful breeding and husbandry will ensure we use the minimum number of animals.

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 basic synaptic, neuronal and network properties are likely to be common to all mammals, and the large body of data available for rodents makes them a good model system for human brain function. Mice allow the use of the latest mouse genome engineering technologies to identify cell subtypes and to allow the expression or removal of specific genes of reporter proteins. Mice are monitored carefully to identify any

deviations from normal health. Where possible we will restrict our genetic manipulations spatially and temporally to only a small population of cells are affected in a particular tissue region and less likely to have a detrimental effect. All surgery will be performed by fully trained staff using appropriate anaesthetic and analgesic regimes to minimise pain and procedures will receive regular review to identify further refinements to minimise animal suffering. Mice will be group housed and receive environmental enrichment wherever possible.

87. Development and plasticity of synapses and networks

Project duration

1 years 6 months

Project purpose

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

Key words

Genetically modified, nervous system, model

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to successfully breed and maintain animal model strains specifically for the study of the development, plasticity and pathology of the nervous system.

The strains to be maintained have specific genetic modifications in receptor trafficking, organelle dynamics and cytoskeletal function. These pathways play key roles in brain development, function and pathology.

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

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

The benefits are that the future use of these animal model strains will contribute to the understanding of the basic mechanisms of brain function and to nervous system disease

processes which could ultimately lead to the development of new therapeutic methods. Previous work with these and similar lines has directly lead to important new knowledge of the function and dysfunction of the nervous system.

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?

Over a one year period it is expected that up to 1000 mice could be produced, though with good husbandry and careful genetic testing it is likely fewer animals will be bred to maintain the model strains.

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 animals will only undertake normal breeding activity. There are not expected to be adverse events and the severity limit is mild. Animals not required for future breeding will be humanely killed to permit generation of cell cultures and the study of brain, organs and body tissues.

Replacement

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

Live brain tissue and intact animals are essential for studying brain development and the properties of nerve cells and their connections. Moreover the study of interactions between different cell types in the brain such as neurons and glia can only be done on tissue from intact animals.

The models maintained under this project provide insights into how cross-talk between the various cell types in the brain and between brain cells and the muscles and organs works.

Reduction

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

With careful breeding and husbrandry, only the minimum number of animals will be bred to maintain healthy strains.

Biopsies will be taken from animals so that the genotype of the animals can be established

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 animals to be bred under this licence have direct relevance to the study of nervous system function and dysfunction in disease. Where possible animals surplus to breeding will be used to generate cell cultures or tissues for other laboratory studies. In this way we will maximise the utility of all animals created during the project.

Project	**88. Development of a single dose vectored Taenia solium vaccine**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	1 Year 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)	Pig Tapeworm infection not only causes disease in pigs worldwide, but also is a major cause of epilepsy in humans in large parts of the world as a result of eating infected pork. There is a single licenced vaccine for use in pigs to control this parasite, which is highly effective in preventing pig (and thus human) disease however, it requires multiple vaccinations which is logistically difficult in developing countries. This project aims to develop and test a new vaccine, for which a single dose will be required to protect pigs, and therefore provides a massive improvement to the current licenced vaccine.	
What are the potential benefits likely to derive from this project (how science could be advanced or	If successful, a single dose vaccine which can protect pigs from Taenia Solium infection, would be able to reduce the risk to humans from eating infected pork,	

humans or animals could benefit from the project)?	and therefore reduce the cases of human tapeworm infection. Currently, infection of humans with this tapeworm causes roughly 30% of all epilepsy cases in developing countries, with no effective treatments available in these areas. A successful single dose vaccine would drastically reduce the prevalence of human infection in these countries.
What species and approximate numbers of animals do you expect to use over what period of time?	This study will be conducted in pigs, and it is estimated we will use 18 animals, over a period of about 10 weeks.
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?	Pigs will be vaccinated intramuscularly and blood samples taken at intervals. There are no adverse effects expected over and above short lived mild discomfort associated with vaccination and blood sampling. All animals will be euthanized at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	An intact immune system is required in order to assess the potency of this vaccine. This cannot be predicted using non protected animal models or using computer modelling.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical advice will be sought in order to ensure the minimum number of pigs will be used to achieve the objective in this PPL.
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.	During the pig studies, all animals will be housed in open barns, with fresh straw bedding, with continuous access to food and clean water in groups , the size of which will be determined by the study design.

Project	**89. Development of gene and cell therapy for neurodegenerative conditions**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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 aim of the project is to develop new treatments for blindness and for childhood dementia, conditions for which there are no treatments available yet.	
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)?	At the end of the project there should be clinical studies ongoing that test the newly developed therapies in patients. In the long run, these should become established treatments that improve the quality-of-life of patients with inherited blindness, and prolong the lifespan and improve the quality-of-life of patients with childhood dementia.	

What species and approximate numbers of animals do you expect to use over what period of time?	Most studies will be using mice, either normal animals, or animals that have an inherited disease. Occasionally, rats or rabbits may be used as the larger eye will allow a more precise surgery. Before patients can be treated, new therapies need to be tested in a second animal; to this end, we will also use rabbits. We are currently investigating new treatment strategies for approximately 12 different eye and brain diseases, a program of work for which we use about 6000 rodents and 30 rabbits each 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?	A variety of natural and artificial mouse strains with inherited blindness exist. Because rodents are nocturnal animals who mostly use other senses (smell, touch), the blindness has little impact on the animals' welfare. The animals with forms of childhood dementia can develop problems such as paralysis and shaking. We ensure that animals are not kept alive when these problems start to be visible to avoid the animals suffering. The new treatment will be administered into the brain or in one eye of the animal, while it is under anaesthetic. When the treatment has taken effect, we can determine how well the brain or eye in the animal is functioning. These functional tests do not require any surgery and are designed to have minimal impact on the wellbeing of the animal. At various time points after treatment, animals will be humanely killed to determine with greater precision the progression of the disease and the effects of that the treatment had.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although the new treatments are tested in cultured cells prior to use in animals, the treatment effect can only be proven in animals, as the diseases we aim to treat are complex disorders, involving communication between multiple cell types, as well as the blood supply and the immune system. Current knowledge and technology are insufficient to model all these interactions, either in a dish or using computers, well enough to reliably predict treatment outcomes.

2. Reduction Explain how you will assure the use of minimum numbers of animals	In the REDACTED we have developed methods that enable us to grow human retinas (light sensitive structures from the eye) in dishes. Using them, we can test some of the effects of the new therapies on the light sensitive cells without using animals. We always try to improve our testing methods. In the past years we have developed a new way to measure inflammation in the eye that means we can follow 1 group of animals for a longer period, thus reducing the number of animals tested. As the animals do not suffer more than mild discomfort at this stage in the protocol, the longer follow-up of a single group does not cause undue stress for the animals.
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 treatment studies for inherited diseases require an animal model of that disease, most studies will be performed in transgenic or mutant mice. We continually review our experimental plans and our procedures with the aim to minimise the impact on the animal wellbeing. For example, a recent refinement is a novel method to induce blood vessel growth in new-born mice. The new method has a shorter period of oxygen exposure for the mothers and therefore less potential for discomfort. As a second example, in the past most vision tests were based on creating a hostile environment for animals that would give them a strong incentive to act in a predictable fashion. These tests have now been removed from our project plans and we arestarting to use a new vision test, developed REDACTED, based on the flight/freeze response in mice. This is a natural behaviour for the animals that has a far lower impact on their wellbeing. It development is driven by the 3Rs principles. Finally, we continue to develop better production techniques for our new therapeutics, to improve their purity and further decrease the (already low) risk of inflammation. Where appropriate, the refined methods are published in scientific journals to encourage their use by other groups.

Project	**90. Development of germ cells in the mammalian ovary and testis.**	
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)	Optimal development of germ cells, sperm and eggs, is vital for successful fertilisation, embryo development and for the health of any resulting offspring. In turn, this can have effects on the generations to come. As such, we need to know how exposure to drugs might affect the production and viability of sperm or eggs. Work here aims to determine how such drugs might affect germ cells and whether it is possible to protect the germ cells from such damage. Much of the work uses tissue culture techniques, with part of the research devoted on the continual	
	improvement of such methods.	

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)?	Information about how drugs might affect the fertility of patients or the children of patients is of benefit both to the patients themselves and also for their health care professionals
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, around 3,500 over the duration 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?	Most of the proposed work will have only mild adverse effects, for example breeding of genetically modified mice with some specific cell types labelled. Often, animals will have no invasive procedure carried out while they are alive, the procedure required only to obtain tissue. The maximum severity is classified as moderate. Moderate severity procedures will be carried out only in a small number of animals, either where we carry out surgery with analgesia or where low doses of substances such as chemotherapy drugs or arsenic are injected into animals. At the end, animals will be killed, and they or their offspring observed/examined or their tissue cultured, or transplanted tissue collected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We replace the use of animals in experiments wherever possible, and routinely develop and use tissue culture methods to replace such experiments. In some cases, we can eliminate the need for animals completely by using established cell lines. However, most of our experiments require us to obtain gonadal tissue from animals, since the various cell types in both ovaries and testes interact closely with each other, often dependent on each other, requiring the use of tissues. Some experiments are needed to check that results obtained from culture experiments do reflect the in vivo situation

2. Reduction Explain how you will assure the use of minimum numbers of animals	We routinely and regularly check that we are using the appropriate number of animals and tissues for our experiments, thus avoiding unnecessarily large numbers being used while also avoiding carrying out experiments with small numbers that would not allow us to draw statistically significant results. We do this both through use of power analyses and also drawing on past results
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 as our models. The reproductive systems of mice develop in a similar way to those of humans, and working on mice also opens the possibility of using genetically altered animals, together making them an ideal model. Where we do examine the reproductive systems of large mammals, licenced work is needed only where we transplant such tissue into mice. Most of our work involves collection of tissues for culture. Most transgenic animals used in our research have little if any discernible feature affected by the transgene. On the occasions that surgery is required, we work quickly and watch the animals closely to minimise any pain or suffering, killing the animals humanely if this is judged to be too high

Project	**91. Development of neuromodulatory interventions for treating of 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)	**Overall aim:** The overall aim of the project is to develop non-surgical tools to adjust brain activity and also to use them to discover ways of treating diseases which affect the brain, such as Alzheimer's disease and Parkinson diseases ('neurodegenerative diseases') without the use of drugs. **Specific objectives:** 1. To develop tools to adjust brain activity without surgery. 2. To test the role of brain activity in the development and progression of neurodegenerative diseases. 3. To develop non-surgical ways of slowing/reversing the	

	development and progression of neurodegenerative diseases.
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 global number of people living with dementia, mainly Alzheimer's disease, will increase from 50 million in 2018 to 152 million in 2050, this is an increase of 204 % (https://www.dementiastatistics.org/statistics/global-prevalence/). There are currently no known cures for dementia. Parkinson's disease is the second most common age-related neurodegenerative disorder after Alzheimer's disease. An estimated seven to 10 million people worldwide have Parkinson's disease (https://parkinsonsnewstoday.com/parkinsons-disease-statistics/). The cost to carers, healthcare systems and social services in terms of dementia care is enormous. Most importantly, neurodegenerative conditions are incredibly distressing and traumatic for sufferers. This project develops tools that will enable new ways for scientists to investigate the healthy and diseased brain and in the long term it will support the development of new treatments with reduced risk to patients. This project generates knowledge that will help our understanding of the role of brain activity in the development and progression of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, and uses the knowledge to develop treatment methods.
What species and approximate numbers of animals do you expect to use over what period of time?	We will used healthy animals and animals that model certain features of neurodegenerative diseases, such as Alzheimer's disease. The absolute maximum number of animals we will use is 4000 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 study will use wild-type mice (as found in nature) including old mice, and also use mice that have been genetically altered to produce a model of Parkinson's disease or Alzheimer disease which will be kept into old age. The animals will undergo surgery of moderate severity during which miniature probes to adjust and record the brain may be implanted. This means that the mice will be quiet and move less for a day or two after surgery. The animals will be given multiple types of pain relief after surgery. Animals might lose a bit of weight but will typically regain that weight within two to three days. In the event of infection or at the end of the procedures, the animals will be humanely killed in consultation with the veterinary staff. After full recovery from the surgery, the animal may undergo sessions in which the activity in the brain will be adjusted using magnetic/electric/ultrasound fields and measured/imaged

	using the miniature probes implanted during surgery. In addition, the animals may undergo behavioural tests to assess learning and memory and this may involve food restriction to motivate them. Throughout the adjustment/recording periods, the mice will be closely monitored for abnormal behaviour or changes to things such as heart-rate, temperature or breathing rate, and if the animals appear to be unwell, the experiment will be ended, and the advice of veterinary staff will be sought. In the event of infection or at the end of the procedures, the animals will be humanely killed. Substances may be given to animals in order to find out more about the diseases and will then be humanely killed so that brain tissues can be analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will use computer models and experiments in the laboratory to gain as much information as we can, without using animals. It is essential to test our findings for safety and to find out how well our theories work in animals, because we do not have detailed knowledge of how the brain works yet. In addition, any new tools we invent that can be used to investigate the brain activity have to be tested in animals before they can be used in humans. Alzheimer's disease involves numerous complicated physiological processes that we do not understand and cannot replicate in the laboratory. It is therefore necessary to perform the investigation in mice whose brains in many ways resemble the human brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals we will repeat tests in the same animal and collect data from multiple regions of the brain. In addition, we will use advanced computer methods to maximize the information we gain from each experiment and to refine the experimental plan. Finally, we will perform all experiments in special surgical procedure rooms, using sterile techniques, which will increase the quality of our data and minimise discomfort to the animals. We will also consult statisticians as necessary to ensure that we only use the minimum number of animals needed to obtain meaningful results. We will re-use our data for multiple studies wherever feasible.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are among the simplest mammals that can be used to study brain functions. Mice are the species of choice in biomedical research because it is easy to alter their DNA to create 'models' which cause or make them more susceptible to various disorders found humans such as Alzheimer's and Parkinson's diseases. To minimise suffering, after the initial surgery, the animal will be given pain relief and allowed to fully recover from the surgery before any further procedures are carried out. The most common adverse effect from the surgery would be infection, which is expected to occur in a very few animals (<1%). The animals will be closely monitored throughout and treated humanely in consultation with veterinary staff in the event of pain or infection. Otherwise, the animals may be quiet and less active for a day or two following surgery, after which they return to normal behaviour.

Project	**92. Development of new combinatorial cancer immunotherapies**	
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 this study is to develop new combination therapies to treat cancer. Cancer vaccine can be used to treat existing cancer. Over the last two decades, cancer vaccines have been tested in patients with very limited clinical success. This may due to the fact that these vaccines were used as a stand-alone therapy in patients with advanced cancers, so an anti-tumour immune response could not be triggered. It is important to note that cancer vaccines work by stimulating effective immune responses, like the cytotoxic CD8 T cells in the body to fight cancer cells. The effective immune responses could be improved by	

	combining cancer vaccines with other immunotherapies. Also, most cancer vaccines tested so far were based on vaccine platforms that cannot induce cytotoxic CD8 T cell responses, which are cells that actually kill tumours. This would be improved by selecting a vaccine platform that is better suited to the induction of CD8 T cells. One of our approaches is to use weakened viruses (i.e. vectors) whereby a protein from the tumour of interest is expressed from the virus to stimulate the cells to recognise and attack the tumour. Our collaborators have now shown that these types of vaccines are superior to other vaccine platforms in that they induce very strong cytotoxic CD8 T cell responses in both animals and humans. We will extend this approach with the aim to develop combinatorial cancer immunotherapies that would work better in killing tumour cells. These therapies will first be tested in mice, the results will provide better support for clinical development of new cancer therapies.
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 benefit of this project is to validate novel cancer therapy approaches and bring them for early clinical development. In the process of developing new cancer therapies, this work will expand our understanding of the immune responses generated by vaccination and immunotherapy treatment. Once we have a better understanding on which immune responses are required for protection against cancer, we can translate what we learnt from animal study to human. The different therapeutic approaches we developed here should be broadly applicable to the design and development of treatment against a variety of cancer. We have good links with clinicians and private companies to enable us to rapidly translate the findings to clinical trials.
What species and approximate numbers of animals do you expect to use over what period of time?	All our work uses mice as they are the best immunologically studied animal species and have proved to be excellent indicators of immunogenicity in terms of identifying regimes with improved immunogenicity. We estimate to use a maximum of 14000 animals over 5 years for this plan of work. The majority of these animals will be used for efficacy studies.
In the context of what you	The proposed experiments employ well-

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?	characterized experimental techniques and should not cause more than moderate degree of suffering to the animals. The expected adverse effects will be mainly attributed to withdrawal of blood, inoculation of tumour material and development of tumours. The mice can experience some discomfort due to subcutaneous tumours. In typical experiments, mice will be injected with the tumour cells, then administered with cancer vaccines and drugs to treat the existing tumours. In the unlikely event that the severity of any procedure threatens to exceed the “moderate” level, the animals at risk will be humanely killed. At the end of the experiments or when animal displaying overt signs of disease, they will be humanely killed immediately by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no adequate alternative other than using animals to study the immunogenicity and protective efficacy of human vaccines and immunotherapies. The immune response to vaccination and therapies involves complex interaction of body systems which cannot be replicated in tissue culture. However, we use in vitro tissue culture techniques involving tissue taken from our animals after death, to perform experiments by manipulating the cells without having to use a live animal. Cancer development and tumour response to treatment are also complex processes involving interactions that take place between a tumour and the host. . Lower animals such as fish have a less well known and well developed immune system making them not suitable to use for testing cancer therapies targeting the immune system. Therefore, we need to use animals such as mice in our research for studying cancer and therapies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where necessary, before undertaking an experiment we will perform a statistical analysis to ensure that the correct numbers of animals are used to enable robust conclusions to be drawn. Also, strains that are not used for experiments will be cryopreserved to reduce the number of breeding animals. Moreover, we will continue to explore new methods that allow more information to be obtained per experiment and thus reduce the number of animals studied, e.g. imaging of tumours

	over time. Finally, we will be testing as many conditions per experiment as possible, but without compromising scientific validity of the data, in order to use just one control 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.	Mice are the lowest species of mammal which can be used to study cancer and immunotherapy. Mice are the only mammal in which transgenic technology in altering the adaptive immune system works reliably, and their genome, biology and physiology has been widely studied and documented. There are large number of established tumour models in mice that closely mirror tumour development in humans. When novel procedures or treatments are undertaken, pilot studies with increased monitoring will be performed initially, and the outcomes of these will be used to amend future work. We typically use low severity tumour models involving the injection of tumour cells under the skin to induce cancer. If we do not know the model well, small scale pilot experiments will be performed to establish the optimal dose of tumour cells required to achieve the development of palpable tumours within a reasonable time period.. These models do not cause metastasis and clinical signs of tumour burden. However, sometimes we need to use higher severity or more specific tumour models to validate the efficacy of the cancer therapies, including metastasis tumour models and inducible tumour models. Aseptic techniques will be used throughout the experiments. We plan to trial more refined methods e.g. the use of mini pump to replace multiple injections. Mice with tumours will be monitored regularly and as disease progresses, the frequency of monitoring will increase to ensure undue suffering does not occur. When necessary, imaging will be used to monitor internal tumour growth. We will terminate the experiment as soon as a valid scientific outcome is reached.

Project	**93. Development of novel agents for cancer therapy**	
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)	In the process of developing new drugs for cancer treatment we need to test novel compounds in animal models of cancer to ensure they are safe and effective before being trialled in patients. It is very important that before we test a drug in an animal model of cancer we do studies in mice to find the most appropriate dose of drug to test. These studies are often in normal mice and often involve just one injection of drug before the mouse is culled. In these studies we do not expect to see any adverse effects of the drug. After the animal is culled we will collect blood and tissues to measure levels of particular proteins or other molecules that can tell if the drug has had it its desired effect; this is	

	called measurement of “biomarkers”. Testing the levels of biomarkers and levels of the drug itself are crucial ways in which we check that a drug administered to an animal reaches the tissue in which we want it to have an effect and reaches high enough concentrations in that tissue to be effective. These types of studies are called dose-setting experiments as they enable us to select the appropriate dose and frequency of dosing to use in mouse models of cancer. This aim of this project licence is to allow dose setting studies to be performed. It is important to note that drugs tested in animals will already have been through a series of in vitro experiments in cells to show that the drug is potent and selective, but only by testing in mice will we establish whether it is suitable for dosing in animals and ultimately in patients.
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)?	These studies should form the basis of future testing for compounds in clinical cancer studies in patients. Biomarker approaches validated in these experiments will be translated into clinical measurements that can be used in patient studies. This “translational biomarker” approach is critical to ensure that novel compounds are effectively tested in the clinic and their effects understood.
What species and approximate numbers of animals do you expect to use over what period of time?	Only mice will be used for these studies. Over a period of 5 years, we anticipate using a maximum of 1200 animals.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Studies performed under this license will not exceed moderate levels of severity. Some experiments using novel compounds may involve multiple dosing which may occasionally lead to unexpected adverse clinical signs being observed. However, experiments will be designed to ensure that compound exposure is given in a stepwise manner to minimise the impact of unexpected toxicity. All animals will be humanely sacrificed by a Schedule 1 method at the end of procedures.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In this programme of work testing in human cells will be used to extensively evaluate compounds in terms of efficacy, toxicity and likely bioavailability (i.e. exposure in animals). However, it is only in whole animals that the complexity and interplay between biological systems can demonstrate if a compound will be absorbed after dosing and have an effect on processes of cancer that are expected to translate into effectiveness in patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies with compounds will be performed using a robust and reproducible design including randomisation, suitable vehicle controls, consistent dosing, sampling and analysis methodology. This approach gives the best opportunity to generate clear decision-making data on compound effects and efficient progression into mouse models of cancer with minimal use of animals.
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.	This licence uses wildtype and immunocompromised mice that are no greater than moderate severity. It is essential that we use immunocompromised mice in order for non-mouse cell lines to grow tumours successfully without rejection by the host. Wild-type mice (which have a normal immune system) may also be used in studies where the immune response is thought to play a key role. To minimise suffering, all mice on procedure will be constantly monitored and humanely sacrificed when exhibiting signs of altered health status and/or tumour burden. All users will be fully trained in monitoring tumour development for each model and will be signed as competent prior to initiating their own *in vivo* studies. Our animal unit is proactive in environmental enrichment, provides fun tunnels and nesting materials in cages, and employs non-aversive handling methods to minimise animal stress.

Project	**94. Development of Novel Analgesics**	
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 main aim of this licence is the identification of new drugs for the treatment of inflammatory pain (in which the pain is caused by tissue damage) and neuropathic pain (where the pain is due to damage directly to nerves). Inflammatory pain includes such diseases as back pain. Examples of neuropathic pain include the pain felt after shingles. Both these types of pain can be severely debilitating and have effects on people's day to day lives. These conditions are reported to be one of the most prominent causes of disability worldwide and have been estimated to affect up to 28 million people in the UK alone .The figures for	

	those people in the UK suffering from what is reported to be moderately or severely limiting pain indicate that approximately 7.9 million people will fall into this category. Pain at this level will cause distress to people when carrying out normal day to day activities such as walking, standing/sitting, washing etc. Across the world, the economic burden of chronic pain has started to be recognised by national governments including the UK, US and Australia through the production of national strategies to deal with the issue. The annual cost of back pain to the UK British exchequer is estimated to be in the region of £5 billion per annum. Prevalence rises with age with up to 62% of those over 75 reported to suffer from a painful condition. The treatment of both inflammatory pain and neuropathic pain is currently poor and is considered a major area of unmet need. Despite drugs being available for the treatment of these conditions, the majority of patients do not achieve effective pain management. Existing therapies have major side effects (sedation, nausea, vomiting) which can limit their effectiveness and also discourages patient compliance. Clinicians generally agree that even small improvements in safety and/or tolerability of therapeutics for pain relief would be a good thing. Any new therapies would be expected to have better profiles than existing treatments.
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)?	New treatments for inflammatory and neuropathic pain and an increased understanding of the mechanisms responsible for these conditions
What species and approximate numbers of animals do you expect to use over what period of time?	5000 mice and 400 rats over the five year lifetime of the 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?	Of the animals used under this licence approximately 50% would be expected to experience only mild severity i.e. short term mild pain such as having an injection. The other 50% would be expected to experience moderate severity due to surgery. A moderate procedure involves an animal suffering short-term

	moderate pain, or longer-term mild pain. The experiments we plan to perform mostly involve producing an insult which we will reverse using new compounds. In order to achieve this, in our tests of inflammatory pain, we will administer substances either into the footpad or directly into the knee joint which act to cause an inflammatory response similar to that seen in diseases such as osteoarthritis. We will then measure how much weight can be put on each rear leg through measurement in a weight bearing machine. Once we have seen a stable change in the injected paw or leg we will administer new compounds to reverse this change back to normal. Alternatively we may cause damage to the sciatic nerve to mimic neuropathic pain and try to reverse the effects produced with new compounds. Here we will squeeze the rear paws to measure how much pressure we can exert before the animal withdraws its foot. Once again, when we have seen a stable change we will try to reverse this change by administering new compounds. If we see positive effects then these compounds may go on to be tested in people with inflammatory or neuropathic pain. At the end of the studies we may take blood samples or spinal fluid to allow us to look for the amount of drug present in them or to examine any changes in other substances such as enzymes or other proteins. This will help us make decisions on how to proceed in further tests. We have expertise in using animals in these experiments on this licence and the incidence of adverse events has been reduced to an absolute minimum such that now they are rarely, if ever, seen. However, should any unexpected effects such as lameness or the inability to put weight on the paw be seen we will humanely euthanize the animal to minimise unnecessary suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Preliminary studies will be carried out in a range of in-vitro cell assays. Any substance which is selected for testing will have been examined in a number of these in-vitro tests to ensure that it has the required selectivity at the target site and has the desired affinity for the target.

	Furthermore, for the majority of substances there will be a very high degree of confidence that the pharmacokinetic profile will be suitable for investigation. Where available, comparisons will be made with data obtained from other substances in the same class to determine which better satisfies the criteria for development. However, pain is a highly complex process requiring an input from many parts of the nervous system and so, for this reason, in-vitro testing alone is not enough to determine if new medicines will be effective analgesics. The use of animals is the only way to determine if new medicines produce an analgesic effect.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All studies are examined by a qualified statistician who approves the study design. These designs are regularly reviewed to ensure best practice. This will ensure that minimal animal numbers are used and the correct analysis is carried out to give the best possible interpretation of the results. Statistical analysis will be used to show significance of the results and we will use statistical experts in order to ensure the correct analysis is used. Statisticians will also be consulted, where appropriate, regarding the design of studies, and the most powerful type of analysis that should be employed to analyse results. Current experience indicates that group sizes of 7-10 in rats will be used. The numbers of animals used in studies in which mice are employed are generally slightly higher, due to the larger variability found in this species, and will therefore usually be between 10 and 15. In order to control bias and variation mice animals are sourced from on supplier. Only one sex of animals is used enabling us to control, as far as possible, between experiment variability. Drug treatments are randomised based on pre-dose readings by dividing animals into relevant groups with approximately equal scores. Measurement of treatment effects are carried out with the operator blind to treatments in order to minimise bias.
3. Refinement Explain the choice of species and	Choice of species All the studies carried out under this licence will

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.	be in rats and mice, predominantly the latter. There is a wide literature supporting their use in models of chronic pain, such as those outlined here, which will be used to provide a background to the studies prior to instigating any work. Whilst most studies will be carried out in mice, having the ability to also test in rats will allow us to provide information e.g. efficacious doses which can be used in future studies. Such studies will be required following the selection of candidate drugs and are carried out in rats. We may also use genetically modified mice in all procedures e.g. receptor knockout mice. Animals such as these will enable us to develop a better understanding of pain pathways and also provide information as to the selectivity of test substances. Choice of experiments The models we have chosen to use in this licence include those which we believe are the minimally invasive to the animal yet will provide us with most information. We have consulted with experts . in order to pick the most appropriate models where possible and intend to use those which model important features of the disease states. They have been extensively studied in the literature and have been validated using compounds which are in clinical use for the treatment of inflammatory and neuropathic pain. Minimising suffering All of the models in this licence are designed to mimic some of the symptoms observed in the human conditions and so some level of pain is inevitable. However, experience gained by the project licence holder has shown that the level of pain (indicated by an increased pain response experienced by the animals is not such as to cause any major changes in the welfare of the animals. For example food and water intake are normal, animals show normal growth curves and the general observed health and behaviour of the animals is unaffected by the treatments. Tests have revealed that the level of locomotor activity seen in treated animals is also no different from that observed in untreated animals. The insults used to induce any

	inflammation/ hyperalgesia have been assessed to be the minimum which can be carried out to cause a significant reproducible biological effect without causing excess adverse events. In all new studies undertaken the level of insult will be assessed prior to conducting further studies.

Project	**95. Development of Novel Deubiquitinylating (DUB) Enzyme Inhibitors for Fibrotic, Mitochondrial and Neurodegenerative Diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	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)		
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals	This work will support the generation of novel DUB inhibitor drugs that can uniquely target specific rare diseases or diseases that are difficult to treat. Due to the specificity that we are able to achieve, these new drugs will offer fewer side-effects than	

could benefit from the project)?	conventional treatments creating better quality-of-life for patients. Our new treatments may also be given in combination with existing treatments or novel treatments. In the process of developing the novel therapeutic approaches we will greatly advance DUB scientific knowledge, and are already active contributors to this field, publishing in high impact scientific journals and attending and contributing to scientific conferences.
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use mice or rats bred for laboratory research as part of this project licence. In 5 years, we expect to use approximately 3,000 mice and 440 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?	Based on experience and veterinary advice, animals might experience mild pain from injections or during blood sampling, but this will be short-lived. Some animals will be used in surgical procedures, but these will be conducted under anaesthesia and pain relief will be provided. On occasion some animals will be housed individually for up to 24 hours to allow collection of urine. Single housing will impact typical animal social experiences, however animals will be returned to group environment after the stated period of time. The majority of animals are expected to make a full recovery. If animals show signs that depart from normal behaviour or feeding/drinking patterns then they will be assessed and, if necessary, killed humanely by schedule 1 method. Additionally, there may be occasions where animals are fasted for short periods of time, or are fed altered diets, however these procedures are anticipated to be mild in severity of impact to the animal. Throughout this project every effort will be made to ensure that moderate severity limit will not be exceeded for animals under this project licence. It is possible that some animals will experience unexpected side-effects, if these cannot be treated or do not improve, then we will ensure the animal doesn't suffer and is killed humanely by schedule 1 method. To minimise the number of animals affected, we will use very small numbers in experiments where a new chemical entity is being administered for the first time to an animal. Under these situations animals will be administered small doses to start with, which will then be slowly increased to manage the risk of unexpected side-effects. Each administration will be followed by

	close and regular assessment and observation to ensure that if unexpected side-effects are observe, the experimenter is able to respond quickly. Animals will be given pain relief where appropriate and will not be allowed to suffer lasting pain or harm. At the end of every experiment, animals will be killed by an approved humane method. Unique genetically-engineered mice may be provided to other researchers to prevent duplicating the same mice.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro and ex vivo assays will allow us to address important early-stage criteria such as enzyme potency, selectivity, off-target effects and to make predictions about the compound's metabolic stability before it is dosed in vivo. While the predictive capacity of these in vitro approaches is continually improving they cannot currently replace animal studies. This is because new drugs need to be tested in complex, biological systems with integrated functional pathways and this requires the use of animals. Sole reliance on in vitro biochemical and cell based assays are not suitable to determine and identify key properties required of therapeutic agent in a complex biological system. Pharmacokinetics and biodistribution are examples of crucial endpoints that require integrated responses of the body to a drug that include components of absorption, distribution, metabolism and excretion. In vitro systems are getting better at predicting absorption and metabolism with Caco-2 cell flux and primary hepatocyte metabolic profiling, however they are not perfect. Moreover, distribution and excretion of a drug or its metabolites is impossible to accurately predict with in vitro methodologies. Indeed, it is recognised that co-ordinated use of both in vitro and in vivo allow robust translatable predictions to man that would not be achievable by in vitro or in vivo studies alone In our specific case additional complications arise from the mechanism of action of our compounds as they are designed to act as reversable covalent inhibitors of DUB enzymes. This means that the compounds have the potential to bind to the target enzyme for longer than might be expected. The potential time disconnect between test compound

	concentration and initial effect cannot be replicated to sufficient detail in cellular or sub-cellular experiments without testing this in experiments involving the integrated system of a living animal. Moreover, potential covalent interactions with unidentified off-target proteins could result in physiological impact that would not be predicted from in vitro assessments. Additional complexity is created by the biology of the disease state. At least one of our targets is only expressed on the outer mitochondrial membrane and is involved in the processing of mitochondria over time. This flux of degradation and removal of damaged mitochondria and proteins and de novo synthesis of new mitochondria in a cell can be tissue specific, e.g. kidney, heart. While some cell systems allow us to study the general mitochondrial processing these systems have recognised limitations that result from lack of feedback or homeostatic processes from surrounding tissues. In addition, genetically altered mouse strains with expression or specific deletion of DUBs allow the testing of the significance/relevance of our approach to the different disease targets. Laboratory rodents are the lowest suitable species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Measures to keep animal numbers to a minimum: Strict screening criteria (see Figure 3) with clear stop/go decisions to ensure only our best compounds progress to in vivo studies. Adapting the study type - studies employing serial micro-sampling, reduced time-course, or cassette dosing will reduce animal numbers; consequently, full time-course studies will be performed downstream where fewer compounds are tested, reducing animal numbers further. Avoiding duplication - if data is available elsewhere, some experiments may not be necessary; however, some may be replicated for validation purposes. Whenever possible, genetically modified animals will be obtained from researchers/suppliers to avoid re-creating these lines and to utilise their knowledge.

	Minimal numbers approach in the severe limit protocol with stepped dosing. Experimental design principles - pilot studies and statistics: Each of the protocols described herein are established and standardised throughout the drug discovery field with well accepted animal numbers required for robust translational predictions. Pilot studies will be employed when working with compounds that have not undergone in vivo testing before. These will be in minimal numbers of animals using dose-escalation procedures to ensure animals are never unintentionally administered non-tolerated doses of a given drug. Sources of variation and bias will be reduced whenever possible through after-action reviews of studies to ensure future learnings. Moreover, advice will be sought from Establishment Licence Named Individuals, e.g. NVS and NACWO, so we are able to follow current best practice. Factorial experimental design and blocking will be applied whenever possible and animals randomised to treatment groups. Appropriate statistical methods will include independent t-tests and ANOVA on sequential and end-point data sets, regression analyses for time-courses and Log Rank tests for assessment of survival time (using surrogate endpoints). Frequently used models will be monitored for robustness and integrity (e.g. using Manhattan trend plots). Every 6-12 months statistical power analysis of conducted experiments will be conducted to ensure that we are able to identify meaningful effects at 5% significance level with a power of 80%, and to adapt experimental design if our studies are over, or under powered. Separate power calculations may be necessary when considering experiments with male and female animals or both sexes together, and this will be taken into account. Sources of advice:

	'The Design of Animal Experiments: Reducing the Use of Animals in Research through Better Experimental Design' (Festing et al., Laboratory Animal Handbooks No. 14, Royal Society of Medicine Press: London). Biostatistical support will be obtained through consultancy agreement and statistical advice will be sought, and study design discussed before experiments start.
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 are extremely well characterised biological model systems, with a wealth of information supporting their use to develop therapeutic agents that are very similar to the one that we are developing, small molecule enzyme inhibitors. While mice and rats may not completely reflect all the aspects of disease in humans, when experiments are designed in the correct manner, they are able to produce high value predictive information that is critical for clinical development. We will look at all times to supplement data from animal experiments with data produced in cell-based system to refine our predictions of clinical outcome. A compelling example of this is in the drug metabolism space where metabolism of our potential new drugs by human and mouse isolated liver cells, coupled with an analysis of circulating drug levels in a mouse will allow us to accurately predict what human circulating drug levels will be upon administration of a given dose. For surgical procedures and recovery we will ensure that recognised best practice will be followed at all times, including aspetic technique. We will ensure that appropriate housing, access to food and analgesia is given during recovery to minimise any unnecessary suffering. Lowering the risk of administering an unsafe drug or unsafe levels of a drug to patients is on of the main reasons for testing drugs in animal models. We will strive to focus on animal welfare in these studies and will make refinements wherever possible, including the latest innovations in environmental enrichment, refinements in dosing techniques and applying best practice for surgical techniques, pain relief and anaesthesia. All staff involved in the conduct of these studies will be highly trained and experienced in the technical aspects of the procedures undertaken. Moreover, we will

	continuously learn from our experiences in mice and rats to tailor and refine our experiments to maximise the value that is derived from the smallest number of animals used. Consideration of the 3Rs is an integral part of planning experiments and these will be implemented throughout our project.

Project	**96. Development of personalised anti-cancer strategies**	
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 main objective of this project is to use mouse models of cancer to identify new drugs or combinations of drugs to treat cancer patients. Research will also be carried out to better understand how cancer spreads in a well characterised mouse model, so that new drugs can be developed to stop the spread of cancer. Finally, research will be carried out on early disease generation, to see if we can find cells or molecules in the blood stream which allow doctors to detect the cancer sooner so the patient can be treated quicker. This will be done in both test tubes and in mouse models.	

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 research carried out in the project is likely to tell us whether new drugs will work in certain cancer patients. The outcome of this research would then allow doctors to have more confidence to test these drugs in patient clinical trials, with the ultimate aim of finding drugs that can be approved and used to treat patients on a regular basis as an approved drug treatment for cancer. The research should allow us to better understand why some patients cancers respond to a drug whilst others do not. From this, further research could then identify new drugs that might work in those patients who do not respond to current drugs. The research should also allow us to better understand how some cancer types spread throughout the body to other organs. Further research could then identify new treatments for these patients with the ultimate aim of improving patient care.
What species and approximate numbers of animals do you expect to use over what period of time?	12,700 adult 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?	Continuous improvement in husbandry and experimental procedures minimize actual or potential pain, suffering, distress or lasting harm. Some mice may be given a drug to test its tolerability, whilst others may have cancer cells implanted under the skin or directly into organs. Some mice will have cancer cells injected into the blood stream, brain or the heart chambers. Mice will be monitored regularly to asses cancer growth using either callipers (for superficial tumours) or the latest imaging methods for internal tumours. Imaging will be undertaken under a general anaesthesia from which the mice will be allowed to recover. Some of the mice will be given novel drugs, some irradiation treatment and some will receive conventional anti-cancer treatment. On occasions it may also be necessary to look at combinations of these three treatment options. The expected adverse effects of procedures used in this research are mainly weight loss, a change in normal behaviour and a loss of condition of their fur. The mice will be closely monitored by trained and competent scientists to make sure they do

	not show symptoms of suffering such as weight loss of >20%, or loss of well-being or appearance. In the unexpected event that they do suffer more than this they will be humanely killed by trained staff. At the end of the experiment, all mice will be humanely killed, and in some circumstances selected tissues and body fluids taken for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cancer is a complicated disease, with cancer cells interacting with lots of other cells in the body, e.g. blood vessels; cells of the immune system. Drugs also interact with other cells in the body – the best example of this is a cancer patient's hair falling out when they are given a drug. Therefore to get a true reflection about how a cancer and the patient is going to respond to a drug it is important to carry out the research in an animal and not in a test tube. By doing this research in mice, it gives the best chance that the finding will be relevant when the drug is used in patients. Before we do this, however, we are able to screen the best drugs by doing experiments in a test tube, so we know which ones might have a better chance of working and therefore not using mice unnecessarily.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before a large experiment is carried out a small study (pilot experiment) will be done, to make sure that the cancer cells used in the mouse model are growing properly and to estimate the least number of mice that need to be used for the full, scientifically and statistically justified, experiment to get reliable information. Using new technologies and techniques will permit the minimum number of mice to be used. For example, new techniques in the laboratory allow us to digest a tumour using enzymes, leaving us with pure tumour cells. We can then inject these cells instead of using surgery to implant a tumour piece. Injecting cells instead of pieces means that we use fewer mice for each experiment.
3. Refinement	Mice are used in our research as they are more

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.	closely related to humans than other species such as flies or zebrafish, which don't have lungs. The conditions under which experimental mice are kept are designed for the least possible disruption of natural behaviour and the highest possible quality of life. Continuous improvement in husbandry and experimental procedures minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of mice is unavoidable. For example, if a tumour is surgically removed, under recovery anaesthesia, from a mouse, the wound is stitched closed instead of using surgical clips. This causes less pain and/or irritation to the mouse and ensures the wound heals quickly. In addition analgesia will be used whenever a surgical intervention is utilised.

Project	**97. Development of PET radiotracers for molecular imaging**	
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)	The aim of the project is to develop molecular imaging probes to use in a medical scanning procedure that can be diagnostic or help to understand disease. The probes will help select the best treatment for the patient. The work will validate molecular imaging probes that are radiolabelled for positron emission tomography imaging for their use as imaging biomarkers in both animals and man. Biological markers of disease can be imaged in a scanner using molecules with radioactive tags. Once these molecules have been designed the protocols are developed for radiolabelling the molecules and evaluating them in vitro and in vivo for their properties.	

	There are particularly exciting current developments in combining cancer imaging and therapy where the molecular imaging probes that localise at tumours can be modified and administered with a therapeutic isotope (beta or alpha emitting) after imaging with positron or gamma emitting isotope. The probes developed in this work will be a starting point for these new treatments. The objectives of this project: 1. To establish the key properties of imaging probes in rodents such that if successful, they can be used in animals and man. 2. To validate successful imaging probes from objective 1 for use in the assessment of cancer (diagnosis, treatment selection and response) by testing them in suitable rodent models.
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 goal of this project is to develop new ways to measure the properties of disease using imaging probes in medical scanning. The scan can then be used predict how a patient will respond to treatment. This means that the best treatment can be selected and the molecular imaging probe may also be used to monitor if the patient is responding to treatment. If they are not then a rapid change can be made, improving the clinical outcome. Nuclear molecular imaging probes and small animal scanning allow us to study the molecular processes that are the basis of disease with earlier detection and characterisation. This is an improvement on imaging the end results of cellular and biochemical changes. The effectiveness of this approach allows high quality statistically relevant data to be collected on a lower number of animals. This work will be translated to clinical studies and also inform the development of candidate therapeutic compounds. It will obtain validating data for discussion with clinical colleagues and planning of clinical trials.

What species and approximate numbers of animals do you expect to use over what period of time?	Rodents (mice and rats) with 500-1000 animals over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For biodistribution and metabolite studies, animals will (generally) be injected with an imaging probe (under anaesthesia) and either be scanned or sacrificed at a later timepoint. Animals may also be injected with existing drugs or specific blocking agents to see how this affects where the probe binds. For studies in tumour bearing animals, immunocompromised mice will be inoculated with human cancer cells/tumour material to generate tumours for analysis. These will then be imaged, then subject to therapy and imaged again at one or more timepoints to assess the relationship of the image information and any response to the therapy. There are no adverse effects expected for imaging with radioisotopes, and anaesthesia regimes used will be well established with the physiological state of the animal monitored at all times. Where necessary, food will be withdrawn to improve the molecular imaging agent uptake. Possible events such as infection in immunocompromised animals and weight loss resulting from tumour implantation will be avoided by the use of individually vented cages and regular animal monitoring. Only established cancer therapies will be used at previously reported doses to avoid toxicity. Tumour growth will be measured twice weekly and the humane endpoint will be reached if the tumour diameter exceeds the specified limits.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-	Tests using cells will be carried for all new imaging probes before animal work. However, they cannot tell us if a new probe will be able to report on a biological target in a whole organism.

animal alternatives	Animal work is necessary to validate the probe in a whole organism, before translation to “first in human” clinical trials. Replacement is therefore not possible. To see if probes can report on cancer treatment response, animal models must be used as it is not yet possible to model the very complex tumour environment and how this may change in response to treatment in any other way.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers of animals are kept as low as possible to give meaningful results by using statistical techniques and match guidelines on animal research. Imaging probes will be validated in cell and tissue studies to ensure only the most effective imaging probes are used in animal imaging 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.	All protocols will be carried out in accordance with the UKCCCR guidelines on the welfare of animals in cancer research, with specific reference to imaging studies. Mice have been chosen as they allow the growth of a range of tumour models that best reflect the clinical situation in man, and are the species with the lowest ‘neurophysiological sensitivity’ in which such well-characterised models exist. There are many studies comparing data between mice and man that show the data gained from mice is ‘translatable’, i.e. can be used to base clinical trials on. For biodistribution scanning that needs heart uptake data, rats provide a larger organ for imaging and it is possible to correct for the heartbeat to provide clearer images of uptake.

Project	**98. Developmental and de novo generation of haematopoietic stem cells**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Bone marrow transplantations into patients with blood cancer (leukaemia) or blood-defective patients have demonstrated the medical value of blood stem cell (HSC) regenerative therapy. However, the lack of a sufficient number of donor (or patient-specific) HSCs remains the overriding constraint to treatment. This is because very few people donate these rare bone marrow or umbilical cord blood HSC for clinical use. Also, even if more donors could be found, it is important that the donor HSCs are matched to the patient, to avoid the patient's body from rejecting the transplanted donor	

	cells. It is unknown how the body makes HSCs. We do know REDACTED that they are made only during a brief period of time and are made from the cells that line the blood vessels at early stages of embryonic life. If we can determine how these valuable stem cells are made, we should be able to engineer HSCs outside the body. The objectives of this study are to identify the fragments of DNA (genes) that play a role in HSC generation and use these genes to make HSCs from other cells in the adult body (for example, the cells that line the blood vessels could be an abundant source) and very importantly, to make HSCs that are matched to patient. To do this we will use mouse models in which the HSCs are marked by molecules that can be detected under fluorescent light. We will also use mice that have defective HSCs to help us identify and study the genes that are important in the production of HSCs in the mouse embryo. The fluorescent and defective HSCs will be examined under the microscope to observe their movements in the embryo. For this, thick embryo sections in the region of interest (the dorsal aorta) are examined microscopically[19]. This vessel lies too deep within the embryo for the microscope to image it and thus imaging is performed on thick sections in which the cells stay alive during imaging. Putative HSCs will also be sorted from the rest of the cells of the embryo and injected into adult mice, to test whether they are real HSCs that can produce all different cells of the blood system. In some cases, we will inject these cells into young mice to determine the potency of the HSCs. We will look for what genes are turned on in the HSCs in attempts to identify new HSC genes. Once we have identified the key genes for HSC generation in the mouse embryo, we will introduce these genes into mouse and human blood vessel cells so as to induce them to become HSCs. If we are successful, we will optimize this method in order to produce large numbers of patient-specific HSCs.
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)?	Each year around 1,800 people in the UK will need a haematopoietic blood stem cell transplant. Most of these patients will not be transplanted since there are not enough donors of HSCs and these donated HSCs are not matched to the patient. The potential longer-term benefits likely to derive from this project

	are in the ability to produce patient-specific HSCs. This should allow for clinical transplantation of all patients needing a replacement for a defective blood system. Since the cells have come from the patient, they are exactly matched and should not be rejected. The short/medium-term benefits of this project will be on the generation of new knowledge on the isolation of blood stem cells and its precursors and on the precise combination of active DNA fragments and proteins required to make healthy blood stem cells. We will also gain an understanding of these, all molecules often dysregulateddirect the production of in blood cancers. Furthermore, we will generate new mouse models that will help the scientific community studying the blood system.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse models of that allow the study of HSC blood stem cell generation. Mice are easy to breed, have a blood system and blood stem cells very similar to those in humans. Mice can be transplanted with blood stem cells in the same way that human patients are transplanted in the clinic. In addition, mouse strains exist in which they that are defective in blood stem cell production, allowing us to determine what genes are important in blood stem cells. Approximate numbers of mice to be used in the next 5 years are based on our usage in 2017. For breeding and maintenance of mice in 2017, 2500 mice were used (1500 wild-type + 1000 genetically-modified) and 175 mice were used as in transplantation recipientsexperiments. As the number of projects in the lab has increased since 2017, we estimate a 15% increase in numbers of mice needed in breeding and maintenance for the coming 5 year period (3200 mice/year for a total of 16,000). While this may seem like a large number, our experiments use mutant mice that must be kept in a heterozygous state (homozygotes are late embryonic lethal)of which, at best, only half have the right genetic make-up to can be used for our experiments. This significantly increases the number of het x het matingsbreedings we must do to produce embryos for our experiments and requires rather large cohortslarge numbers of females which drop out of the pool upon embryo production. Approximately 2,500 (500/year) out of 16,000 of mice bred will be used for preparation and analysis of haematopoietic stem and progenitor cells. Also we will be performing more

	transplantations (40% increase) examining leukaemic stem cells. The number of transplant recipient mice we will need are estimated to be 300/year (1500 for the 5 year period). Approximately 17,500 mice will be used 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 proposed study involving mice is expected to have only mild to moderate affects. These are related to blood stem cell deficiencies and are found only in early stage mouse embryos. The proposed blood stem cell transplantations in mice are similar to those observed in human medicine. Genetically modified animals will be generated and used for breeding and maintenance of the mouse lines (mild effects). These animals will be used to generate the embryos we need for transplantation experiments. The females will be culled under Schedule 1 methods, to obtain embryos. The mouse colony will be maintained to a minimum number and aged adults will be culled by Schedule 1 methods. For transplantation experiments, the moderate effects caused by irradiation and haematopoietic blood reconstitution (lethargy, weigh loss) will be monitored daily for changes in health status. If monitoring indicates deteriorating health by the clinical scoring system, animals will be monitored twice daily, provided supportive treatment and/or culled and examined for blood failure. Sudden death may occur but it is rare (about 1.4%). Deaths will be reported to the Home Office. Most recipient mice used in transplantation experiments will be culled under Schedule 1 methods at the end of each experiment. Some mice will undergo blood modulatory treatment and then be culled for blood tissue analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our mouse embryo studies are pivotal to understanding how blood stem cells are generated. To date, no research laboratory has been able to generate blood stem cells from cultured embryonic stem (ES) cells or any other cell type. Blood stem cells do not survive in culture – they either change their characteristics, are no longer stem cells, or they die. Hence, we can only study blood stem cells within the context of the whole animal. Only by testing putative stem cells by transplantation into mouse recipients are we able to determine whether

	a stem cell has been generated. We will replace the mouse model when we are finally able to generate transplantable blood stem cells from ES cell cultures or from the gene transfer studies as proposed in our research project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are carefully planned and controlled so that only the minimum numbers of genetically manipulated mice are maintained in the colony. Research staff meet routinely to discuss colony size for each mouse line. When necessary, genotype analysis is by a rapid method to identify genetically modified mice, so as to keep the number of mice in our colony to a minimum. Since we need mostly females for mouse embryo production, the number of males for matings are kept to a minimum. To ensure high breeding efficiencies, females are screened for oestrus cycle before being mated with a male. Mouse lines not in current use are frozen as sperm. For transplantation experiments, we will use statistical methods to allow the maximum acquisition of data, with minimum mouse 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.	Mice are used in these experiments because they are the best and most accessible model for study of blood stem cells. Following World War II, studies on the lethal effects of irradiation have shown that blood stem cells are eliminated upon exposure to high dose gamma irradiation. With the transplantation of donor bone marrow containing blood stem cells, the adult mouse can be rescued and the entire blood system can be restored. This is the gold-standard, and the only, assay for blood stem cell functional identification. Well-characterized strains of inbred and mutant mice and reagents to sort mouse blood stem cells, the short 2-year life span and close similarity to the human blood system contribute to its acceptance as the choice model for almost direct translation to human clinical study. During transplantation experiments to minimize welfare costs to the animals we use a split dose irradiation (3-24 hour interval) to ablate endogenous HSCs, thus reducing stress and adverse effects related to irradiation. Other hematopoietic ablative strategies are well-characterized and used clinically for human bone marrow transplantation therapies and thus minimize harm. To increase comfort and warmth during breeding

	and experimental procedures, mice are given specific bedding materials and other environmental enrichments (chew blocks, tunnels). For animals undergoing blood stem cell transplantation or for animals in leukaemia studies, softened food may be administered to prevent weight loss, and antibiotics are provided in the drinking water to prevent any risk of infection. The research staff are/will be fully trained for all the experimental procedures and will follow recommendations from the animal facility staff for further refinement of techniques.

Project	**99. Developmental and degenerative mechanisms in ciliopathies**	
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 objectives of this project license are: 1. To create and improve treatment to treat patients that carry genetic mutations. We will focus in a condition known, as Bardet-Biedl Syndrome, also known as BBS, were children become blind, have brain defects and obesity. 2. In particular we aim to create new therapies using mice to test the therapies. We will use mice that have been specifically bred with genetic mutations similar to the ones we have in our patients.	

	3. We will rescue the BBS mice models delivering the correct copy of gene that they have mutated or cells than can do the correct function.
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 project is to treat human patients with BBS and other similar disorders. BBS is a genetic condition that affects many organs, causing blindness, inducing obesity and cognitive impairment among many other tissues affected. BBS affect 1 in 100,000 new babies born in the United Kingdom. Children affected by BBS are born with normal vision but they go blind in their teens. So far, there is no treatment available to cure or halt the disease. We aim to find the best possible way to deliver the correct copy of the broken gene and we will use modified viruses to deliver it or replace the broken cells with new ones. Once we can demonstrate our system is working and it is safe in mice we aim to start clinical trials in human patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice for this project. We expect to use 3000 mice, in a period of 5 years. Those include normal mice, known as wild-type mice, as controls and animals with the same mutations as our patients, also known as genetically altered animals, to test the efficacy and safety of our treatments.
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 genetically modified animals that present the same defects as BBS patients. Blindness and obesity is not a condition that are hampering day-to-day living for the mice, as they have already as poor sight anyway. not extremely harmful to the animals, and our procedures are mainly only injections. Our procedures has been well stablished within the scientific community and are only considered of moderate harm for the animals. Therefore, our protocols are only considered of moderate harm to the animals. However, adverse effects could appear. In some cases we will need to anaesthetise the mice and practice eye of brain injections. They could be infections, haemorrhage or unexpected secondary effects of injections and anaesthesia. On top of the injection procedures, there is a lot of information and plenty of data suggesting that regarding the vectors and cells we inject have no side effects and, proving they are very safe. We

	will design the experiments in advanced and we will know all the different tests we will want to check in treated animals after our treatment. After the test, we will kill the animals to further do more analysis and validate our approach to treat the disease. The final expected level of severity will be always moderate. We aim to be vigilant if unexpected severe adverse signs appear to euthanize the animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The human genetic disorders we are working often affect multiple tissues and organs. This means that many different organs and systems of the body are involved in them. On top of that, these organs are the ones with the bigger complexity in our bodies, like the eye and the brain. This makes it impossible with the technology we have right now to use a model different from the animal if we want to study BBS or if we want to test therapies. For that reason, we can only use mice to understand the organ failures successfully. Another aspect we need to think about is that BBS affects the brain and the behaviour of the patients and the animals, and unfortunately, this cannot be reproduced without the use of animals. However, we aim to work in new technologies that are appearing to create structures similar to organs, called organoids. These resemble the eye and brain structures and could be used in the future to test new therapies. These techniques are well advanced for BBS and it will allow us to reduce the numbers of animals and the harm we cause.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals we use for this project we will design the experiments in advance and applied statistical methods. This will allow us to reduce the number of animals at a minimum without compromise the scientific conclusions. Another way to reduce the number of animals is to improve the way we analyse how the animals recover with the treatment. We will use new technologies to image the brain and the eye with better accuracy allowing us to see the results

	better, reducing the number of animals. These new technologies will also allow us to measure the efficacy of the therapy without the need of sacrificing the animals and that will allow us to follow them over time, reducing the number of animals used and killed.
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 will be the animals used in this project. To investigate human genetic conditions mice are the best and only model. Mice are mammals, they share most of the genes with humans, when we mutate a gene in mice it usually shows the same symptoms as the patients. We even have models that have the exact same mutation changed that is found in some of our patients, and they have the same defects we find in humans. Another reason to use mice is that the therapies we are testing work in a very similar way in mice and humans. This allow us to be certain that the results we observe in the lab can be reproduced in humans. The injections we give to the animals in the eye and the brain are not complex surgeries. However, we will be careful monitoring the animals during and after the injections a few times a day as the most probable adverse effect could be infection after the procedure. With the help of the veterinary we will decide if we need to use painkillers or antibiotics if we find animals in this situation. The therapies we give are not expected to have any secondary effect in the animals either, but we will also monitor the animals carefully to see is there are signs of distress or change in their behaviour due to our therapies. This information will be also very relevant to understand if our therapies are safe to use in humans.

100. Developmental and Reproduction Safety Testing of Chemicals, Plant Protection Products, Biocides and Substance added to Food or Feed Products Using Small Animal Species

Project duration

4 years 1 months

Project purpose

- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)

Key words

Regulatory, Safety Assessment, Developmental, Reproduction

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

The drive for new and safer products in conjunction with human population expansion and developments in our habitat, drive the need for more effective solutions, for example, to develop "bee friendly" insecticides, environmentally acceptable weed killers, new disinfectants which counteract microbial resistance or safer (to humans) veterinary medicines, animal feed additives, food ingredients and preservatives.

This project licence authorises the conduct of studies in laboratory rats, rabbits and mice to evaluate the hazard profile of novel chemicals, plant protection products, biocides, food and feed additives and veterinary medicinal products in terms of the risks to reproductive capability, fertility and the development of unborn, newly born and developing animals. In order to make sound regulatory decisions regarding safe human exposure levels to these materials, information is required covering exposure of adult animals and the impact on all ages of development from conception to sexual maturity.

Further aims include validation of new experimental conditions, including the collection of fluids and tissues to support validation of alternative methodologies to refine and reduce the overall use of animals.

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?

During day to day life people are exposed to a wide range of substances at work, in their home, during leisure and other activities. If not properly assessed and controlled these substances can cause significant injury, health issues and/or lead to terminal illness or even death. Developmental and Reproductive Toxicology (DART) studies may be performed dependent upon production volumes as required by legislation on chemicals.

The principal benefit of this project is the generation of safety data to allow regulatory decisions regarding human exposure throughout the reproductive lifetime from the formation of sperm and eggs though to maturation and mating. Without these studies, progression of new products could put the reproductive capacity of humans at risk as production tonnages increase Validation and refinement of test methods may also be completed for specific techniques and may be published to the wider scientific community.

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?

Over the 5 year life of this Project Licence, it is estimated that 4300 mice, 22600 rats, 2050 rabbits will be used.

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Animals will be given the “test material” under investigation in a way which mimics possible human exposure. As the most likely route of exposure is orally the majority of animals will receive the test material either mixed in their food or directly by insertion of a flexible rubber catheter into the stomach, via the mouth. For some test materials the oral route of administration may not be appropriate for example the material is more likely to come in to contact with skin or other body membranes. Most animals are treated daily; occasionally studies may require several doses within 24 hours. The length of study is dependent on the tonnage of the test material produced each year as a higher tonnage increased the risk of repeated human exposure and ranges from a simple study to explore effects on reproduction with a small number of animals to a multigeneration study to explore effects of generational exposure to a compound. Blood and urine samples may be taken to measure the level of test material or its metabolites within an animal’s circulatory system. These may also be analysed to detect any changes in blood or urine chemistry, allowing in-vivo monitoring of body systems and organs for example liver or kidney function.

Neurobehavioural assessments may be carried out to identify potential neurotoxicity by observing and describing behaviour. Many of the endpoints measured on reproduction studies do not adversely affect the life of the animals. For example, offspring may simply be observed for developmental milestones such as eye opening and the development of reflexes and as they grow they may be observed for evidence of sexual maturation, which may be precocious or delayed. Study animals are observed at least twice a day by highly trained technologists who monitor for any signs of discomfort. Other measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects. Effects on reproduction and fertility of a test material are not always evident during the in-life phase of a study and may not impact the animal's wellbeing (for example reduced numbers of maturing sperm and a reduced number of eggs). Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia.

Replacement

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

There are currently no scientific and legally acceptable evaluations of whole body, systemic toxicity that will satisfy regulatory requirements with respect to developmental and reproductive safety of medicinal products and other chemicals other than the use of animals. Wherever possible, validated *in vitro* tests for specific organs are used and valuable information may also be obtained from alternative nonmammalian test species (e.g. fish, amphibians). Where available, review of scientific articles, nonanimal methods and read-across to other animal data such as metabolism, pharmacology and general toxicology information is also utilised to reduce animal use.

Reduction

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

Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies.

Where available, sensitive analytical techniques may be used to reduce animal numbers

(for example by reducing blood volume requirements).

Wherever practicable, the re-use of suitable animals, and by looking across studies, the combination of endpoints e.g. general toxicity, DART, safety pharmacology, mutagenicity etc in studies is considered, to reduce overall animal usage.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Species choice and use of specific animal models is determined by the need to generate regulatory acceptable data. The rodent is the first choice for reproduction studies run using the OECD guidelines. Rabbit provides a second species for evaluation of teratology. Animal studies are only considered where there is a direct legislative or regulatory requirement and after review of all other available information to ensure that no alternative is feasible. A step-wise approach is taken, with higher risk studies, when little may be known about the test material, being performed early in a programme and using only small numbers of animals. As confidence in the data and the level of information grows, longer term studies in larger numbers of animals may become necessary and the design of these studies, whist adhering to regulatory requirements, can be refined and tailored to obtain the most relevant and valid scientific information from the fewest animals and with the lowest level of adverse effects possible.

Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare. Any confinement or restraint is restricted to the minimum required to achieve the scientific objectives of the study and all study plans/protocols are reviewed for adherence to welfare guidelines and best practices by the site’s Animal Welfare and Ethical Review Body (AWERB).

101. Developmental and Reproduction Safety Testing of Chemicals, Plant Protection Products, Biocides and Substance added to Food or Feed Products Using Small Animal Species

Project duration

4 years 8 months

Project purpose

- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)

Key words

Regulatory, Safety Assessment, Developmental, Reproduction

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

The drive for new and safer products in conjunction with human population expansion and developments in our habitat, drive the need for more effective solutions, for example, to develop "bee friendly" insecticides, environmentally acceptable weed killers, new disinfectants which counteract microbial resistance or safer (to humans) veterinary medicines, animal feed additives, food ingredients and preservatives.

This project licence authorises the conduct of studies in laboratory rats, rabbits and mice to evaluate the hazard profile of novel chemicals, plant protection products, biocides, food and feed additives and veterinary medicinal products in terms of the risks to reproductive capability, fertility and the development of unborn, newly born and developing animals. In order to make sound regulatory decisions regarding safe human exposure levels to these materials, information is required covering exposure of adult animals and the impact on all ages of development from conception to sexual maturity.

Further aims include validation of new experimental conditions, including the collection of fluids and tissues to support validation of alternative methodologies to refine and reduce the overall use of animals.

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?

During day to day life people are exposed to a wide range of substances at work, in their home, during leisure and other activities. If not properly assessed and controlled these substances can cause significant injury, health issues and/or lead to terminal illness or even death. Developmental and Reproductive Toxicology (DART) studies may be performed dependent upon production volumes as required by legislation on chemicals. The principal benefit of this project is the generation of safety data to allow regulatory decisions regarding human exposure throughout the reproductive lifetime from the formation of sperm and eggs though to maturation and mating. Without these studies, progression of new products could put the reproductive capacity of humans at risk as production tonnages increase Validation and refinement of test methods may also be completed for specific techniques and may be published to the wider scientific community.

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?

Over the 5 year life of this Project Licence, it is estimated that 4300 mice, 19600 rats, 2050 rabbits will be used.

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Animals will be given the “test material” under investigation in a way which mimics possible human exposure. As the most likely route of exposure is orally the majority of animals will receive the test material either mixed in their food or directly by insertion of a flexible rubber catheter into the stomach, via the mouth. For some test materials the oral route of administration may not be appropriate for example the material is more likely to come in to contact with skin or other body membranes. Most animals are treated daily; occasionally studies may require several doses within 24 hours. The length of study is dependent on the tonnage of the test material produced each year as a higher tonnage increased the risk of repeated human exposure and ranges from a simple study to explore effects on reproduction with a small number of animals to a multigeneration study to explore effects of generational exposure to a compound. Blood and urine samples may be taken to measure the level of test material or its metabolites within an animal’s circulatory system. These may also be analysed to detect any changes in blood or urine chemistry, allowing in-vivo monitoring of body systems and organs for example liver or kidney function. Neurobehavioural assessments may be carried out to identify potential neurotoxicity by

observing and describing behaviour. Many of the endpoints measured on reproduction studies do not adversely affect the life of the animals. For example, offspring may simply be observed for developmental milestones such as eye opening and the development of reflexes and as they grow they may be observed for evidence of sexual maturation, which may be precocious or delayed. Study animals are observed at least twice a day by highly trained technologists who monitor for any signs of discomfort. Other measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects. Effects on reproduction and fertility of a test material are not always evident during the in-life phase of a study and may not impact the animal's wellbeing (for example reduced numbers of maturing sperm and a reduced number of eggs). Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia.
Replacement

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

There are currently no scientific and legally acceptable evaluations of whole body, systemic toxicity that will satisfy regulatory requirements with respect to developmental and reproductive safety of medicinal products and other chemicals other than the use of animals. Wherever possible, validated *in vitro* tests for specific organs are used and valuable information may also be obtained from alternative nonmammalian test species (e.g. fish, amphibians). Where available, review of scientific articles, nonanimal methods and read-across to other animal data such as metabolism, pharmacology and general toxicology information is also utilised to reduce animal use.

Reduction

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

Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies.

Where available, sensitive analytical techniques may be used to reduce animal numbers (for example by reducing blood volume requirements).

Wherever practicable, the re-use of suitable animals, and by looking across studies, the combination of endpoints e.g. general toxicity, DART, safety pharmacology, mutagenicity

etc in studies is considered, to reduce overall animal usage.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Species choice and use of specific animal models is determined by the need to generate regulatory acceptable data. The rodent is the first choice for reproduction studies run using the OECD guidelines. Rabbit provides a second species for evaluation of teratology. Animal studies are only considered where there is a direct legislative or regulatory requirement and after review of all other available information to ensure that no alternative is feasible. A step-wise approach is taken, with higher risk studies, when little may be known about the test material, being performed early in a programme and using only small numbers of animals. As confidence in the data and the level of information grows, longer term studies in larger numbers of animals may become necessary and the design of these studies, whist adhering to regulatory requirements, can be refined and tailored to obtain the most relevant and valid scientific information from the fewest animals and with the lowest level of adverse effects possible.

Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare. Any confinement or restraint is restricted to the minimum required to achieve the scientific objectives of the study and all study plans/protocols are reviewed for adherence to welfare guidelines and best practices by the site’s Animal Welfare and Ethical Review Body (AWERB).

Project	**102. Developmental and Reproduction Safety Testing of Medicinal Products Using Small Animal Species**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 Years 1 Month	
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 authorises the use of laboratory rats, mice and rabbits to evaluate the safety of medicinal products in terms of the risks to reproductive capability, fertility and the development of unborn, newly born and developing animals. In order to make sound regulatory decisions regarding safe human exposure levels to these materials, information is required covering exposure of adult animals and the impact on all ages of development from conception to sexual maturity.	

	The expansion of scientific and medical knowledge has led to the development of drugs which can treat or alleviate the symptoms of many illnesses but there is still a need to develop medicinal products to diagnose and treat many human conditions such as Heart Disease, Stroke, Obesity, COPD, Respiratory Infections, Cancer, Autoimmune diseases, Diabetes, Alzhiemer's and Schizophrenia, amongst others. When these needs are combined with the growing threat from antibiotic resistant bacteria the advancement of science and the development of new products are as important as ever. The primary aims of this project are to support the development of these medicinal products through acquisition of data to:- 1) Support selection of new candidate molecules for further evaluation and development; 2) Demonstrate the safety-hazard profile of a new medicinal product prior to the initiation of clinical trials involving women of child bearing potential (WOCBP) and in a paediatric setting; 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include the validation of new experimental conditions, including the collection of fluids and tissues to support drug development and validate alternative methodologies to refine and reduce the overall use of animals. Developmental and Reproductive Toxicology (DART) studies may be performed at any time during a development programme for a new medicinal product. In general, there will already be some information on the expected range of effects and dose levels from prior general toxicology tests that will guide the selection of dose levels. This reduces the risk of excessive toxicity, maximises the data that can be obtained and promotes a better outcome for the studies. Nevertheless, characterising the effects at high doses provides valuable information regarding the safety profile of the test substance and ultimately gives the regulatory authorities and clinicians the confidence to select suitable dose levels of medicinal products for human clinical trials or to determine safe exposure
	levels/controls for other materials.

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)?	People are exposed to medicinal products as patients, medical professionals, carers and during their manufacture. The products can be biological or non-biological materials and include diagnostic agents or substances associated with drug candidates e.g. metabolites, impurities and drug degradants. The principal benefit of this project is the generation of safety data to allow regulatory decisions regarding human exposure throughout the reproductive lifetime from the formation of sperm and eggs though to maturation and mating. Without these studies, progression of new medicines to early human studies and to patients could not occur safely. Validation and refinement of test methods may be completed for specific techniques and may be published to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the life of this Project Licence, it is estimated that up to 20650 rats, 15900 mice and 3400 rabbits will 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?	The animals used under this licence will be given the test substances in a similar way to that in which they are expected to be given to humans. As most medicines at taken orally the majority of animals will receive the test material directly by insertion of a flexible rubber catheter into the stomach via the mouth. Most animals are treated in this way once per day daily, although studies may occasionally require two or three doses within 24 hours. For some test materials the oral route of administration may not be appropriate; for example, it may be broken down, not tolerated or not absorbed during the digestive process. In these situations, alternative administration routes such as sub-cutaneous or intravenous injection may be more appropriate. Animals may be manually restrained for “bolus” administrations lasting a few seconds or minutes, or they may be habituated to sit in specially designed chairs for longer intravenous administrations. For test materials which will be administered to humans intravenously over extended period of time due to their inherent toxicity or fast clearance from the body it may be more appropriate to surgically implant a permanent catheter (with appropriate

	anaesthesia and analgesia) to allow the animal free movement whilst being dosed in their home enclosure. Test materials intended to treat a localised condition may be administered to that body part or area for example to the skin. Blood and urine samples may be taken to measure the level of the test material or its metabolites within an animal's circulatory system. These may also be analysed to detect any changes in blood or urine chemistry, allowing in-vivo monitoring of body systems and organs for example liver or kidney function. Study animals are closely observed several times a day by highly trained technologists who monitor for any signs of discomfort. Other measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour such as reduced activity. Many of the endpoints measured on reproduction studies do not adversely affect the life of the animals. For example, offspring may simply be observed for developmental milestones such as eye opening and the development of reflexes and as they grow they may be observed for evidence of sexual maturation, which may be precocious or delayed. Humane endpoints will be applied or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively fewer adverse effects. Many toxicological effects of test materials on developmental and reproductive parameters are not evident during the in-life phase of a study and do not impact the animals wellbeing (for example reduced numbers of maturing sperm and a reduced number of eggs). Only through

	microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia.
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 scientific and legally acceptable evaluations of whole body, systemic toxicity that will satisfy regulatory requirements with respect to developmental and reproductive safety of medicinal products other than the use of animals. Wherever possible, validated *in vitro* tests for specific organs are used and valuable information may also be obtained from alternative non-mammalian test species (e.g. fish, amphibians). Where available, review of scientific articles, non-animal methods and read-across to other animal data such as metabolism, pharmacology and general toxicology information is also utilised to reduce animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies. Where available, sensitive analytical techniques may be used to reduce animal numbers (for example by reducing blood volume requirements). This licence includes provision to perform combination studies that span multiple endpoints of the overall stages of reproduction and development. Such combination studies can be beneficial in using fewer animals than required for the separate studies individually.
3. Refinement Explain the choice of species and why the animal model(s) you will	Species choice and use of specific animal models is determined by the need to generate regulatory acceptable data. Where a choice of species is possible, care is taken to select the most

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.	biologically appropriate species, and the species which most closely relates to man. Animal studies are only considered where there is a direct legislative or regulatory requirement and after review of all other available information to ensure that no alternative is feasible. A step-wise approach is taken, with higher risk studies, when little may be known about the test material, being performed early in a programme and using only small numbers of animals. As confidence in the data and the level of information grows, longer term studies in larger numbers of animals may become necessary and the design of these studies, whist adhering to regulatory requirements, can be refined and tailored to obtain the most relevant and valid scientific information from the fewest animals and with the lowest level of adverse effects possible. Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare. Any confinement or restraint is restricted to the minimum required to achieve the scientific objectives of the study and all study plans/protocols are reviewed for adherence to welfare guidelines and best practices by the site's Animal Welfare and Ethical Review Body (AWERB).

Project	**103. Devices and therapies for cardiovascular interventions and 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)	Diseases of the heart and blood vessels remain the commonest cause of death in Western society. Two very common conditions are atrial fibrillation, also commonly known as AF, and heart failure. Atrial fibrillation is where the normal rhythmic electrical activity of the heart becomes irregular and heart failure is where the heart is unable to pump enough blood to meet the demands of the body. Atrial fibrillation occurs in up to 20% of elderly people and it is associated with a dramatic increase in the risk of having a stroke. It is	

	commonly treated using a technique called ablation where electrical energy is used to isolate the affected part of the heart. However, this procedure frequently fails because doctors are unsure whether they have applied enough energy to the heart. In this programme of work we intend to test a new device that will allow doctors to immediately know if the high energy pulses from the ablation device have been successful in preventing the abnormal electrical activity travelling around the heart. Heart failure, like atrial fibrillation, is also very common and occurs frequently after people have had a heart attack which is caused by a blockage occuring in the blood vessels in the heart . Following a heart attack, doctors try to restore blood flow to the area of the heart affected by the blocked blood vessel by inserting a device known as a stent into the blood vessel. However, in some people these stents eventually fail and become blocked again as the body reacts to their presence in the blood vessel. In this second part of this programme of work we aim to test a new stent design where the coils that make the stent springy are arranged in a different way and they are also designed to be slowly absorbed by the body. By being absorbed once the hearts blood vessel has recovered, the stent no longer acts as a source of irritation to the body and we anticipate the blood vessels will be less likely to become blocked again.
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 has two major parts: In the first part we will be investigating a new device that will help doctors determine if a procedure that they perform to treat patients with atrial fibrillation and other rhythm disturbances in the heart is performing optimally. Specifically we will be testing if a light based detection system incorporated into these devices which are known as ablation catheters, can be used to tell when the ablation catheter has applied enough high frequency energy to the part of the heart being treated. This is important because in order to ensure the best patient outcomes the doctors aim to stop the abnormal activity in the part of the

	heart being treated from influencing the rest of the heart without excessively damaging the heart and causing serious complications associated with this type of treatment. However, at the present moment in time, doctors either treat the part of the heart they are interested in for a fixed amount of time or until a certain temperature is reached in that part of the heart without actually knowing if this has successfully treated that part of the heart. With the proposed device doctors will know exactly when to stop treating the heart and are therefore more likely to successfully treat the underlying condition (e.g. atrial fibrillation). Additionally, by knowing when to stop applying high frequency energy to the heart, doctors will be much less likely to damage the heart walls and thereby protect patients from the one of the main risks associated with this type of surgery. 2. In the second part of the study we will also be testing a new device for the treatment of patients who have had a heart attack or, in whom, the blood vessels supplying the muscular parts of the heart have become narrowed or partially blocked. In these patients a spring like device known as a stent is normally placed in the blocked or narrowed vessel in order to keep the vessel open and to re-establish blood flow to the affected part of the heart. However, there are some common problems associated with conventional stents which, over time, lead to the vessel becoming blocked again. Here, we will be testing if a new stent design made using a material that is absorbed by the body over time, is better at preventing the blood vessels from blocking again. If we find that this new stent design is less prone to causing vessel blockage than conventional stents then these can be rapidly adapted for use in patients. This will lead to fewer long-term complications associated with stent placement in the blood vessels of the heart. Additionally, because the stents are designed to be absorbed over time and therefore disappear from the body, patients may not need to be treated with drugs for as long after they have had the stent implanted compared to the current situation with conventional stents.
What species and approximate numbers of animals do you expect	The programme of work will use sheep models and the initial studies establishing proof of concept effectiveness in both strands of the

to use over what period of time?	project will require approximately 32 animals and we anticipate this work will be completed in about two years. Farm animals have much more similar heart properties to man than rodents and are therefore more relevant. If the initial studies are successful then follow-on studies may be undertaken subject to amendments to the programme of work and we would expect these to last for a further 3 years. Such follow on studies will be designed in light of the outcome of the present studies and animal usage determined in light of the acquired knowledge.
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 overall severity level for the two arms of the study will be different; in the first part all the invasive procedures are all performed in terminally anaesthetised animals. In the second arm, animals will recover from anaesthesia following surgery and will be monitored for a period of up to 5 months before undergoing a final surgical procedure performed under non-recovery anaesthesia. The severity limit of this second arm will not exceed moderate. In both work packages of the study, animals will also undergo a limited number of investigations to assess the function of their heart; this will entail blood sampling, echocardiography and ECG measurements. These procedures are non invasive and do not require sedation; animals are gently restrained by the assistant whilst the operator takes the measurements. These investigations are the same as occur in patients and are not expected to cause any form of lasting harm or discomfort and would either be categorised as sub-threshold or potentially mild. For both arms of the study the adverse effects that we may encounter are similar and include stroke, heart attack and abnormal rhythms in the heart. Whilst each of these adverse effects is potentially of a severe nature, if they were to occur they would arise whilst animals are anaesthetised and undergoing procedures. Through careful surgical, physiological and anaesthetic monitoring evidence that these adverse events had occurred would result in the humane killing of the animals such that no animal would experience these symptoms whilst conscious. However, given that animals will be being treated with drugs that prevent these adverse events and procedures will be performed

	by experienced operators we do not anticipate an incidence rate of more than 1% each. All animals are maintained in group housing within the facility and provided with enrichment including food toys, protected areas and rubbing posts.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	● In order to assess the applicability of these novel devices it is necessary to undertake in vivo experiments that closely mimic the real-life settings in which they would be employed clinically. This requires that we use the same surgical approaches and assess the suitability of the devices in situations where the heart is working and experiencing all the neurohumoral inputs and controls the heart experiences in the body. ● computer models are not clever enough to accurately predict the complex signalling outcomes involved in the inflammatory response, the only way we can investigate this is in a situation where all the elements of the inflammatory system are present and working. ● even with blood perfusion it is not possible to maintain functioning organs (human or animal) ex vivo for the amount of time required to complete these experiments and recapitulate the bodies responses which occur naturally over the time frame of months rather than minutes. ● before these devices could be translated to the human setting we have to demonstrate that the novel devices are at least as effective as conventional devices in animal models
2. Reduction Explain how you will assure the use of minimum numbers of animals	● consideration of experimental design and good practice before undertaking in vivo experiments; we will work with an independent statistical consultant to ensure appropriate design strategies are

	implemented ● the use of a large animal model allows multiple 'tests' to be undertaken simultaneously in the same heart thus reducing the overall animal numbers required to achieve target outcomes To elaborate how experimental design is pivotal to reducing animal numbers we will exemplify the stent arm of the programme of work: o animals are randomly assigned to the two treatment groups based on pre-surgical assessments (e.g. age, weight, cardiac indices assessed by echocardiography, blood biochemistry) o each group will receive one stent type and be monitored post operatively. o Depending on within and between animal variability it is estimated that 9 animals per stent will allow a difference in neointimal thickness of between 19 and 28 µm to be detected. o Differences between groups with these sample sizes are detectable at the 5 % level and with at least 80 % power.
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.	● procedures are refined to be as minimally invasive as possible ● we follow the same approaches that are employed clinically ● the choice of animal model means that devices and approaches require no modification to be suitable for use experimentally ● animals are housed in social groups and are provided with enrichment objects in the housing environment ● where animals are individually housed post-operatively, this is for the minimum amount of time to ensure full recovery from surgery and wound integrity. Additionally, the singly housed animals are in sight of

	and can still interact with their group peers. ● animal welfare is assessed at least once daily by experienced care staff and the investigators involved in the programme of work ● there is an open and bilateral relationship between the investigators and all named personnel thus ensuring animal welfare and well-being is at the forefront of studies

Project	**104. Diabetes mechanisms and treatments**	
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)		
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By understanding more about the disease process in these translatable animal models, there is the potential to develop novel therapies that can treat the disease and its complications and to identify patients who will respond best to particular therapies. Further the project will look for an overlap in mechanisms across complications and look for potential biomarker predictors of pain and pain relief. The project	

	will generate data to support or refute the clinical development of at least 3 novel therapies for painful neuropathy and glucose sensitivity as well as isolate active molecules from plant extracts currently taken in REDACTED that could lead to a patent and controlled clinical studies and therapy. This project will continue to refine these rodent models of diabetes by characterising new translatable, predictive markers and establishing social housing welfare benefits to the animals. The project data will be of interest to both research and clinical communities and lead to peer review publications and presentation in the lay media. Ultimately in the long term this work will be of potential direct benefit to diabetic patients since the novel therapies will treat those aspects of the disease that will assist in patients returning to a normal lifestyle
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 years of the proposed licence we estimate that we will use: Rat: 2750 Mouse: 250
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 proportion of the techniques used under this licence (e.g. blood sampling, drug administration) are minimally invasive to the animals and therefore classified as mild. Some animals will develop chemically induced diabetes leading to high blood sugar levels, thirst, urinating more and spontaneous neuropathic pain and these will be classified as moderate. Some animals will have surgery to implant a transmitter device under the skin or electrodes under scalp to record e.g. body temperature or brain signals. Some animals will be fasted overnight in the same way a diabetic patient needs to fast before a blood test to establish glucose and insulin tolerance. Some behavioural assessments will require separation from cage-mates. Separation will be as short as reasonably practicable. They are expected to recover quickly and will be given painkillers and post-operative care just like people re-covering in hospital. There are limits to the number and frequency of any injections, blood sampling and behavioural assessment that any one animal can experience. Overall, the severity of this license is expected to be moderate. At the end

	of the experiments the animals will be humanely killed and tissues may be used for biochemical investigations. Many functional tests will be carried out in isolated tissues/cells after the animals are killed. We anticipate only a small number of animals may show unexpected adverse effects that exceed the expected severity of the protocols and where do so they will be killed by a humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals have to be used in the studies on diabetes and complications such as neuropathic pain, because these are chronic complex conditions where the whole organism with an intact nervous system is required in order to measure for e.g. a painful response. Animal models of diabetes can be used for the study of diabetic complications that are seen clinically. No cellular systems are in existence that can replicate the whole functioning organism. Therefore, there is no feasible alternative that would entirely replace the use of a living animal that would allow the objectives to be met. However, data generated using computer modelling and cell systems is essential to meet the specific objectives and benefits of this project. We will investigate anti-diabetic properties of novel therapies *for* e.g. human bioengineered pancreatic cells before testing in diabetic animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be kept to the minimum required through good experimental design. Careful planning of each study will ensure that tissues from the same animals can be used for several types of experiments maximising data output limiting animal use to a minimum. Further, efficient multiple tissue use-age integrates behavioural with biochemical pharmacological data from each animal thereby strengthening our understanding of the relationship between mechanisms and complications

	For most experiments, sample sizes have been set using power analysis and refined under previous project licences, generally using a significance level of 5%, a power of 80-90%, to detect a difference between groups of 25%. We will continue to monitor group sizes and modify as appropriate based on their analysis.
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.	At all times our protocols will reflect the most refined techniques as determined by the published literature, the advice of colleagues and our own experiences. The majority of experiments carried out under this project licence will be in rats, as the induced diabetic rat models are widely accepted as good models of human disease as they develop many complications seen in type 1 and 2 patients, including neuropathic pain and insulin resistance and show sensitivity to anti-diabetic drugs such as metformin. Both diabetic models mimic many of the complications observed in the human condition, so some level of neuropathic pain is inevitable. However, experience from previous project licences has shown us that the level of pain experienced by diabetic animals is not such as to cause any major changes in the welfare of the animals compared to the diabetic animals that do not develop neuropathic pain. Testable reflexive sensory stimuli will be applied for the shortest time practicable and the animal will always have direct control over the duration of the sensory stimulus applied, by removal of its foot. To minimise welfare costs to diabetic animals the reduced body temperature will be minimised by group housing animals and extra drinking water will be provided and cage bedding changed more frequently due to thirst and increased urination. Animals will be closely monitored and if any become unwell they will be culled and an examination performed to identify the cause of death and to inform subsequent experiments.

Project	**105. Diagnostic miRNA biomarkers for livestock**	
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 will be to develop novel tests for assessing overall health and disease risk in cattle using quantification of biomarkers in biological fluid samples. The study will focus on production diseases, namely mammary, metabolic and reproductive health, which are highly prevalent in modern dairy herds and have a huge impact on both industry economics and the welfare of dairy cows. Specific Objectives will be to identify in milk and/or circulating plasma samples robust biomarkers that can be used for early diagnosis of mastitis, metabolic disease and pregnancy (Objective 1), as well as early-life prediction of long-life health and productivity (Objective 2). Using this information, point-of care tests will	

	be developed for early disease diagnosis and identification of at-risk animals using proprietary technology developed by industrial partners that will use quick, accurate and direct detection of biomarkers in cattle biological fluid samples.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work proposed in this application will directly address a clearly identified need by the global dairy industry for improved tests for early diagnosis of mastitis, metabolic status and pregnancy status in dairy cattle, as well as for early identification of animals at risk of disease. It will do so by generating the necessary knowledge for the development of diagnostic test based on biomarkers. Current interactions with industrial partners will facilitate translation of the developed tests into commercial diagnostic tools for farmers. Medium to long-term benefits of such assay will be significant improvements in dairy cow health and welfare that will translate into more productive animals and higher profits for dairy farmers and the wider dairy supplier chain, as well as better food products for consumers. Moreover, the scientific knowledge on health biomarkers that will be generated will be useful for understanding biomarker biology in general and for developing diagnostic applications for other domestic species.
What species and approximate numbers of animals do you expect to use over what period of time?	To achieve our Objectives, we expect to use about 300 cows 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?	Blood sampling will be the main regulated procedure that will be carried out in this study. It is a mild procedure that generally does not have any adverse effects. We expect that at the end of the study all animals will return unharmed to their milking herd of origin
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal	The identification of physiological biomarkers of tissue function and dysfunction can only be performed by controlled in vivo screening in the species under study, in this case, cattle. No in-

alternatives	vitro or other non-in vivo system currently available can account for the complexity associated with a live animal system and can therefore not be used to achieve our Aim to identify suitable biomarkers for the development of diagnostic assays for live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Adequate power calculations will be performed before each experiment to determine the minimal number of animals needed to obtain statistical meaningful results that can be used for the purpose of developing diagnostic assays as intended. Abundant data from previous studies on identification of predictive biomarkers for different production and disease traits in cattle is available and will be used for such calculations. In addition, advice on experimental design will be sought from an experienced Statistician if required. Experiments will be planned so they can be published in accordance with the NC3Rs' ARRIVE guidelines
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.	This project will seek to obtain physiological data that will be used to produce diagnostic tests specifically for cattle so it cannot be performed in a species other than cows. Of all body compartments, blood constitutes an excellent and easily accessible source for reliable assessing changes in systemic levels of biomarkers and therefore blood samples will be collected. For assessing changes in biomarkers in the mammary gland environment (i.e., associated with mastitis), milk will be collected rather than blood. Blood sample collection is a mild and routine procedure with minimal risk and to which animals become well accustomed.

Project	**106. Diet composition and nutrient use efficiency in sheep**	
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
	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 aims of this programme are 1) to increase our understanding of the dietary choices made by sheep, and 2) investigate how their diet influences nutrient use efficiency and animal performance.	
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 allow an improved understanding of the interactions between sward composition, diet and animal performance. This will improve the output of ruminant products and help reduce the environmental impact of ruminant agriculture. For example, an improvement in the efficiency of use of forages for animal growth will reduce	

	the amount of methane (a greenhouse gas) emitted. An improved understanding of the impact of grazing on semi-natural communities will support the development of grazing guidelines which support the recovery of related habitats.
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep – approximately 400 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?	In order for the data to be representative the conditions in which the animal will be studied are designed to be as close as possible to those used in commercial livestock production systems. Any suffering would alter the behaviour of the animals and render the results unrepresentative. Most animals will be used in short-term experiments that may last for between 2 to 4 months. The procedures involved carry a mild level of severity. At the end of the procedures the animals will be re-homed in the establishment's flock, or will be sent to slaughter as part of the normal supply chain for human consumption.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In grazing and growth studies it is not feasible to gain the results satisfactorily from any method not entailing the use of animals. These studies integrate behavioural, ingestive and metabolic parameters. The data cannot be collected using *in vitro* techniques, and therefore animals must be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All work carried out will follow protocols of work that ensure the maximum amount of information is obtained from the minimum resources required to be statistically valid. Use of changeover design experiments, where appropriate, efficiently controls random variation and therefore fewer animals can be used. Where changeover designs cannot be used (e.g. growth studies or experiments with fresh forage) more animals may be required and/or the technical constraints of the experimental

	design have to be accepted.
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.	Nutrition studies of farm livestock need to be applicable to farming conditions, and therefore work investigating meat production in sheep requires the use of those animals housed under normal commercial practises. The highest standards of animal health and welfare must be maintained throughout any experiment in order for results of the work recognised by other scientists and farmers to be transferable to productive farms.

Project	**107. Dispersal through fitness landscapes in a social bird: from individuals to populations**	
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
What's the aim of this project?	The overall aim of this project is to determine the causes and consequences of individuals' dispersal decisions in relation to spatial and temporal heterogeneity in their environment.	
Why is it important to undertake this work?	Dispersal of organisms from where they are born to where they breed results in the flow of genes through populations. At a broad scale this gene flow may have consequences for speciation, and at a fine scale it affects patterns of relatedness within a population, which in turn affects the risk of inbreeding and the expression of cooperative behaviours. However, dispersal is hard to study because many individuals disperse beyond the boundaries of study sites, and it is hard to distinguish this long-distance dispersal from	

	death. This project will use a uniquely detailed long-term dataset on dispersal in a marked population of a highly social bird with very limited dispersal, the long-tailed tit Aegithalos caudatus. We will use dispersal data from 26 years of study, combined with further field observations, experimental manipulations and population modelling, to investigate the causes and consequences of individuals' dispersal decisions.
What outputs do you think you will see at the end of this project?	The study will provide new information on the causes and population-level consequences of a key demographic parameter, dispersal, in a natural population. Outputs will be published in peer-reviewed journals (circa 5-10 papers expected) and through attendance and presentation of research at national and international conferences. Recent outputs on related topics using this study system have been published in Nature Communications and PNAS, as well as in leading subject-specific journals.
Who or what will benefit from these outputs, and how?	There are four main beneficiaries. 1. General public - Birds have a fascination for the UK public, as evidenced by the mass membership of the RSPB (>1 million) and the vast number of households that feed birds (c. 12 million). Therefore the project is likely to attract significant media attention. 2. School students - An impact programme has also been funded by REDACTED to engage with biology students at a local sixth form college with the aim of nurturing academic students from disadvantaged socioeconomic backgrounds to enhance their opportunity to study at Russell Group universities. 3. Conservation biologists - Effective conservation requires assessment of the mechanisms through which spatial and temporal environmental variation influence biodiversity, an issue lying at the heart of this proposal. We have strong links with the RSPB, British Trust for Ornithology and local wildlife trusts and will ensure that advances we make are brought to a wider audience of environmental practitioners and policy advisers. 4. Academics - This project lies at the interface between behavioural ecology and population ecology. Therefore, it will appeal to a broad spectrum of natural scientists, who will be reached through publications in leading international journals and through presentations at national and international conferences.

Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We aim to publish at least five or six academic papers in leading journals from the project; a recently published paper in a high impact journal (PNAS) demonstrates the general interest and significance of research in this area. Results will also be presented at national and international conferences. Pathways to impact include existing links with a local sixth form college and conservation NGOs. The first school visit relating to this project has already occurred and further learning and mentoring opportunities are planned.
Explain why you are using these types of animals and your choice of life stages.	The long-tailed tit is a model system for the study of social evolution. This project builds on the findings of long-term study (initiated by the applicant in 1994) of the behaviour and ecology of this species, providing a robust rationale for the objectives described above. The regulated procedure required (blood sampling by brachial venipuncture) is mild and has no adverse effect on the animals, whose welfare is paramount for the collection of long-term behavioural and life-history data. Blood samples will be taken from nestlings (11 days old), juveniles and adults, according to the time of capture.
Typically, what will be done to an animal used in your project?	A bird will be captured in a mist-net (adult or juvenile), extracted and placed alone in a clean cotton bag; or, taken from a nest (nestling) and placed in a clean cotton bag with other members of its brood for a maximum of 20 minutes. Individuals will be taken from the bag, ringed and biometric data recorded prior to the regulated procedure. For brachial venepuncture, the bird will be held in the ringer's grip with the left wing extended to expose the brachial vein. The underwing will be swabbed with ethanol and a small puncture made in the brachial vein using a microlance to produce a drop of blood. Blood (10-25 microlitres) will be drawn up into a heparinized capillary tube and a small piece of cotton wool will be held against the underwing to staunch any blood flow. Birds will then be released (or replaced in their nest in the case of nestlings) following assessment of their wellbeing. Total handling time for each bird is typically <5 minutes.

What are the expected impacts and/or adverse effects for the animals during your project?	Mild stress from capture and handling. Mild pain from brachial venepuncture. Any effects are short-term, with no medium- or long-term consequences.
What are the expected severities and the proportion of animals in each category (per animal type)?	Mild severity for all sampled individuals.
What will happen to animals at the end of this project?	set-free
Why do you need to use animals to achieve the aim of your project?	The use of wild birds in their natural environment is essential for the aim of the project.
Which non-animal alternatives did you consider for use in this project?	There is no alternative to the use of animals for the study of dispersal decisions.
Why were they not suitable?	NA
Enter the estimated number of animals of each type used in this project.	other-birds: -
How have you estimated the numbers of animals you will use?	The aim is to individually mark and genotype all members of the study population. The study population typically comprises 50-70 breeding pairs with annual production of 120-200 offspring. The maximum number of birds processed in a year is c.250, so over 5 years, it is estimated that a maximum of 1250 birds will undergo the regulated procedure.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	It is essential that as many birds in the population as possible (typically >95%) are individually marked and genotyped to determine the social environment for all individuals for which dispersal data are collected. Therefore, there can be no reduction of animals used within the strictly defined study site.
What measures, apart from good experimental design, will you use to optimise the	The aim is to sample all members of the population, requiring regular capture effort from March to June

number of animals you plan to use in your project?	each year.
Which animal models and methods will you use during this project?	Long-tailed tits will be used for this project. Brachial venepuncture is the standard technique for obtaining blood samples from small passerines, causing only short-term, mild discomfort to the animal. To minimise disruption to parents during processing of nestlings, broods will be ringed and blood-sampled in two batches so that c.50% of the brood (typical brood size is 7-10 nestlings) is always present in the nest. Thus, should parents return to the nest when nestlings are being processed they will always find some nestlings present. To minimise distress to nestlings on cold days, the bag containing nestlings that have been removed from the nest will be kept on a warm hot-water bottle to remove any risk of chilling.
Why can’t you use animals that are less sentient?	The long-tailed tit is a model system for the study of social evolution. This project builds on the findings of long-term study (since 1994) of the behaviour and ecology of this species, providing a robust rationale for the objectives described above.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	I will be informed of advances in the 3Rs via communications from my own establishment, although for the reasons given it is most unlikely that these can be implemented in the project.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	The regulated procedure required (DNA sampling) is mild and has no long-term effect on the animals, whose welfare is paramount for the collection of long-term behavioural and life-history data.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	NA

Project	**108. Dissecting the metabolic control of the immune-inflammatory response**	
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)	Inflammation is the body's response to infections and tissue damage and helps to alert the immune system and ensure the danger is resolved. However, if inflammation is excessive and chronic in nature we now know that it is harmful and contributes to major diseases including heart disease, osteoarthritis and dementia. Importantly obesity is a major risk factor for these diseases but we do not understand why. The aim of this project is to determine how obesity and a diet high in saturated fatty acids	

	(FAs) predisposes an individual to a harmful pro-inflammatory response, which we have shown previously persists well beyond the point of weight-loss, both in young and old mice. Lifestyle factors such as diet are known to have these long term effects by modifying our DNA, a process called epigenetic modification. Therefore we want to specifically determine the epigenetic changes that occur in the immune system in mice on a high fat diet and see if specific beneficial nutrients, namely omega-3 FAs, can reverse these epigenetic changes and prevent the harmful pro-inflammatory responses of the immune system. Objectives 1) To determine the reversibility of the epigenetic modifications induced in immune cells by fat overload; 2) To assess the effect of fat overload and subsequent weight-loss on epigenetic modifications and immune cell responses in older mice; 3) To study the biology of specific proteins, and their control of the immune response before and after fat overload.
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 in this area will advance scientific understanding of the processes that negatively influence the immune response during obesity, specifically the causes of raised inflammation which drives the poor health outcomes of obesity. It will focus on epigenetic modifications which mediate the long term effects of obesity on how immune cells respond (specifically T cells and their receptors which sense danger such as infections). In this way, it will help to identify new strategies to modulate these processes and restore a more beneficial immune response, which is relevant to preventing diseases such as cardiovascular diseases, cancer and diabetes, for which obesity is the biggest risk factor.
What species and approximate	7500 mice

numbers of animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We propose to use mice to study the immune response (inflammation) in obesity and its links to heart disease. Some of our mice may experience moderate severity symptoms including greasy skin and bowel irritation. On our prolonged diet protocols, some mice may exhibit weight loss, altered grooming behaviour, and reduced mobility. These resemble the symptoms of human obesity. In some experiments, animals will experience interventions such as injections in the abdomen or blood, or blood sampling from the tail vein. Here animals might experience temporary pain due to the injections but will return to normal behaviour rapidly. For animals that undergo the abdomen or blood injections, extra caution will be taken when performing injections to prevent injury to other organs or haemorrhage. All animals are humanely killed at the end of our experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To limit our animal usage we have always first used in vitro cell based techniques for our studies to provide information about molecular and cellular mechanism(s) involved in the pathogenesis of disease and to identify possible new therapies. This utilisation of in vitro assays is an important replacement of in vivo experiments. That said, our in vitro findings and hypotheses about disease mechanisms must ultimately be validated in animal models as the immune system is a highly complex and integrated system and its functioning can only be fully tested in a whole organism. This step in animals is also crucial if we are to ultimately progress our findings to patient benefit, for example if the omega-3FAs are able to reverse the negative effect of the high fat diet we can go on to design and carry out a clinical trial. There are no other in vitro or in vivo alternatives to this work. Continued review of the scientific literature will also be undertaken on a regular basis in order to

	identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will keep numbers down by performing in vitro experiments, by not repeating experiments already published, by performing pilot experiments, by using well trained personnel, We will use the NC3R's experimental design tool to aid experimental design and consult trained statisticians before using any new protocols. All staff performing animal experiments will attend appropriate training on key aspects of experimental design. We will publish in open access journals that support the ARRIVE guidelines for reporting.
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 all protocols, we utilise approaches that minimise the animal's potential suffering. The likelihood of adverse responses is minimised for agents by choice of dose rationale informed by prior in house pilot investigations and in vitro studies and experience from collaborators or published science. We will also systemically review each experiment on completion to see what lessons can be learned from the study in terms of endpoints (scientific and humane) and any animal welfare issues that may have arisen during the experiment that could then guide any subsequent experiments.

Project	**109. Distinguishing realistic environmental risks of plastics**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 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)	Contamination of marine environments by plastic debris is a visually obvious and undesirable consequence of the dramatic increase in production and use of plastics over the last 50 years. Pieces of plastic have been found in marine environments worldwide and the need to establish levels of contamination associated with environmental impacts has become a priority to inform policy. Micro- (<5 mm) and nanoplastics (< 1,000 nm) particles are manufactured (e.g. microbeads) or may be formed by fragmentation of larger plastic pieces and will inevitably accumulate in the environment. Experiments have indicated the abundance and persistence of plastic particles presents potential risks to marine	

	organisms. However, there is a lack of specific assessments of risk from different types and sizes of plastics particles and this is an urgent priority as there is concern that these small particles might present unexpected toxicity in organisms because some 9in the nano size range) may be transported across cell membranes and may persist within the gut and interfere with processes of the digestive system. This project is focused on providing critical information to enhance the environmental risk assessment of plastic particles, and is based on low concentration, real-world, exposure scenarios. In particular, we will investigate the effects of plastic size on the kinetics and the tissue distributions of plastics in trout. The outcomes of this work will be to provide risk assessors with information to evaluate any realistic risk, which plastic particles may pose in marine ecosystems.
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 “redacted” project aims to provide information to guide environmental protection initiatives, including risk assessments. Our work will also investigate the potential bioaccumulation of plastic particles and co-contaminants in marine organisms. In addition, it will investigate the bioavailability of sorbed co-contaminants after ingestion of plastic particles. The specific benefits of this application for a Project Licence for work on fish are: 1) To provide novel data on the effects of particle sizes on uptake, accumulation and retention of small plastic particles by a vertebrate. 2) To provide novel data on the effects of co-contaminant transport by plastics to a vertebrate. Understanding uptake from water and via food and any subsequent retention of plastic particles, as well as the potential for these particles to transport co-contaminants, will inform subsequent toxicological research experiments and ultimately inform risk assessment. Hence the data will inform further work within the “redacted” project and in the medium term, via conference presentations and peer reviewed publications, it will inform others working on the effects of nanoparticles on wildlife, as well as those attempting to evaluate any effects on human health. Collectively, over a longer timeframe, the data will inform risk assessments on the potential environmental risk of plastic particles, which is of key importance to developing appropriate policy. For example, there

	are policy requirements relating to microplastics within the EU Marine Strategy Framework Directive and legislation has been introduced or is pending in several nations in relation to the release of plastic particles from products such as cosmetics. Understanding the potential environment impacts of small plastic particles is of key relevance. Many scientists believe these larger items will fragment into smaller particles including into nanoplastics. Hence the proposed project has the potential to have very broad reach and significant impact.
What species and approximate numbers of animals do you expect to use over what period of time?	4,800 rainbow trout over approximately 1 year of study
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?	No adverse effects have been reported for similar concentrations used in previous experiments. Severity of this project is expected mild/moderate. Animals are expected to feed voluntarily and to survive the experiment. All fish will be killed by the Schedule 1.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Use of live fish rather than cell cultures or perfused organ preparations is necessary for this project because the integrity of the organism is essential for us to examine the distribution of plastics across the whole organism and thus to address the research questions under investigation. In addition, toxicological experiments to investigate effects on gut physiology and overall fish health require intact and integrated organ systems. Standardized ecotoxicity tests will be based on juvenile fish and all aspects of toxicology from absorption, tissue distribution/accumulation, metabolism/toxicity and excretion, will be under investigation. This cannot be replicated *in vitro*
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experiments will be performed with one fish species and with the minimum numbers needed to collect data which are statistically robust. Following ARRIVE guidelines, the minimum statistically viable sample size, randomized and blind, will be used within this project. Experimental

	designs are based on the minimum number of fish needed for robust statistical analyses of the effects under investigation.
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.	Rainbow trout are cultivated in aquaculture and are commercially important and environmentally relevant. They live in fresh and seawater (i.e. 13-17°C). Rainbow trout have a complete digestive system including a stomach and 'complete' digestive tubing. Their size is ideal for quantitative whole-body autoradiography with a good resolution of tissues within the images. Moreover, rainbow trout have been used as an important fish model in several eco-toxicological studies, which will inform our understanding of the physiological mechanisms acting in the digestive system. Fish will be housed under normal conditions and only exposed to acute (1 day) or long-term (up to 21 days) microplastic, nanoplastic and potentially toxic chemicals in food or water at environmentally relevant concentrations. The fish will then be followed for up to 15 days before being killed to investigate contaminant levels in their tissues. We do not expect the fish to experience any clinical harm, though if toxicity were to occur we would expect to see vomiting, postural and behavioural changes such as erratic swimming behaviour (ataxia), equilibrium loss, rapid operculum movement (tachyventilation), or increased flashing of flanks. Such behaviour will be monitored closely and if any visible signs of severe pathology or infectious disease are noticed the veterinary surgeon will be consulted, and if necessary the experiment will be terminated.

Project	**110. DNA methylation and the development of blood 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)	While all cells of our bodies contain the same DNA, chemical alterations to it can result in important differences in how these cells function. In many cases, these chemical alterations can themselves be inherited as cells multiply. One important type of modification is the addition or removal of methyl groups. Specific enzymes that promote these chemical reactions have been identified. Very importantly, mutations in the genes of some of these enzymes have been found to be associated with certain human blood cancers (leukaemias), suggesting that incorrect methylation of DNA may be an important step in developing these diseases. However, little is known about the	

	molecular mechanisms by which these mutations can cause disease In this project, we aim to study mutations in the mouse equivalents of these genes in order to understand their function in health and in diseases such as the leukaemias.
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)?	Not all forms of human leukaemia can be treated adequately with current methods and some rare forms are very aggressive indeed. New ways of treating these diseases may arise from understanding why mutations in genes involved in the chemical modification of DNA are particularly associated with them.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, approximately 5500 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?	The great majority of the animals will be maintained and killed humanely (for the analysis of tissues and cells) without showing any outward signs of adverse welfare. If animals do become unwell, a blood test will be administered immediately and then they will be killed humanely. In some cases, we shall transplant cells that might be of higher risk of developing leukaemia into recipient mice. To do this successfully, we need to knock-back the recipient's immune system, using radiation (much as bone-marrow transplants are performed in humans). Animals receive extra welfare support throughout the post-irradiation phase, when they might otherwise be susceptible to gut problems or infections.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The development of leukaemia is a complex multi-stage process, involving interactions between a number of different types of cell. It cannot at present be recapitulated without using intact animals.
2. Reduction Explain how you will assure the use	Matings will be carefully planned to ensure efficient crossing of the mouse strains and regulated to meet the needs for experimental

of minimum numbers of animals	animals. We will apply detailed considerations of sample size and appropriate statistical tests to ensure that the experiments will provide a robust result to test our hypotheses.
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 genes in which mutations are associated with human leukaemias are also present in the mouse genome and, at least in some cases, they are known to result in similar diseases. The mouse therefore appears to be a robust model system in which to study the connections between mutation and disease. In our experiments we are particularly interested in the very early steps in disease progression and therefore the majority of the animals will show no outward signs of adverse welfare at all. We have no need to let disease, once it has been detected, develop to the point at which it might cause significant welfare issues.

Project	**111. Dropping the Needle: Non Invasive Drug Delivery to the Eye**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 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 demonstrate that we can deliver drugs to the back of the eye using eye drops rather than injections	
Why is it important to undertake this work?	Age-related macular degeneration (AMD) is a leading cause of vision loss in Europe and the US. Current therapy for AMD is intravitreal injections into the patients eye on a monthly basis for a minimum of 5 years. This is difficult for patients as it is extremely distressing for them. It also has side effects such as retinal detachment which requires surgery on the eye to fix. These side effects come from the method of delivery (injection) rather than the actual therapeutic. The solution we propose with this work is to deliver these drugs as eye drops rather than as injections. This will relive a lot of the problems associated with this	

	treatment regime, save the NHS money, while also empowering patients to allow them to take control of treating their own condition.
What outputs do you think you will see at the end of this project?	The primary output of this work will be the information on the MPPA system demonstrating its efficacy as a novel ocular drug delivery technology. Specific academic outputs will be to publish the findings. This is important for the development of the project but also to provide a knowledge base for other academics working in this field. Specific product outputs will be to support the development of the intellectual property already filed and allow it to translate it into a commercially viable proposition for our organisation. We are already discussing this technology with interested pharmaceutical companies with the intention of progressing to clinical trials as soon as possible using data obtained from these in vivo experiments
Who or what will benefit from these outputs, and how?	In the short term the benefits would be to provide a high impact publications and work for the academics involved raising the profile of early career researchers. This work will also potentially support a an application to the MHRA for a clinical trial of the delivery vehicle. In the longer term this work has the potential to have an immense impact on the lives of patients with age-related macular degeneration. This technology will empower patients to control their treatment by applying it themselves at home rather than attending hospital once a month. This is especially key for patients who develop the disease while still at work as it will remove the need for them to take a day off work a month to have their treatment. This also becomes pertinent as with the ageing population there may be too many patients with the disease for the NHS to provide the treatment easily as the number of specialist trained ophthalmologist to provide injections will not be available. A second beneficiary is health care providers, particularly the NHS. Anti-VEGF therapy injections for age-related macular degeneration are estimated to cost 1 % of the total NHS drugs budget. If the MPPA technology was successful it would relieve this pressure on the NHS significantly reducing this cost burden of treating age-related macular degeneration.

Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We will maximise the outputs of the work by collaborating with academics and companies working in this field to maximise the use of the data we obtain. We will rapidly disseminate the outcomes of the tests whether, negative or positive to inform the academic community and support other researchers developing technologies in this area.
Explain why you are using these types of animals and your choice of life stages.	We have selected rodents as we have the expertise both in the group and at the University to support this work. We have chosen rats as they have a larger surface area on the cornea which will maximise the delivery of the therapeutic into the eye. We have selected adult rats as age-related macular degeneration is an adult disease. Adult eyes are also larger and therefore we will have a larger tissue area for analysis than earlier life stages.
Typically, what will be done to an animal used in your project?	Animals will be restrained and have a single eye drop applied to each eye on one occasion. They will then be rehoused for 20 minutes - 48 hours. The animals will then be killed using a schedule 1 killing method and the tissues harvested. Each animal will only have procedure once.
What are the expected impacts and/or adverse effects for the animals during your project?	The potential impacts include possible ocular surface irritation from the drops. The adverse events predict for this experiment would be ocular irritation from the drops. Control measures would be taken to irrigate the eye with warm saline and provide pain medication. Irritation persisting for more than 24 hours will require a humane endpoint using schedule 1 methods. It is possible that inflammation could occur from the application of drugs currently used by injection when applied topically. If this occurs the animals will immediately be killed using schedule 1 methods. This will occur in less than 2% of cases.
What are the expected severities and the proportion of animals in each category (per animal type)?	All animals will experience mild severity.

What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Blood flow and blood pressure to the eye can alter the ability of drug delivery technologies to deliver drugs into the eye. Unless the system is tested in animals we cannot be sure that the technology will be successful before a clinical trial.
Which non-animal alternatives did you consider for use in this project?	We have a range of screening tests to determine the efficacy of the technology prior to use in animals. Firstly, we use a complete cell monolayer and apply the MPPA and therapeutic to determine the ability of the MPPA to deliver drugs across a cell membrane. Once this has been established we move onto testing the MPPA in biological membrane. We isolate a membrane from a chicken egg and use it a specific testing apparatus to determine that the MPPA can deliver the therapeutic across the membrane. Finally, we use ex vivo porcine eyes obtained from local slaughterhouses. We use the entire eye and apply the MPPA and therapeutic to the corneal surface and then determine if the therapeutic can reach the posterior segment. Only molecules have been successful in these screens are the MPPA tested in vivo.
Why were they not suitable?	An ex vivo eye does not have a blood flow and is not affected by blood pressure. This can affect the ability of the technology to deliver to the posterior segment. The ex vivo model works excellently as a supporting screen to select molecules for in vivo testing but as the eye is dissociated from the blood flow it cant replace in vivo testing.
Enter the estimated number of animals of each type used in this project.	rats: 900
How have you estimated the numbers of animals you will use?	We have carried out statistical modelling. We have also carried out this work previously and had the numbers peer reviewed as part of the publication process.
What steps did you take during the experimental design phase to reduce the number of animals	We have taken advice from statisticians and spoken to MHRA to determine if the experiments will be robust to support moving the technology forward to clinical trial while reducing the number of animals required in each experiment.

being used in this project?	
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	We will design experiments to utilise as much tissue as possible at each timepoint. We will also use experimental design to ensure that both eyes can be used in each animal, this requires optimisation as delivery to one eye can affect the other.
Which animal models and methods will you use during this project?	The animal model is refined to only use topical administration. This ensures that the animals will only experience suffering in the mild catagory.
Why can't you use animals that are less sentient?	Age-related macular degeneration is only seen in adults, so using immature life stages will not provide robust scientific data for the project. Also using rats at a younger age may require more animals so that enough tissue can be obtained to analyse as the tissue is smaller in the younger animals. Insects or fish are not suitable as they do not have the appropriate ocular structure equivalent to the human eye.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We will stay informed about advances in 3Rs by staying abreast of literature in the field to allow us to update protocols where appropriate. We will also attend and present our work at conferences to obtain as much information to feed into this protocol as much as possible.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	We will ensure that all necessary monitoring will be carried out. If any adverse signs are observed then monitoring will be increased and pain management can be applied. Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use. We will use SyRF the free online platform for researchers to perform a systematic review and meta-analysis of animal studies. https://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf
What published best practice guidance will	All experiments will be carried out according to the ARVO best practice guidelines for the use of animals in vision

you follow to ensure experiments are conducted in the most refined way?	research. Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment. The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines

Project	**112. Drug Discovery for Neglected Diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project seeks to discover better and safer treatments for parasitic diseases that afflict millions of poor people and their domestic animals living in tropical and sub-tropical regions of the world. Current drugs are unsatisfactory for a variety of reasons, including high cost; unacceptable (often serious) side effects to adults, pregnant women and children; the need to be given by injection rather than by mouth; and increasing treatment failures, often due to the emergence of drug-resistant strains of parasite. The diseases we study include malaria, which causes about 600,000 deaths a year, mainly in	

	young children in Africa, and three “neglected diseases” (African trypanosomiasis, leishmaniasis and Chagas’ disease) which collectively cause about 50,000 human deaths a year. Trypanosomiasis is also a serious animal health problem in Africa. Better treatments for these diseases would lead to better health and greater productivity thereby helping to alleviate poverty in developing nations.
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 aid the discovery of potential new medicines. The data generated in this project will be essential to the development of new medicines as they provide a means to select substances for full preclinical development and thence into clinical trials. Three new potential drug treatments have entered clinical trials from my previous licences.
What species and approximate numbers of animals do you expect to use over what period of time?	Model systems for the study of these serious human and animal diseases have been established in mice, rats and hamsters. The total number of animals to be used over the course of the project has been estimated based on a prediction of the likely number of new substances for investigation. It is anticipated that up to 4800 adult mice, 500 adult rats and 200 adult hamsters will be used over the 5 year duration 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?	Our interventions are of two types. We shall test the efficacy of novel compounds to treat the disease state in the rodent model systems. Of course, we shall only do this when there are robust data from laboratory studies to suggest that the compounds are active and not generally toxic. In these studies animals will have discomfort from dosing the potential medicine (this is usually done by injection into a vein or by giving it via a tube passed down the throat and into the stomach). If the medicine is toxic (rare) the animals may lose weight, start to have muscle tremors or become very subdued – animals showing these signs would be humanely killed. If the drugs don’t work at killing the parasites, or the animals are in a group that don’t receive a treatment, they can show clinical signs of the disease as described below. Secondly, in order to have parasite preparations that are

	representative of those that are infectious in animals, for use in these laboratory studies, we shall have to harvest parasites from infected animals. These are standard procedures, where the severity can be precisely controlled and in which unexpected events should be very rare. Our rodent models of infection closely mimic the course of the human diseases and the outcome of treatment with the currently available medicines. The early symptoms of these infections are flu like (fever, loss of appetite, general malaise), but, like human patients, can be ultimately fatal if not adequately treated. By frequent monitoring of the health of infected animals and by measuring parasite numbers in small blood samples, we can usually withdraw animals from the studies and kill them humanely before signs of serious illness or death from the disease occur. However, our initial studies on T. cruzi (the causative agent of Chagas' disease) have demonstrated that these observations and tests are not always predictive of the stage of disease, mainly because these parasites hide in the tissues of the body, rather than circulating in the blood. We have therefore applied advances in whole animal non-invasive imaging technology to measure the total parasite load and thereby reduce the severity of the protocol for developing new medicines for Chagas' disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Compounds that show promising activity in laboratory tests may still prove to be poor agents *in vivo* and therefore there is still no alternative to using animals to select promising compounds for further preclinical testing and clinical trials in humans or the "target" animal species (e.g. cattle). It is indeed a legal requirement to demonstrate safety and efficacy in animal models before clinical trials can begin. Parasites are sometimes difficult to obtain in sufficient amounts from *in vitro* culture and have to be obtained from animals. The nutritional environment in tissue culture is different to that of the animal host and it is important to confirm that parasite adaptation to *in vitro* conditions has not affected virulence in animals or susceptibility
	to experimental compounds.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful experimental design is used to determine that the correct number of animals (neither too many, nor too few) is used to obtain biologically significant results. Generally, for efficacy studies, based on previous experience, group size will usually be about 5 animals. Compounds found to be active will be re-tested at serial dilutions to determine the minimal effective dose. This will usually require up to 5 groups, each with 5 animals, based on a statistical method known as the resource equation method that is used to calculate a reliable effective dose. If we used fewer animals or less doses for potential treatments there is risk of not adequately and robustly defining the minimal dose that works or the amount of substance the pathogens have been exposed to. This information is used to predict the correct human dose and what the safety margins are. In some circumstances we may be able to share parasites produced by other labs, reducing the numbers of animals we require for parasite production.
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 rodent models of infection closely mimic the course of the human diseases and the outcome of treatment with the currently available medicines. The early symptoms of these infections are flu like (fever, loss of appetite, general malaise). As noted above, observation and measurement of the numbers of parasites in small blood samples can usually be predictive of the onward course of the disease and therefore can allow the animals to be humanely killed before the disease state worsens, whilst still giving us the scientific information we need. Chagas' disease is an exception, so the model of Chagas' disease uses bioluminescent imaging to monitor the parasitaemia (i.e. where the parasite has been changed in the lab so that it will glow under the right type of light stimulus) and allow the early humane killing of animals on a study if the treatment is not working while still providing robust data.

Project	**113. Drug Metabolism and Pharmacokinetics**	
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 work to be carried out under this project will be used to select the most promising substances for further development as medicines for serious human and animal diseases. The studies are designed to generate information on what the body does to a substance as a function of time (pharmacokinetics) and the Absorption, Distribution, Metabolism and Excretion (ADME) of new substances in rodents. This will inform our decision making on substance progression within drug discovery programmes for any disease area, directing what changes to a substance a medicinal chemist may need to do to further improve it.	
Why is it important to undertake this work?	New medicines are urgently required for serious diseases, particularly parasitic diseases affecting the poorest of the poor and their domestic animals living in endemic areas of	

	Africa, Asia and South America. The work under this licence is intended to allow the most efficient pathways from drug discovery to real benefits in patients to be followed, by weeding out substances as early as possible that would otherwise fail. This will also prevent the unnecessary use of animals in testing such substances at the later stages of their development.
What outputs do you think you will see at the end of this project?	This project will allow the optimisation of active leads and identification of potential preclinical development candidates for a range of diseases. In particular, with tropical disease research a high priority , substances will be identified under this project licence as suitable candidates for the treatment of serious neglected parasitic diseases.
Who or what will benefit from these outputs, and how?	This project seeks to discover better and safer drug treatments for a range of diseases, including a number of parasitic diseases that afflict millions of people and their domestic animals living in tropical and sub-tropical regions of the world. Here, current drugs are unsatisfactory for a variety of reasons, including high cost; unacceptable (often serious) side effects to adults, pregnant women and children; the need to be given by injection rather than by mouth; and increasing treatment failures, often due to the emergence of drug-resistant strains of parasite. No suitable vaccines are available and other control measures are often ineffective. The parasitic diseases under investigation include malaria, which causes about 600,000 deaths a year, mainly in young children in Africa, and three “neglected diseases” (African trypanosomiasis, leishmaniasis and Chagas’ disease) which collectively cause about 50,000 human deaths a year. Trypanosomiasis is also a serious animal health problem in Africa. Better drug treatments for these diseases would lead to better health and greater productivity thereby helping to alleviate poverty in developing nations. The data generated in this project will be essential to the development of new drug treatments as it provides a means to select the best substances for entry into full preclinical development and thence into clinical trials. Preclinical development candidates brought forward under this programme of work will then undergo further development in partnership with pharmaceutical companies and/or agencies such as the “redacted”. From our previous licence covering this project, three substances were

	delivered and are now in clinical trials. The short to medium term benefit of this project is to rapidly advance drug discovery programmes for a broad range of diseases, delivering high quality candidate molecules for progression through regulatory toxicology and entry into clinical trials. With high attrition in drug development, it is critical a number of candidate molecules are identified and progressed in order to deliver on the long term benefit, which is delivery of new, safe, affordable marketed drug treatments that will have a major impact on the health and wellbeing of millions of people.
Will this work be offered as a service to others?	Yes
How will you look to maximise the outputs of this work?	The outputs of this work will be high quality substances suitable for progression toward treating patients across a range of diseases. These substances will be partnered with pharmaceutical companies or other organisations such as the drugs for neglected diseases initiative to ensure the path toward the patient is as quick as possible. At an appropriate time, detail will be published in high impact journals.
Explain why you are using these types of animals and your choice of life stages.	Adult rodents (mainly mice or rats) are the most characterised vertebrate group from which suitable data can be obtained on how the substance of interest behaves in the body. Mouse is primarily the rodent species of choice as it is often the animal model of disease in those diseases where we have active drug discovery programmes, low amounts of substance are required to conduct studies (mg quantities) and full blood concentration-time profiles can be obtained from individual animals for decision-making purposes. In some cases where alternative rodent species will be used as the disease model (e.g. the hamster for visceral leishmaniasis) then the pharmacokinetics will be determined in that species to support these studies. Rat is most often used as the rodent species in regulatory toxicology testing of a new substance so pharmacokinetics will be determined in this species to support those studies.
Typically, what will be done to an animal used in your project?	The majority of studies performed under this project will be single administration, low dose pharmacokinetic studies with serial blood sampling up to 24 hours. Typically compounds will be administered via the intravenous or oral route. However, other routes of dose administration may be

	needed. For compounds which are either poorly absorbed or highly cleared it may be necessary to evaluate systemic exposure following administration via peripheral routes such as intraperitoneal or subcutaneous, in order to assist the pharmacologist with the design of dosing regimens. DMPK studies to support topical and intranasal targets may also be carried out under this licence. Typically for intravenous administration, animals will receive a slow intravenous injection. Some studies will involve a multiple dose regimen, but this will be limited to up to three times daily, dependent on dose route, typically for up to 5 days. It would be required, for example, to assess the induction of drug metabolising enzymes or the exposure and tolerability of a substance following repeat dose administration. For tolerability studies and dose escalation studies, where exposure is assessed with increasing dose (typically 3 dose levels), animals will be closely monitored for any nonspecific/unexpected adverse effects and if limiting clinical signs are observed, animals will be promptly killed by a schedule 1 method. This is very rare. On occasion, osmotic pumps may be implanted subcutaneously with or without vessel cannulation to deliver sustained exposure of a substance in order to more rapidly (thus saving animal numbers) achieve in vivo proof of principle of the substance series and/or biological target. Once implanted, these pumps require no external connections or intervention during the entire delivery period. Their unique design helps save critical time by eliminating the need for frequent animal handling and repetitive injection schedules. Different blood sampling techniques will be used depending on the type of study and the data required. • For the majority of studies blood will be taken following venepuncture from the tail vein (saphenous vein from hamsters). Blood sampling at more than one timepoint is often achievable using a single venepuncture, thus reducing total number of venepunctures required for a full PK time-course. Animals may be brought in from commercial suppliers with one or more vessels cannulated and acclimitised before use. Since cannulation is a difficult procedure and we use it rarely, the benefit of using animals prepared by surgeons who are performing the surgery regularly generally outweighs the harm of transport.

	• Singly surgically cannulated animals may be used for sampling where information is required in the same animal (e.g. in definitive cross-over, comparative formulation or dose escalation studies). Blood sampling from a cannula may reduce the stress on the animal due to a reduction in animal handling, a reduction in the number of venepunctures and removal of the need for exposure to a warm environment to facilitate sampling. • Dual surgically cannulated animals are also used if the dose is administered by intravenous infusion where the pharmacology or formulation precludes the use of a bolus and/or the intravenous profile requires further definition. The second cannula for blood sampling will reduce the stress on the animal due to a reduction in animal handling, a reduction in the number of venepunctures and removal of the need for exposure to a warm environment to facilitate sampling. • If a sampling cannula becomes blocked a pharmacokinetic profile may be completed using peripheral vein sampling as long as the animal remains in good health. Obtaining the samples would enable the objectives of the study to be achieved, data quality will not be compromised and the use of additional animals in a repeat study will be prevented. • Where it is scientifically beneficial to take serial blood samples following two or more dose administrations to a cannulated rodent (e.g. for a definitive cross-over, a comparative formulation or a dose escalation study) the volume of blood required will not exceed 15% of total blood volume (TBV) collected in a 4-week period and no more than 10% TBV at any one time. Typically, rodent acute organ perfusion studies allow an estimation of the distribution, retention and/or metabolism of substances within organs of interest that may be a target for activity and/or responsible for removal of the substance and are used to answer specific, programme-dependant questions. The entire procedure is typically carried out under terminal anaesthesia. However in some instances conscious animals may be dosed prior to terminal anaesthesia, for example with heparinised saline or a transporter inhibitor, to ensure optimal circulation, tissue perfusion or investigation of the effect of enzyme or transporter mechanisms on substance disposition.
What are the expected	ADME/PK studies are generally short term (24 hours or

impacts and/or adverse effects for the animals during your project?	less), low dose acute studies which limit the number of adverse events observed. Dose ranges selected are designed to ensure that the exposure to substances is below that which elicits an off-target or target related pharmacological response. Adverse effects of dosing and sampling are not expected. In a very small number of cases, animals will be bought in with a cannula placed in one or more major blood vessels that were implanted in a surgical operation. After the animal's recovery, they will be transported to our establishment and the cannula used to allow dosing and sampling to take place over a period of time, without having to subject the animal to repeat injections. Similarly, an osmotic minipump may be implanted surgically, performed in house, to permit the long-term dosing of an animal without any further intervention being necessary. All surgical procedures will be carried out under general anaesthesia, with pain relief and post-operative support being provided to all animals. Based on our previous licence the percentage of animals undergoing these techniques is extremely low and certainly <1% of total numbers.
What are the expected severities and the proportion of animals in each category (per animal type)?	The severity categories in this project are mild or moderate. Based on the actual severity procedures reported in our previous licence, we expect approximately 4% of mice or rats will be moderate with the remainder mild.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Animals need to be used because there are no reliable alternatives currently available to achieve the objectives of this project. Individual parameters can be evaluated in in vitro systems e.g. solubility or affinity for metabolising enzymes but these can only be evaluated in isolation. While good progress has been made in recent years with both in silico and in vitro models these approaches have their limitations when predicting what happens to a substance in the whole organism (in vivo) as the fate of a substance in the body can be complex and reliant on many inter-dependent processes. Consequently, this makes it necessary to evaluate those substances having the most appropriate in silico and in vitro profiles in living animals in order to gain confidence that only the most appropriate

	substances are being progressed toward full preclinical development and predicting what will happen when the substance is given to humans for the first time. Demonstration of safety and efficacy in animal models is a legal prerequisite for human clinical trials.
Which non-animal alternatives did you consider for use in this project?	No reliable alternatives are currently available to predict how a substance behaves in the animal.
Why were they not suitable?	It is currently very difficult to predict how a substance behaves in a whole organism, particularly metabolism and transporter interaction. These help define some of the key parameters we have to understand and optimise in order to deliver a high quality substances with potential to treat a disease.
Enter the estimated number of animals of each type used in this project.	mice: 2600 rats: 725 hamsters: 40
How have you estimated the numbers of animals you will use?	The number of animals to be used is based on the number of active drug discovery programmes we are supporting and animal use metrics from my previous two HO project licences on this.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	The minimum number of animals will be used to generate quality ADME/PK information which enables the objectives of individual studies to be achieved. Numbers are decided based on experience of qualified scientists and their knowledge of the programme of work. Rodent studies typically involve determination of ADME/PK in three animals, per route, per substance, using a single dose acute regimen. This is deemed sufficient to enable valid decisions to be made. Exceptions to this would generally be where a tissue concentration-time profile is required or when using the hepatic portal vein model which requires composite sampling and enables estimation of the fraction absorbed and hepatic extraction. Statistics are not employed in ADME/PK studies as the low number of animals used does not support this type of analysis. Indeed, for pharmacokinetic studies it will not usually be necessary to use numbers of animals required to achieve statistical significance as the parameters determined in the

	studies are mainly used as a guide for substance progression or termination.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	The same substance may be administered to the same animal on multiple occasions. This experimental design may be utilised when assessing intravenous and oral pharmacokinetics (crossover studies), performing dose escalation studies or assessing formulation options for one substance to reduce inter-animal variability which in turn increases the confidence in the data whilst reducing the number of animals required. Early “snapshot” PK (reduced blood sampling time-points) will be used where appropriate for preliminary screening work thus reducing stress on the animals due to a reduction in animal handling, a reduction in the number of venepunctures and removal of the need for exposure to a warm environment to facilitate sampling. Dosing of multiple substances (as a cassette, simultaneously; typically n=5) to individual animals may be utilised when information on a large number of substances is required, thus significantly reducing animal usage. The total dose and/or expected pharmacological effects should be comparable to those of a single substance. Where a cannulated animal has been the subject of a pharmacokinetic study in which it experienced no significant adverse welfare effects, it may be re-used in further pharmacokinetic studies. This will reduce the total number of animals that have to undergo the cannulation operation without impacting significantly on the lifetime severity experienced by any one of them. Computational (in silico) models may be built and refined using information from PK studies. As these improve, we expect a further reduction in the use of animal experiments in future. Higher throughput in vitro methods such as in vitro metabolic stability are validated using data from animal studies and in those drug discovery programmes where there is a good in vitro:in vivo correlation, these in vitro technologies will be rigorously applied and lead to a reduction in animal studies.
Which animal models and methods will you use during this project?	ADME/PK studies are generally short term (24 hours or less), low dose acute studies which limit the number of adverse events observed. Doses selected are designed to ensure that the exposure to substances is below that which elicits an off-target or target related pharmacological response. Adverse effects of dosing and sampling are not

	expected. On occasion, a multiple dose regimen may be required to achieve and maintain steady-state concentrations in blood/tissue or allow investigation of the effect of substances on enzyme (i.e. induction or inhibition of CYP450) or transporter mechanisms and therefore any potential changes in the pharmacokinetic profile of a substance. On occasion it may be necessary to administer, or co-administer, compounds of known pharmacological effect. These compounds may be enzyme or transporter inducers or inhibitors and it may be necessary to administer these prior to, and/or concurrently with test substances over several days, by an appropriate route/regimen. Occasionally it may be necessary to assess the tolerability of a substance following repeat dose administration and/or administer higher doses of a substance e.g. to confirm adequate systemic exposure to support safety assessment studies. In such cases there is a risk that some adverse effects may occur as a result of off-target pharmacology. These will be minimised by several factors: 1) precedence with respect to the substance or substance class at doses up to or exceeding that to be used, 2) close monitoring for clinical signs and 3) NVS/NACWO advice if required as to whether the animal will be euthanized. Typically compounds will be administered via the intravenous or oral route. However, other routes of dose administration may be needed. For compounds which are either poorly absorbed or highly cleared it may be necessary to evaluate systemic exposure following administration via peripheral routes such as intraperitoneal or subcutaneous, in order to assist the pharmacologist with the design of dosing regimens. DMPK studies to support topical and intranasal targets may also be carried out. Typically for intravenous administration, animals will receive a slow intravenous injection. Some studies will involve a multiple dose regimen, but this will be limited to up to three times daily, dependent on dose route, typically for up to 5 days. It would be required, for example, to assess the induction of drug metabolising enzymes or the exposure and tolerability of a substance following repeat dose administration. For tolerability and dose escalation studies, animals will be closely monitored for any nonspecific/unexpected adverse effects and if limiting clinical signs are observed, animals will be killed by a schedule 1 method. This is rare. On occasion, osmotic pumps may be implanted

	subcutaneously, with or without femoral or jugular cannulation to deliver sustained exposure to a substance in order to more rapidly (thus saving animal numbers) achieve in vivo proof of principle of the substance series or biological target. Once implanted, these pumps require no external connections or intervention during the entire delivery period. Their unique design helps save critical time by eliminating the need for frequent animal handling and repetitive injection schedules. Different blood sampling techniques will be used depending on the type of study and the data required. • For the majority of studies blood will be taken following venepuncture from the tail vein (saphenous vein from hamsters). Blood sampling at more than one timepoint is often achievable using a single venepuncture, thus reducing total number of venepunctures required for a full PK time-course. Animals may be brought in from commercial suppliers with one or more vessels cannulated and acclimitised before use. Since cannulation is a difficult procedure and we use it rarely, the benefit of using animals prepared by surgeons who are performing the surgery regularly generally outweighs the harm of transport. • Singly surgically cannulated animals may be used for sampling where information is required in the same animal (e.g. in definitive cross-over, comparative formulation or dose escalation studies). Blood sampling from a cannula may reduce the stress on the animal due to a reduction in animal handling, a reduction in the number of venepunctures and removal of the need for exposure to a warm environment to facilitate sampling. • Dual surgically cannulated animals are also used if the dose is administered by intravenous infusion where the pharmacology or formulation precludes the use of a bolus and/or the intravenous profile requires further definition. The second cannula is used for blood sampling, thus reducing the stress on the animal due to a reduction in animal handling, a reduction in the number of venepunctures and removal of the need for exposure to a warm environment to facilitate sampling. • If a sampling cannula becomes blocked a pharmacokinetic profile may be completed using peripheral vein sampling as long as the animal remains in good health. Obtaining the samples would enable the objectives of the study to be achieved, data quality will not be compromised and the use of additional animals in a repeat study will be

	prevented. • Where it is scientifically beneficial to take serial blood samples following two or more dose administrations to a cannulated rodent (e.g. for a definitive cross-over, a comparative formulation or a dose escalation study) the volume of blood required will not exceed 15% of total blood volume (TBV) collected in a 4-week period and no more than 10% TBV at any one time. Rodent acute organ perfusion studies allow an estimation of the distribution, retention and/or metabolism of substances within organs of interest that may be a target for activity and/or responsible for removal of the substance and are used to answer specific, drug discovery programme-dependant questions. The entire procedure is typically carried out under terminal anaesthesia, however in some instances conscious animals may be dosed prior to terminal anaesthesia, for example with heparinised saline or a transporter inhibitor, to ensure optimal circulation, tissue perfusion or investigation of the effect of enzyme or transporter mechanisms on substance disposition.
Why can't you use animals that are less sentient?	Rodents (mainly mice or rats) are the most characterised vertebrate group from which suitable ADME/PK data can be obtained. Mouse is primarily the rodent species of choice as low amounts of substance are required to conduct studies (mg quantities) and full concentration-time profiles can be obtained from individual animals for decision-making purposes. Mouse is also more often the disease model species. In some cases where alternative rodent species will be used as the pharmacology model (e.g. the hamster for visceral leishmaniasis) then the ADME/PK will be determined in that species to support these studies. Rat is most often used as the rodent species for regulatory toxicity testing so ADME/PK will be determined in that species to support these studies. Genetically altered or mutant animals may be used to investigate specific ADME properties, e.g., the CF1 mdr1a deficient mouse or the HRN^{TM} mouse. These are not expected to exhibit altered welfare .
How will you stay informed about advances in the 3Rs,	Regular monitoring of NC3Rs website for updates. If a refinement to a method that is used in this project is noted, through scientific meetings, when performing literature

and implement these advances effectively, during the project?	searches, or if raised by the named information officer, we would investigate whether it is appropriate to our models and if yes, adopt as practice.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	The advice of the NACWO/NVS may be sought on matters of animal day to day care and welfare. Animals will be housed in groups where possible and environmental enrichment provided. In situations where it is required to either lightly restrain, wear jackets (e.g. to protect surgically implanted cannulae) and/or to restrict the extent of normal movement of animals (e.g. for the collection of urine/faeces in metabolism cages), acclimatisation before experimental use will be required. This will minimise any stress induced by the procedures thus improving animal welfare and also the data obtained if the stress induced is felt to adversely affect the scientific results. If the use of metabolism cages is required, animals would be housed for no longer than 3 days per study. In addition, rodents may be held in a warm environment for a short time prior to intravenous dosing and blood sampling from a superficial blood vessel. Furthermore, the following general principles will be applied: a) In some studies it may be necessary for animals to be housed singly for a period of time, particularly during recovery from surgery or when given a topical application. This may result in animals showing visible signs of anxiety. To reduce the visible signs of anxiety and any long term adverse events, additional environmental enrichment will be provided unless it would interfere with the scientific outcomes. b) In some studies, it may be necessary to withhold food and/or water, prior to dosing, for up to 24 hours in adult rodents. This may be, for example, to ensure consistency of absorption of orally administered substances. It is considered that withdrawal of food or water for this short period will not cause appreciable distress. Highly trained staff conduct established standard procedures with associated high quality animal welfare practices.
What published best practice guidance will you follow to ensure experiments are	In addition to information and guidance published on the appropriate NC3Rs website link (https://www.nc3rs.org.uk/animals-drug-discovery-and-

conducted in the most refined way?	development) : Baumans V, Brain PF, Brugére H, Clausing P, Jeneskog T, and Perretta G (1994). Pain and distress in laboratory rodents and lagomorphs: Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Pain and Distress accepted by the FELASA Board of Management November 1992. Lab Animals 28(2), 97-112 Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM and van de Vorstenbosch C (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. J. Applied Tox., 21 (1), 15-23 Removal of blood from laboratory mammals and birds. First report of the BVA / FRAME / RSPCA / UFAW Joint Working Group on Refinement (1993). Lab.animals 27(1), 1-22 Hem, A., Smith, A.J., Solberg, P (1998). Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guineapig, ferret and mink. Laboratory Animals, 32 (4), 364-368 Morton, DB and Hau J (2011) Chapter 18: Welfare assessment and humane endpoints. In Handbook of Laboratory Animal Science, 3rd Edition, Volume 1 Essential Principles and Practices. Hau J and Schapiro, SJ (eds), CRC Press LLC, USA, pp 535-572 Joint Working Group on Refinement (2011) A guide to defining and implementing protocols for the welfare assessment of laboratory animals. Laboratory Animals 45: 1-13 Institute for Laboratory Animal Research (ILAR) (2008) Recognition and Alleviation of Distress in Laboratory Animals. Washington, DC: National Academies Press Institute for Laboratory Animal Research (ILAR) (2009) Recognition and Alleviation of Pain in Laboratory Animals. Washington, DC: National Academies Press. See http://dels.nas.edu/animal_pain/ Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., LaCroix-Fralish, M.L., Matsumiya, L., Sorge, R.E., Sotocinal, S.G., Tabaka, J.M., Wong, D., van den Maagdenberg, A.M.J.M., Ferrari, M.D., Craig, K.D., Mogil, J.S., 2010. Coding of facial expressions of pain in the laboratory mouse. Nat. Methods 7, 447–449.

Project	**114. Drug trial to target the mechanisms of a cardiomyopathy**	
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?	Arrhythmogenic cardiomyopathy (ACM) affects on average 1 in 2000 individuals in the general population and is a major cause of sudden death in the young. Although we know the genetic basis of the disease, we still do not understand how the mistakes in the DNA (mutations) promote abnormal heart rhythms (arrhythmias) and heart injury predisposing young people to dying suddenly. This lack of understanding makes therapeutic interventions very challenging. We have identified four pathways as driving forces in ACM progression. In this protocol we aim to inhibit these pathways in two well-characterized mouse models of the disease. This will significantly improve our comprehension of the disease. More importantly though, it will open the route for development of mechanism-based therapies in patients.	

Why is it important to undertake this work?	Previously we identified a drug that can completely stop the disease in a number of experimental models. Unfortunately, the drug is toxic and hence intolerable for patient use. This work showed us, however, that if we block one particular enzyme, we can block ACM. In this protocol we will try drugs that inhibit the exact same enzyme, which have already been given to patients for other diseases and are known to be safe. Up until now inflammation was thought to be a consequence of the ACM destructive process. We now have strong evidence that it is in fact a primary, driving force. If we administer an anti-inflammatory chemical substance to an ACM mouse model, we can stop disease progression. In this protocol we aim to understand the exact mechanism through which inflammation promotes ACM and study the efficacy of widely-used anti-inflammatory drugs. The mTOR pathway is vital in regulating the cell cycle. We have robust in vitro evidence that this pathway is upregulated in ACM and that blocking it can mitigate the disease. We now want to inhibit mTOR in mouse models using drugs that are widely used by patients. Finally, microRNAs are small molecules that regulate several functions within a cell. There is increasing evidence that dysregulated levels of microRNAs play a major role in human disease. In a recent study, we showed that two microRNAs are massively increased in our ACM mouse models. In this protocol we aim to inhibit them in order to establish whether their over-expression is causally related to disease or whether they are merely biomarkers. Collectively, these studies will tremendously improve our understanding of disease mechanisms and ultimately pave the way for clinical trials.
What outputs do you think you will see at the end of this project?	As detailed above, this project will give us valuable information on understanding the mechanisms of pathogenesis of arrhythmogenic cardiomyopathy and sudden death. It is also anticipated to provide the basis for future clinical trials. The results obtained will be published in high impact journals and presented in major cardiology conferences.
Who or what will benefit from these outputs, and how?	Our work will benefit the scientific community - in terms of new information that is lacking in the field- and above all benefit the patient. it is anticipated that the results will be published prior to completion of the PPL, and possibly

	clinical trials can follow shortly after. The “redacted” has longstanding experience and support in designing and implementing clinical trials and follows hundreds of families every year.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We already have longstanding scientific collaborations with experts worldwide and we intend to share all new knowledge with all field experts either through publications or through presentations. This shall include possible unsuccessful approaches to avoid unnecessary experiments being duplicated by other groups.
Explain why you are using these types of animals and your choice of life stages.	All scientific insights were initially derived from cell culture models. Unfortunately, however, such models cannot replicate the heart physiology in vivo. We now need live animals to study the efficiency of drugs on whole organism levels and organ structure/function. Similarly, although computer simulation and mathematical models may aid in the understanding of disease pathways, they do not provide the possibility of testing drug efficiency. Nevertheless, we shall keep seeking alternative models throughout the duration of the project. We will use two mouse models of disease, each harbouring a different mutation causing ACM. Groups of 6 animals per strain will receive different pathway inhibitors. A single group will not receive any pharmaceutical agent and serve as a positive control. Few mice per strain will be used merely for breeding purposes and maintenance of the colony. A total of 370 animals will be bred over the 5 year period, 240 of which will be used in experiments We have selected time points for completion of all experimentation way before the time that the animals are known to show any clinical signs of disease. We are using the minimal numbers possible to obtain physiologically significant results (and hence avoid the chance of having the repeat any experiments) as well as account for animals that may need to be removed from the protocol. Moreover, to minimize numbers even further, we are trying 4 drugs concurrently using only a single control (no drug) group. Numbers in experimental groups and breeding schemes have been calculated based on extensive previous experience, expert statistical advice and multiple power

	calculation softwares to reduce animals used.
Typically, what will be done to an animal used in your project?	The animals will initially be used merely for breeding purposes. Pharmaceutical agents will be administered by a very experienced user causing only minimal discomfort. At specific time-points during the treatment regime, the animals will be subjected to an electrocardiogram (ECG) to evaluate the efficacy of the drugs on heart function. This is performed with the aid of mild anaesthesia to prevent any discomfort. At the end of the treatment, the mice will be transferred to an imaging facility where their hearts will be imaged also under mild anaesthesia. At the end of this, they will be humanely euthanized.
What are the expected impacts and/or adverse effects for the animals during your project?	None of the above-listed procedures is regarded as severe or is anticipated to cause excessive distress to any animal. The untreated animals, however, may show signs of cardiac dysfunction prior to completion of the protocol (rare). These include but are not limited to hunched posture, lethargy, anorexia and dehydration. If such a case occurs, a veterinary consult will be sought immediately and the animal removed from the protocol.
What are the expected severities and the proportion of animals in each category (per animal type)?	The maximum severity is moderate. We have designed the experiments in such a way so that all experimentation will be completed prior to the animals showing any sign of disease. Still, if any animal, for any reason does show any sign of concern, humane end points will immediately be applied.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Unfortunately it is not possible to achieve the objectives of this project without the use of live animals. In vitro systems have helped tremendously both in deciphering the signalling pathways involved in disease pathogenesis and in shortlisting pharmacological agents with the potential to limit disease onset and progression. We are now, however, at the point where we need to assess the effect of drugs on cardiac function and structure.
Which non-animal alternatives did you consider for use in this	Our cell culture model shows structural abnormalities in the form of apoptosis and relocalisation of proteins. Some groups have even used cell culture models to show fat

project?	accumulation. Still, useful as they may be for initial screening, these parameters cannot substitute the need for live cardiac function imaging. Similarly, in the past we have used cellular electrophysiology to obtain action potential recordings from our cell culture models. Although these measurements showed us the changes in the sodium and potassium currents, they cannot substitute the need for an ECG that will record arrhythmias. Changes in action potential morphology does imply alterations in electrical channel communication but cannot be readily translated into an increased risk of sudden cardiac death. Wherever possible, knowledge obtained from our live animals will be confirmed and expanded in vitro.
Why were they not suitable?	The parameters assessed by in vitro and computer models cannot fully model the structure and function of a beating heart and hence cannot replace the need for live animals.
Enter the estimated number of animals of each type used in this project.	mice: A total of 370 animals will be bred over the 5 year period, 240 of which will be used in experiments
How have you estimated the numbers of animals you will use?	experimental design is included as an attachment as well as EDA reports and power calculation reports.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	The power of the experimental design is that it allows the researcher to obtain solid experimental data with the minimal number of animals necessary. The answer is not always to use very few animals as this may introduce a great variation in results, decrease their validity and require the repetition of the exact same experiment. We have designed our experiments so that 4 agents will be given to 4 groups of mutant animals at the same time. This strategy, emphasized to us during the PIL and PPL courses, will allow us to use a single control group that wont be receiving a pharmacological regime. We have years of experience in designing animal experiments. The proposed design has been reached with the aid of professional statistical advise. If needed, during the duration of the project additional advise will be seeked from expert statisticians with experience in the cardiovascular field. Invivostat was used for the power calculations (http://www.invivostat.co.uk). The final report is included as an attachment (Attachment 2). Briefly, to obtain valid results

	we need 6 mutant animals allocated to each pharmacological group with 6 mutant animals being allocated to the control (vehicle) group. If each drug was to be tested in a separate experiment, we would need 6 mutant animals to be treated and 6 mutant animals to act as vehicle controls at a time. For a total of 4 drugs, we would have used: 4 x 6 = 24 vehicle controls. With our 'concurrent drug design', however, we significantly reduce this number from 24 to 6 controls. If 4 mutant animals were to be allocated in each drug group, the power of the experiment to detect a 50% change from controls would have been 87%. Given the variation that exists between individual experimental units (mice), expert statistical advise deemed this percentage low to avoid the need to repeat the experiment in the future. With 6 mutant animals allocated per group, however, the power of the experiment to detect a 50% change from control rises to 97%. 8 mutant animals would have increased the percentage to 100% but we do not think this is statistically different from 97% so we decided to use the absolute minimum number possible that is 6 mutant animals per group. The drugs, need to be administered to wildtype (non-GA) animals as well. This will ensure that the drugs alone do not affect any of the endpoints measured in the study. Power calculations were made with the aid of invivostat. As shown in Attachment 2, just like with the mutant animals, the minimal number of non-GA animals needed to be allocated in each group is 6. Experimental design assistant (EDA) reports are included as separate attachments. One report, shows the detailed breakdown of one cycle of experiments for the Dsg2 line and the second report showed the detailed breakdown of one cycle of experiments for the JUP line along with appropriate controls. Each of these explains every step for the testing of 4 substances. Each needs to be repeated one more time for the testing of an additional 4 substances (please see attachments: EDA Dsg2 and EDA JUP).
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use	As outlined in the background section, we previously showed that a GSK3b inhibitor, SB216763, completely abolishes the disease phenotype in both JUP and Dsg2 mice. In this original study, we measured exactly the same endpoints that we propose herein. The cardiac ejection

in your project?	fraction is a measure of cardiac function. Animals treated with SB216763 showed a 23% increase in ejection fraction compared with vehicle-treated controls with a standard deviation of 11. We inputted the observed changes between the drug-treated and vehicle-treated animals into the Power and Sample Size software with the following outcome: ‘‘The response within each subject group was normally distributed with standard deviation 11. If the true difference in the experimental and control means is 23, we will need to study 6 experimental subjects and 6 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.9.The Type I error probability associated with this test of this null hypothesis is 0.05’’. This software outcome is in agreement with the power calculations provided by Invivostat and suggested by the expert statistician consulted. However, this study only tested one pharmaceutical agent. Because we are planning on testing 4 agents concurrently we can use a single control group of 6 as opposed to 4 separate groups of 6. The software analysis is included under Attachment 2. Secondly, the animals to be used are extensively phenotypically characterized. This means that no baseline measurements are required and the animals can immediately enter the pharmacological regimes. Thirdly, we will allocate the animals randomly to treatments and the “redacted” will be blinded to the genotype and pharmacological treatment of each animal while obtaining imagining measurements. This will alleviate any potential source of bias that could decrease the power of the experimental results deeming their repetition necessary. Since sex does not seem to significantly influence the ACM phenotype in mice, animals from both sexes will be used so no animals will be wasted from a litter at all. Finally, the same animal will be used for all end points! Each animal will be given a drug, subjected to serial ECG recordings, then to imaging and finally to pathological examination. This continuous use of every animal ensures the least possible number of mice. All animals used will be inbred, stemming from a small number of litters, housed in the same environment under the same conditions. This will ensure decreased variability in the results obtained. We shall only use drugs that have been extensively given to mice before as treatments for different diseases. This will ensure that there is extensive information on routes of administration, precise dosages and possible adverse

	effects. This way we shall not sacrifice any animals to optimize a drug regime and we will use the minimum number of animals possible. The big advantage of using inbred mouse strains is that all animals will be genetically highly similar and the phenotypic manifestations of disease we will observe and measure will only be attributed to mutation status and the drug regime we are administering to a given animal. This ensures high power, high fidelity experiments with minimal variation due to non-experimental factors.
Which animal models and methods will you use during this project?	We will familiarize ourselves with the animals on a daily basis, which will make handling them much easier and decrease their anxiety in our proximity. We have years of experience handling animals peacefully and confidently. We shall not handle the mice by their tails and will use the cupping or tunnel method instead. We are familiar with the schedule of our animal facility and shall not attempt to inject an animal immediately following a cage change to avoid increasing its stress even further. We shall coordinate with the animal technician so the injection can take place at the same time as the cage changing/ cleaning. Although the animals will be subjected to anaesthesia (AB) for less than 15 minutes for the ECG recordings, we will still apply an ointment to their eye to prevent potential dryness and discomfort. They shall also be placed on a warm platform to prevent any potential heat loss. Because anaesthesia may lead to dehydration, certain procedures require the administration of a small bolus of warm saline subcutaneously before the animal recovers. Since we will not maintain anaesthesia for more than 15 minutes, this precaution is not considered necessary and shall only be used as per NVS advice. The animals will be kept in a warm, dimly lit and quiet environment until they are fully recovered from anaesthesia and only then they will be transferred back to their holding room. When we are performing multiple ECGs on a given day, the animals shall be allowed to recover in groups to prevent the feeling of isolation. They will then be monitored very closely (up to 3 times per day) until their normal behaviour has been restored. The use of anaesthesia itself for ECG recordings is only to reduce the anxiety and the stress of the animals that will be handled. Gaseous anaesthesia was chosen because it has a very short induction time, a very short recovery time, and gets minimally metabolized so it has minimal side effects and it

	allows for multiple uses throughout the lifetime of an animal. The ECG device to be used was purchased solely based on the fact that it can record traces wirelessly without the need for hypodermic needles. This is the only device we know of currently on the market that can record an ECG without even the need to tape electrodes on the limbs, therefore causing absolutely no pain or suffering to the animal. Since the pharmaceutical agents need to be administered multiple times, it is of pivotal importance to us to minimize cumulative suffering. This is achieved by: mostly using substances that do not need to be injected daily, alternating the injection sites around the abdomen, delivering a very small bolus (up to 100 microliters) of non-irritant carrier material IP and not IM to avoid the pain caused by injecting a muscle. Injections also eliminate the laborious nature of food and/or drinking water drug additive studies which require constant recording and tracking of weight or volume displacement respectively. Furthermore, this route of administration will eliminate the need to perform radioisotope analysis of circulating drug concentration in mice sera to prove that the correct concentration of the drug was consumed. We shall use as small a gauge needle as possible according to the Laboratory Animal Management and Welfare, “redacted” 4th edition, to cause minimal distress. Both the principal investigator and her senior postgraduate researcher are highly experienced in injecting mice and can do so with confidence and smooth, safe movements that provide the animal with security and calmness. The animal will be held upright during injections and the skin on the lower abdomen will be tightened without causing discomfort so as to facilitate the smooth and fast entry of the needle. No animal shall be dropped back into its cage following and injection. It shall be gently and slowly lowered down to the cage floor and allowed to leave the cupped hand on its own. We shall observe the mice for a few minutes following the injection as well as additional times during the day to make sure there is no sign of bleeding. We will use the remaining tissue from ear clipping identification for genotyping purposes therefore not requiring an additional regulated procedure (tail clipping) that could potentially distress the animal. We shall administer each drug through an IP injection. Although this approach might cause momentary distress, it is a much milder approach to implanting a drug delivering mini-pump, which would subject the animal to the risks associated with abdominal surgery. Although we recognize that mice are more active at night, and they may be less

	disturbed if they are injected towards the end of the day – we feel the need to be able to observe the animals more closely following the injections while the animal facility is fully staffed. Accordingly, the animals will be injected in the morning on the days that substances need to be administered. We shall maintain a large distance between the animal being handled and the rest of the colony to avoid possible ultrasounds distressing the remaining animals. We shall apply some pressure using clean gauze on the injection site immediately after pulling the needle out. We shall use different sterile needles between animals. Each injection lasts less than 10 seconds. Accordingly, we do not deem it necessary to use a restraining device of any sort as this might distress the animal more than the injection itself. All drugs will be used at pharmaceutical grade. If we need to use a drug that is currently in clinical trials and is not commercially available in pharmaceutical grade, we shall use it at the highest grade available (>98% pure, HPLC, and filter sterilised). We will try and use substances that do not need to be administered on a daily basis. The most common frequency of administration shall be once a week for the period of the 12 weeks. No animal will be left isolated unless for very brief periods of time while recovering from anaesthesia. No animal shall be denied of environmental enrichment at any point. Following consultation with the animal technical staff, 'rewards' may be provided to animals that undergo injections such as strawberry/ blackcurrant flavoured jam. This has been shown to increase cooperation of rodents and minimize distress. All cages will be clearly labelled with the PPL number, the protocol identifier, the responsible PIL's name, the date each protocol has been initiated and comments pertinent to the specific animals housed inside. Records shall be maintained for at least 5 years. Throughout the duration of the study, the principal investigator and her senior postgraduate researcher shall not plan to be absent concurrently. If such a need arises, however, it shall occur following prior consultation with the named people of the animal facility and not during the periods where protocols 2-3 shall be taking place. The distance the animals have to travel from the primary to the secondary facility is less than 1 hour. However, we shall make sure that they have a very comfortable journey according to all regulations and we shall provide plenty of food and water (in the form of hydrogel).

Why can't you use animals that are less sentient?	A zebrafish model of arrhythmogenic cardiomyopathy was used before for the discovery of the initial agent (SB216763). However, the very different physiology of the heart of a zebrafish (a single atrium and ventricle) makes it impossible to extrapolate results from this model to the patient. A mammal is required. Moreover, although imaging will be performed on animals that are terminally anaesthetized, we need multiple ECG recordings to assess if arrhythmias are eliminated by a given pharmaceutical agent. To avoid stress, however, ECG recordings will be kept very short (under 15 minutes) and they will be performed under anaesthesia.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	understandinganimalresearch.org.uk has frequent updates on advances of the 3Rs such as the summary of the Oxford 3Rs day. The National Centre for the Replacement, Refinement and Reduction of Animals in Research (www.nc3rs.org.uk) provides information on all the latest advances that will allow scientists to put the 3Rs into practice. The website of the Royal Veterinary College (www.rvc.ac.uk) also provides updated information on how to implement the 3Rs in research projects hence ensuring animal welfare. Finally, the Animal Facility Named Persons work closely with all researchers on a daily basis and always keep the project leaders and workers up to date with new information regarding implementation of the 3Rs.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	All refinement measures listed above have been reached through experience in this type of project as well as consultation with experts. Further refinement measures, however, can be taken if deemed necessary through very close monitoring of the animals and keeping up to date with the literature.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	The Laboratory Animal Management and Welfare "redacted" 4th edition. The Laboratory Animal Science Association (LASA) guidelines is another great source of information on conducting the most refined animal experiments. The Animal Welfare Information Centre is a reference source for the recognition and alleviation of pain and distress in animals. Similarly, the Universities Federation for Animal Welfare Science is a scientific charity providing information on the long-lasting welfare of animals including animals involved in experimentation.

Project	**115. Dynamics of the hypothalamic-pituitary-adrenal axis**	
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 hypothalamic-pituitary-adrenal (HPA) axis is a vital system in the body controlling the release of “stress hormones” such as cortisol. Oscillations in these hormones are critical for our health as they control the activity of many important biological functions, and ensure the body is in an ideal state to respond to stress. But stressful experiences early in life, or excessively-large or prolonged periods of stress, can lead to long-lasting disruptions in HPA axis activity, which in turn has consequences for brain function and our physical and mental well-being. The overall aim of this project is to understand	

	how the HPA axis controls hormone secretion; this information will be critical for understanding how and why these hormone patterns change in disease.
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 provide fundamental insight into how the HPA axis controls the release of stress hormones. It also has the potential to influence treatment of patients by providing information that can be used to correct disrupted HPA axis activity in disease, and by providing information that can be used to develop hormonal treatments that mimic the body's own natural rhythmic processes.
What species and approximate numbers of animals do you expect to use over what period of time?	Animals are required as HPA activity depends on interactions between multiple organs. We will use rats (approx. 480 over 5 years), which bear strong similarity to man in terms of HPA activity.
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 studies will be carried out using tissue collected from animals, and some studies will be carried out in animals under terminal anaesthesia. However, most studies will be carried out in awake animals. These animals will undergo surgery (under general anaesthesia) to create small brain lesions, inject substances to alter HPA function, implant cannulas to enable blood collection, and implant devices to record/stimulate the system. Surgical procedures will be performed in a clean environment to mimimise risk of infection. Animals will receive a high level of post-operative care and are expected to recover swiftly. Some animals may be individually housed and connected to a tether to prevent damage to cannulas/devices. Following recovery from surgery, HPA axis activity will be recorded and blood samples collected. Animals may be administered substances that affect HPA activity or exposed to a brief stress (e.g. noise). The adverse effects of these procedures are moderate in severity. At the end of each experiment, animals will be killed by a humane method, and blood/tissue will be collected for analysis.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Oscillations in stress hormones are controlled by complex interactions between multiple organs. To understand how these oscillations are generated, and ultimately why they change in disease, we need to study the system as a whole, which requires the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of animals used by: carefully designing our experiments in collaboration with statsiticians so that we use the least number of animals that will still provide us with reliable results; carrying out initial experiments in cells/tissue, when possible, to develop hypotheses that can then be efficiently tested in freely-moving animals; using mathematical models of the system to study its dynamical behaviour and create testable (achievable experimentally) and robust (having a good chance of success experimentally) hypotheses to then test experimentally.
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 rats as the HPA axis of this species bears strong similarities to that of man and has been better characterized than that of any other species, and the rat responds well to the surgical and experimental procedures we will use. To minimise harm, we will: take advice from the establishment vet when designing experiments; employ skilled researchers to carry out surgical procedures using aseptic technique; provide animals with a high level of care following surgery, including pain relief (analgesics); select stress protocols that minimise suffering whilst meeting scientific aims; use automated systems to painlessly obtain blood samples and deliver substances via cannulas; humanely kill animals if they show signs of suffering.

Project	**116. Ecology of fish**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		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 to: 1. Determine the impact of non-native fish on freshwater ecosystems and freshwater fish communities 2. Determine the consequences of nutrient enrichment, habitat loss and climate warming on freshwater fish communities 3. Identify the cumulative impacts of the multiple stressors of climate warming, habitat loss, nutrient enrichment and non-native fish on freshwater ecosystems and freshwater fish communities	

	Each objective addresses a significant scientific unknown.
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 are the derivation of scientific knowledge that is used to develop policy and procedures that better regulate the management of rivers in the UK for the benefit of freshwater ecology and biodiversity.
What species and approximate numbers of animals do you expect to use over what period of time?	The species are fish encountered throughout UK freshwaters, including some non-native species. These are Roach Rutilus rutilus, common bream Abramis brama, rudd Scardinius erythrophthalmus, dace Leuciscus leuciscus, tench Tinca tinca, brown trout Salmo trutta, Atlantic salmon Salmo salar, European eel Anguilla anguilla, crucian carp Carassius carassius, bullhead Cottus gobio, gudgeon Gobio gobio, 3 spined stickleback Gasterosteus aculeatus; minnow Phoxinus phoxinus; Common carp Cyprinus carpio, gold fish Carassius auratus; sunbleak Leucaspius delineatus, topmouth gudgeon Pseudorasbora parva, fathead minnow Pimephales promelas, pumpkinseed Lepomis gibbosus; black bullhead Ameiurus melas; European catfish Silurus glanis; zander Sander lucioperca; grass carp Ctenopharyngodon idella; European barbel Barbus barbus; chub Leuciscus cephalus Over the 5 year project, a maximum of 4750 fish will be used. An individual fish is likely to spend no more than 100 days under a Protocol.
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 are inhibited growth rates, shifts in behaviour and modifications to the diet of the fish. In conjunction with these are the use of anaesthesia, insertion of tags for identification and the effects of environmental manipulations. The expected level of severity is mild in all protocols except one which is moderate. The welfare of the fish is measured according to a scoring of the behaviour of the fish during daily observations. This is in relation to their respiration, feeding, swimming behaviour and response to external stimuli. Severity is also measured according to the weight loss of an individual fish (maximum of 10 % loss for all protocols) The animals will either be killed at

	the end or released back into the wild where they have been used at a POLE where this is permissible.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives cannot be used in the project as the work is specific to investigating how different stressors impact the ecology of UK fish species. This requires measurement of their behaviour, diet and feeding interactions and growth rates, all of which require the use of live fish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Minimum numbers of animals will be used in the project by ensuring each experiment if designed with the advice of a statistical expert. That expert will advise on the number of treatments, replicates and fish being used, with multi-factorial designs used where possible to minimize numbers. The aim of the statistics is to indicate the minimum number of animals required to provide statistically robust data.
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 used in the project are all present in UK freshwaters, with their status as either native or non-native, and have been identified as being important ecologically by regulatory authorities. Consequently, they are refined for use in the project in order to meet the project objectives. To minimise harm, the fish to be used experimentally will be sourced from aquaculture facilities, with the “redacted” facility used wherever possible where the fish are maintained to high husbandry standards that are usually free from any confounding factors, such as parasite infections. Animal handling will be minimised to periods of data collection when they will be under general anaesthetic. Where temperature and environmental conditions are being varied within a trial then these will be within the range of those experienced by the animals in natural situations. Where fish are being tagged using Passive

	Integrated Transponder tags or elastomer/ visible implant tags, and/ or a tissue biopsy is being taken, then these procedures will be completed on the fish under general anaesthetic using MS-222. Where fish are being tagged using acoustic tags or radio tags implanted into the peritoneal cavity, then these procedures will be completed on the fish under general anaesthetic using MS-222, with only a personal licensee with training and competence in surgical techniques completing the procedure. Appropriate periods of acclimatisation to tank and pond conditions will be used and where tagging is used to monitor fish performance, those used will be the most appropriate and least invasive. The use of a behavioural and weight loss scoring system within daily observations of fish in tank and pond conditions also minimises welfare costs to the animals through clear definition of endpoints. In work completed at POLES, fish will only be released back into the wild once they have recovered from procedures, as demonstrated by their response to external stimuli, ability to swim and to maintain their normal body position.

Project	**117. Effects of Novel and Other Compounds on the Gastrointestinal System**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is twofold. Firstly, to evaluate effects of test substances (including drugs and chemicals) which may induce side effects on the gastro-intestinal system. This is to check whether these compounds are safe if exposed to humans or safe enough to undergo further testing before potentially being tested in humans. Secondly, to determine the efficacy of drugs interacting with the gastro-intestinal system, and to see if they are better than existing drugs, or have a better side effect profile. These may be	

	drugs for conditions like stomach ulcers, diarrhoea, irritable bowel syndrome, colitis and constipation. Whilst these conditions are usually debilitating rather than life threatening, they still cause a degree of pain and suffering for millions of people worldwide.
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 of this project would include the discovery of new treatments for gastrointestinal diseases (examples given above), and the confirmation of the safety of new treatments or chemicals with regard to gastrointestinal side effects prior to first administration in man. Work under this Licence will also show which compounds are not suitable to move forward into patients due to them not being able to moderate the clinical condition examined, or they are not safe to go into humans, for example. Work performed under this licence may identify more effective drugs for example to those already on market, with fewer side effects and that work better than existing drugs. Some studies will be required by regulators to help them decide whether potential drugs both work in their chosen indication, and are likely to be safe in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	The maximum expected usage of animals on this project is 16000 rats, 5500 Mice and 1200 ferrets. This would be over a five year period. However, it is highly unlikely that these numbers will all be reached, as the work performed will be performed at one species per study per test item. Mice and rats will predominantly be used in these studies. These species are used because they respond to gastrointestinal agents in a similar manner to humans and the data produced will help model gastrointestinal conditions that occur in humans, predict how well the potential medicines will work in humans, and predict the potential side effects of chemicals and medicines in humans. In a limited number of studies, ferrets may be used, but they will only be used when the experiment required specifically requires these species, due to the inability of mice and rats to produce the required response, or to support other work being done in ferrets.

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 experiments performed under this licence will be short term (much less than 24h) using rats and mice, and will involve animals being dosed with a drug once, usually by using a tube into the stomach, or an injection into a vein or by an injection under the skin for example. In some cases, we may also use a marker to see how test substances affect the passage of an inert substance like charcoal down the intestines for example. This would also usually be dosed by a tube into the stomach. These procedures are carried out by people with lots of experience in dosing animals this way, so they cause as little distress as possible. A lot of the experimental measures we look for are carried out after animals are humanely killed, but in some cases we have to take single or multiple blood samples. This is the equivalent of having a blood sample taken by a nurse at a doctor's surgery. For studies that last longer than 24h, sometimes we use other substances to induce a disease state (e.g. Dextran sulphate sodium DSS for a colitis model) which is administered usually in the drinking water. These inflammatory bowel models (mimicking diseases like colitis and Crohns disease) take several weeks to cause their damage to the large bowel, and during this time, we carefully monitor the animals at least twice a day, to see they are not suffering too much. We often give supportive measures, like extra bedding and wet food to make sure their weight is stable, and monitor their bowel output to monitor for excessive damage (e.g. looking for blood in stools). Rarely, we have to surgically implant animals with cannulae (for blood sampling or dosing into a vein), or very occasionally with drug delivery devices, to help with our experiments. This surgery is performed by trained and competent people, under anaesthesia, and we use pain killers and antibiotics after surgery, as agreed with a vet. This is the same sort of treatment you would get if you went into hospital for an operation. We also use ferrets in one type of experiment, and usually we will give them a drug to make them vomit, and see how a test substance affects this. Whilst this is mildly distressing over a short period of time, it doesn't cause any lasting harm. We also can use these animals in future experiments, as long as we

	satisfy certain conditions, and a vet says the animals are fit to undergo studies again.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The gastrointestinal tract is a complex organ system performing a wide variety of functions essential to life, such as secretion, digestion, absorption, excretion and defence. There is no adequate model to replace the whole animal experimental model, as the complex mechanisms under investigation cannot be adequately modelled in non-animal preparations. Before any animal experiments are carried out, drugs are tested in test tubes to assess things like toxicity, metabolism, and how well they bind to their target receptors, for example. Many of these compounds don't go forward into animal testing, because they are simply not good enough to become drugs based on the results of tests like these.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data may not be available, the literature (scientific publications) will normally be consulted to help decide the group size. Statisticians are often consulted particularly where the study type is not routine, as they can use calculations to estimate the correct number of animals needed to get a meaningful result. Where possible, common control groups will be used in order to minimise the numbers of animals used. For less established experiments, a preliminary study may be conducted in which smaller numbers of animals may be used to generate data in order to ensure that the experiment operates as we would expect and to generate some data which may be used to get a better study design. From such pilot studies, the variability of the measurements are used by statisticians to determine the required number of animals per group required to identify whether the test

	substance actually has an effect in a main study. Variables that may affect the study are kept constant wherever possible to make sure the experiments stay the same time after time. This actually means the data is more reliable and meaningful, and easier to make assumptions about In some studies, we are able to re-use ferrets that have previously undergone procedures. In this instance, the re-use of any given animal depends on the result of a veterinary examination being satisfactory .The vet knows what procedures have taken place on these animals, including both their severity and frequency and ensures that all legal requirements and welfare needs are met before such an animal is re-used on study By re-using animals when they are fit to do so, we reduce the overall numbers of animals we use.
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.	Wherever possible, experimental outputs are collected under terminal anaesthesia or at post mortem to reduce the burden on the animal used in the protocols. In some circumstances other samples (like blood) may be taken from the same set of animals to give the maximum amount of data for the fewest number of animals. Highly trained staff use a rigid framework of welfare assessment to allow early detection of animals showing signs of discomfort or distress. We use pain relief as standard with anaesthesia and after procedures where relevant, e.g. surgically implanted models, although these are rare. We sometimes, although rarely, use animals that have had their genetic material altered, e.g. such that they are predisposed to developing a disease type or if a particular gene is important say in a specific component of the gastrointestinal tract in which we have interest in. Although we may use various species on this project, rats and mice are the standard species used as they are of the lowest sentience. We would only use other species where their physiology means that they would be the best animal to use modelling a specific disease type.

	For example, Ferrets are the best models to use in models where we look at anti-sickness drugs, because mice and rats are unable to retch and vomit. This means we will use mice and rats unless other species will provide a better answer to the scientific questions we are asking due to their physiology being more suitable, or their reaction to a condition we are trying to induce being more like you would see in humans. Dosing and sampling procedures will be undertaken using a combination of dose volumes, routes and frequencies that of themselves will result in no more than low levels of discomfort and no lasting harm and will be the minimum consistent with the scientific objectives. Many of the procedures carried out produce only minor levels of discomfort, due to the nature of the procedure, and the skill of the person performing it. For example, an animal having a blood sample taken would feel the same level of discomfort as a patient in a doctors surgery having a blood sample taken. We have made several refinements in one of our surgical models, by enhancing our aseptic surgery technique, reducing the number of animals we use per day and improve the pain relief we give. Food and water withdrawal will be kept to a minimum This is not routinely carried out and only occurs in very specific circumstances when it is an integral part of individual study requirement, which is unavoidable sometimes when you are looking at the GI tract.

Project	**118. Effects of social environment and resource availability on phenotypic trait expression in cichlids and guppies**	
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
	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)	**Cichlids** Our understanding of how competitive traits such as signals or aggression evolve is currently limited due to the neglect of females in studies of competition and the failure to consider how the behaviour of one sex may affect selection on competition in the other sex. To address this gap, we aim to use lab experiments to determine the costs, benefits and constraints associated with female competitive traits, including those arising from interactions between the sexes.	

	Guppies Scientists are unable to accurately predict the course of evolution in the wild. This is problematic as we are altering the natural environment in such a way that it results in evolutionary change. New theory has been developed that is proposed to provide better predictions by taking into account how the availability of resources may shape the evolution of traits. Here we aim at testing this theory using laboratory experiments carried out on Trinidadian guppies.
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)?	**Cichlids** Determining the costs and benefits to females of competition will improve our understanding of how selection shapes female competition across the animal kingdom, allowing better prediction of when and how sex differences in competitive traits will evolve. Analysis of third-party interventions will lead to a better understanding of the selection pressures such interactions exert on animals, which have to date been almost entirely neglected. **Guppies** If our experiments demonstrate that the new theory does provide improved predictions, we will be able to develop a much better understanding of how environmental change is predicted to impact the natural world, and how species will respond. This will help us design policy and management practices that have predictable impacts on affected species. If our tests to do not support the theory, this too will be useful as we can advise that it should not be used to make predictions.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use cichlids (*Lamprologus ocellatus*) and Trinidadian guppies (*Poecilia reticulata*) in our experiments. We will use a maximum of 400 cichlids and 65800 neonate and adult guppies 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?	1) Anaesthesia and marking (cichlids and guppies) To tell individual fish apart, we need to mark them with visible implant elastomer (VIE) tags, which consist of a silicone-based coloured liquid that is injected under the skin, where it subsequently hardens. We need to anaesthetise

	the fish to mark them, as this cannot be done on free-moving individuals. Additionally, anaesthesia is also required to sex, measure, weigh and photograph guppies, since this again cannot be done on freely-moving individuals. Cichlids will be anaesthetised typically once and never more than twice during their lifetime, while guppies will be anaesthetised between 2-6 times and never more than 7 times over their lifetime. The level of suffering is mild: anaesthetised fish recover within minutes after being placed in a recovery tank. No adverse effects of the procedure are expected. Both the anaesthetic and marking procedures have been developed and applied routinely in fish laboratories around the world. Any fish, however, that have not recovered from anaesthesia after 10 minutes will be euthanised via Schedule 1. The appearance and behaviour of all other individuals will be monitored closely following recovery from the anaesthetic using a health-scoring sheet. (2) Predator exposure (cichlids only) Adult cichlids caring for newly-hatched offspring will be exposed briefly to a second cichlid species that predates juvenile cichlids. The aim of this exposure is to determine how mothers change their allocation of time and energy to competition with other females when they simultaneously face the risk of their offspring being eaten. The level of suffering is mild: females will only be exposed for a brief period (maximum 30 minutes) and will be separated from the predator species by a barrier, preventing the risk of injury arising from physical interaction. Furthermore, the predator species poses no risk to the adult cichlids, so while females will be motivated to defend their offspring they will not experience any additional stress arising from fear that they themselves will be eaten. If, however, upon exposure to the predator stimulus, any individual does exhibit a fear response, the trial will be terminated immediately and the predator removed.

	Thereafter, fish will be monitored closely until they have resumed normal coloration and behaviour (this is expected to occur rapidly). (3) Alteration of diet (guppies only) Guppies will be food-restricted in order to determine the sensitivity of different traits to resource availability. The level of suffering is mild: the amount of food that guppies will receive in the food-restricted treatment matches that experienced in the natural environment and while reduced food levels will result in delayed maturation and reduced numbers of offspring, it will be sufficient for the fish to maintain growth, development and reproduction. No adverse effects of food restriction are thus expected. Any fish, however, that loses weight between measurements will be removed from the study, further monitored, and euthanised if after a further week the individual is continuing to lose weight. At the end of experiments, fish will either be euthanised using an overdose of anaesthetic (Schedule 1) (guppies) or added to the stock population for breeding or use in non-regulated work (guppies and cichlids). Guppy neonates will be euthanised via a non-Schedule 1 method (overdose of anaesthetic followed by immersion in fixative).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The questions we seek to answer about the evolution of morphological and behavioural traits can only be addressed using live animals. There are no unprotected animal models with the required combination of features that could be substituted for our study species. For both cichlids and guppies, marking is essential for us to distinguish between individuals and track individual experience – our

	research relies critically on the ability to tell individuals apart and would thus not be feasible with wild or captive fish that had not undergone this procedure. Additionally, for the cichlids controlled exposure to brood predators is essential for achieving a complete understanding of how selection shapes female competition; reliance solely on opportunistic observations of wild populations would not allow us to disentangle responses to brood predators from potential confounding factors such as reproductive state or tenure in the harem. For the guppies, experimental regulation of food intake is essential for determining how the development of different traits depends on the availability of food resources – there is no alternative method for answering this question.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For each species, we explain below how we have determined the minimum number of animals that are required to meet the objectives of each study. **Cichlids** While it is not possible to use the same individuals repeatedly within a single experiment, we can use individuals across multiple experiments, thereby minimising the total number of animals required. A survey of published effect sizes from relevant experiments indicates that a total of 164 fish (44 males & 120 females) used across Parts A-D should be sufficient for detecting effects of interest. To account for potential mortality, infertility, behavioural incompatibility and/or smaller than anticipated effects of manipulations, the total number of individuals that may be anaesthetised and marked will be increased to 400 (100 males & 300 females). **Guppies** The breeding design used in Part A will only have sufficient statistical power if 30 or more males are paired to 2 or more females each, and if 2 or more offspring per female are included in each of the two experimental groups. To account for potential mortality or infertility, these numbers will be increased to 50

	males, 3 females per male and 3 offspring per female and treatment. The total number of fish used will then be 1400. For the artificial selection experiment in Part B, four selection lines and 2 control lines will be needed, and there will be two replicates of each line. A line will consist of 20 males and 20 females, and the experiment will run for 5 generations. The total number of fish used will be 2400. Post-mortem measurements of neonate weight will be limited to a maximum of 4 litters from 2100 females used in Parts A and B. We will euthanise (via a non-Schedule 1 method) and weigh a maximum of 62000 neonates. The total number of guppies (adult and neonate) used will thus be 65800.
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.	**Cichlids** *Lamprologus ocellatus* is an ideal model for studying social conflicts, for several reasons. First, the species occupies small territories, meaning that it is possible to fully replicate the species' breeding territories within aquaria. Second, the fact that females breed within snail shells means that it is straightforward to count the number of eggs and measure hatching success for multiple females within a breeding group. Third, the opportunity to manipulate group membership, as well as the position and number of shells within groups, allows precise control over the intensity of competition among females. This combination of features provides a unique opportunity to experimentally determine the costs and benefits to females of competition and to investigate third-party interventions by males in female contests over the distances that fish typically interact in the wild. The programme of research is aimed at studying competitive behaviours; to minimise risk of harm, experiments have been designed to prevent escalated aggression and the risk of physical injury and have been approved by the local AWERB. **Guppies** We will use Trinidadian guppies because we have an exceptionally detailed

	understanding of their evolution in nature. When their environment is changed through the removal of predators, their population densities increase, and repeatable patterns of evolution are observed across habitats. A key driver of this evolution is known to be a change in resource availability. Although the patterns of evolution are repeatable, we have so far been unable to theoretically predict the observed changes with existing theory, primarily because existing evolutionary theory does not consider resource availability. Our in-depth knowledge of guppy evolution in nature and of the role played by resource availability, coupled with the guppies' short generation time, high fertility and ease of use in experiments, makes them an ideal species for us to use. Both fish populations are housed in light- and temperature- controlled, closed recirculation systems located indoors, where water quality is continually monitored and every fish is inspected daily for signs of ill health. Fish are either housed communally or, if the experimental design does not allow that, individually while maintaining visual contact to other fish. Environmental enrichment includes a sand substrate (for cichlids) and plastic plants, prints of gravel placed beneath aquaria and feeding with live shrimp (for guppies). Our protocol for anaesthetising and marking fish of both species ensures in many ways that levels of distress, suffering and pain will be kept very low, and that freshly marked fish will show no aftereffects 24 hours after the procedure at the latest. For example, anaesthesia will always be induced rapidly and will be maintained for the shortest possible duration, and fish will only be marked when they have reached a minimum body size. Our protocol for euthanising new-born guppies ensures that animals die very quickly with minimal suffering. Our protocol for predator exposure for cichlids ensures that stress experienced upon viewing the predator is very low. The predator does not pose a threat to the adult cichlids and is only presented to them for brief periods behind a

	barrier preventing any physical contact. Our protocol for restricting food for guppies ensures that animals are extremely unlikely to suffer any distress, suffering or pain. Rations are equivalent to those experienced in natural habitats where guppies grow and reproduce successfully. Moreover, we will closely monitor weights of fish on restricted diets and immediately remove any fish found to be losing weight.

Project	**119. Efficacy and safety testing of vaccines**	
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 purpose of this programme of work is to establish the efficacy and safety of batches of vaccine against Bovine Viral Diarrhoea (BVD) and Enzootic abortion of ewes (EAE), in order that the products comply with marketing authorisation in line with European Directive 2001/82/EC. Ensuring these two proven vaccines are commercially available for farmers, offering protection against two key disease challenges facing the industry today, BVD in cattle and EAE in sheep.	
What are the potential benefits likely to derive from this project (how	Vaccination can form part of a control plan to reduce the impact and spread of disease and	

science could be advanced or humans or animals could benefit from the project)?	improve animal health, welfare and productivity. Vaccination can therefore contribute to a reduction in the use of important classes of antibiotics necessary for treatment of severe infections in humans, thereby having a positive impact on animal and human health.
What species and approximate numbers of animals do you expect to use over what period of time?	Batch testing of vaccines must utilise the target animal for that vaccine. The number of animals per batch is determined in the marketing authorisation. As such, over 5 years, it is expected that no more than 180 cattle and 720 ewes will 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?	For potency testing, the animals will be observed and monitored throughout the study and will be vaccinated using the recommended marketing authorisation dose regime. The animals will be blood sampled before and after vaccination to establish that a suitable immune response has been mounted to the vaccine and that a suitable level of immunity has been established. One animal within the group will not be vaccinated and act as a control. The vaccines for Bovine Viral Diarrhoea (BVD) and Ezootic Abortion in Ewes (EAE) have known profiles and no clinical effect of vaccination is expected. However, as with all vaccinations, transient pyrexia and injection site inflammation may occur. For this reason, the rectal temperature, skin fold thickness, appetite and demeanour of animals will be monitored for two weeks after vaccination. Insertion of a needle will incur temporary mild pain for the animal but all procedures are expected to be at mild severity levels. Animals will return to their herds of origin at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project plan is required in order for demonstration of stated immunological effect in target species. It forms part of marketing authorisation for this veterinary medicine product and we will keep this legal requirement under constant review.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Attention to detail in health planning and monitoring prior to animals entering the study and during the study should ensure that the chance of failure due to external factors is minimised. Where possible control groups will be combined to reduce animal usage.
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.	Marketing authorisation requires that the vaccine is tested on the target species. The protocol has been refined to ensure that potential risks to animals at each stage are minimal. Good training in handling, vaccination technique and blood sampling technique should ensure that the potential adverse effects are maintained at the minimal low level. There is a period of acclimitisation into the facilities and the handling area for cattle is of a curved design to allow good animal flow and minimal stress during routine handling.

Project	**120. Electrophysiological and Behavioural Assessment and Treatment of Psychosis**	
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 investigating how psychiatric diseases like schizophrenia affect the normal function of our brain and looking for new treatments. We will make recordings of the brain activity of rats and mice using, for example, wireless EEG systems or by performing recordings from cells in the brain under anaesthesia. We will generate a model of schizophrenia in rats by giving them a low dose of drugs.	
What are the potential benefits likely to derive from this project (how	We aim to aid the development of medical interventions to help treat people who have schizophrenia and other diseases involving psychosis. We are working with drug companies to help them test promising new medicines so that we can identify how best to help	

science could be advanced or humans or animals could benefit from the project)?	and treat people with psychiatric disorders in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice, about 500-1000 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 procedures are usually moderate as we are using special wireless EEG recordings to reduce the number of animals we use, but this means they need to be surgically implanted. We have a lot of experience of this and the animals do not have any trouble with the implants. We use drugs and genetic mutations to make our models of disease which may cause some short-term discomfort to the animals. Our experiments end with us making brain slices to gather as much data as possible from each animal, which is done after the animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to accurately record from neural circuits similar to those found in humans, so rodents are the smallest and simplest animals we can use for this purpose. Psychiatric diseases are complex, but we can look carefully at the behaviour of the rodents and read their EEGs to see how well our treatments are working.
2. Reduction Explain how you will assure	We will use the minimum consistent with our statistical calculations, and maximise the use of each animal through procedures designed to keep brain tissue alive for as long as possible. Our protocols are designed to maximise data collection from each animal meaning we

the use of minimum numbers of animals	need to use far fewer animals to get valid data.
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 are using genetic and pharmacological methods for our models which use the least amount of drugs and the mildest mutations possible. Behaviour in rodents is easily assessed by painless and non-stressful means. Using rodents for plasticity studies means that we only have to trim whiskers to induce plasticity, a painless and non-invasive procedure

Project	**121. Endothelial dysfunction in pulmonary hypertension**	
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)	Pulmonary hypertension is a severe disease which reduces blood oxygen supply in the lung. We aim to develop medicines that that improve delivery of oxygen to blood vessels in the lung.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There is no cure for pulmonary hypertension and the annual mortality is around 10%. An effective medicine is needed to restore normal function to the cells covering the inner side of blood vessels in the lung so that more oxygenated blood can flow through the lung.	
What species and approximate numbers of animals do you expect to use over what period of time?	5 years, approximately 120 rats/year (280 rats/year including breeding), 80 mice/year (240 mice/year including breeding).	

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?	To induce pulmonary hypertension, animals will be exposed to air with low oxygen levels, like the ones people experience when they climb Mount Everest. Typically, animals at lower oxygen levels gain weight more slowly but otherwise do not show any adverse effects. The disease is fully reversible on return to normal air in both mice and rats. This animal model is used to model symptoms that people experience at low altitude. However, many other forms of human disease are not reversible and this is why we have to use other animal models of pulmonary hypertension. Injection of compound Sugen before animals are transferred to low oxygen (Sugen/hypoxia model), thickens the wall of lung blood vessels in a similar way to the one seen human pulmonary arterial hypertension (PAH). Disease develops slowly, over ~13 weeks after animals return to normal air. In another model, a different compound, monocrotaline is given to the animals as a single injection and pulmonary hypertension develops within 3 weeks. This model resembles changes caused by inflammation in various other forms of pulmonary hypertension. Animals tolerate this well and the only visible symptom is that they put on weight slower than healthy animals. We diagnose the disease by measuring blood pressure in the lung and the size of right ventricle of the heart. This is done in animals that are asleep and will not recover, so they do not feel any pain and die while under the anaesthetic. Some discomfort may be experienced by animals injected with medicines, but local irritation induced by injection can be prevented by local anaesthetic. All these animal models of pulmonary hypertension are classified as moderate. Any animal showing signs of poor recovery will be examined by animal care technicians and vet at the earliest opportunity. If the animal fails to respond promptly to simple treatment or its condition deteriorates, it will be humanely killed
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Lung tissues from pulmonary hypertensive patients are not available. We are using cells obtained from patient blood which resemble in many ways function of lung cells, limiting the need to use animal models. We have also initiated work on a new model of human pulmonary artery on a microchip, which will allow testing drugs on cells from pulmonary hypertensive patients and, if successful, reduce or even eliminate experimental use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of cell and tissue culture methods limits the numbers of animals required for the investigation. In addition, the use of tissue samples and histological sections from our biobank of mouse and rat models of PAH previously used in our studies as well as human lung sections limits the number of animals needed for current experiments. Writing a well defined study protocol for every experiment will allow us to reduce the number of animals. We intend to use rats and mice as these animals provide well established, internationally recognised models of pulmonary hypertension. The number of animals required for the study is based on published data from our laboratory and other national and international centres. Experiments will be conducted and recorded to allow publication of results following the ARRIVE guidelines and will utilise randomisation and blinding,where appropriate to minimise biases.
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 intend to use rats and mice as these animals provide well established, internationally recognised models of pulmonary hypertension. Mice show milder symptoms than rats and will be our species of preference, particularly in studies that require genetic manipulation. Hypoxic mouse model (mice kept under reduced oxygen levels) will be our model of preference for initial studies. This model reflects changes in lung blood vessels that would occur in people at high altitude but fails to recreate changes in lung blood vessels seen in more severe forms of

	human disease. Therefore other models may need to be used. Monocrotaline rat model is preferentially used to recreate inflammatory changes in pulmonary hypertension, while Sugen/hypoxia is preferentially used to recreate occlusion of blood vessels seen in human PAH. While these models have their limitations, all the therapies currently approved for treatment of pulmonary arterial hypertension had to undergo development in these experimental models prior to clinical studies. The project will examine the efficacy of treatments in the reversible form of pulmonary hypertension (Hypoxic mice) and only take those that show promise into experiments with Monocrotaline or Sugen/Hypoxia, where disease is progressive and irreversible.

Project	**122. Engineering antibodies for the treatment of 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)	Cancer is the cause of 25% of all deaths in the UK, with a much larger percentage of people affected by this disease. Despite the development of better diagnostic and screening methods, the incidence of cancer continues to increase and many cancers are diagnosed late. Although surgery followed by chemotherapy is frequently used to treat disease at diagnosis, this is often not curative due to incomplete removal of tumour cells that lead to spread i.e. metastatic disease. Consequently, the development of new therapeutic approaches represents an active area of research. One such approach is to combine proteins called antibodies with highly potent drugs that kill cells.	

	Antibodies bind to markers on tumour cells with a high degree of specificity, with substantially lower binding to non-tumour cells i.e. normal tissue. Consequently the linking of potent drugs to antibodies, to generate 'antibody-drug conjugates' (ADCs) is expected to result in preferential delivery of the drug to tumour cells whilst not affecting the normal tissue. In this regard, many chemotherapy drugs are delivered to both normal and tumour cells, resulting in unwanted side effects such as digestive system problems. Thus, the use of ADCs in the treatment of cancer is expected to have significant advantages over existing chemotherapies. However, despite the promise of ADCs, their use has been met with problems related to an inability to deliver sufficient drug to tumour cells. In principle, increases in doses could overcome this problem, but despite their preferential delivery to tumour cells, higher doses result in undesirable toxicity towards normal tissues. As a result, multiple clinical trials involving ADCs have led to disappointing results. Our proposed study seeks to overcome the current problems associated with ADCs for the treatment of breast, prostate cancer and multiple other cancer types. Recent knowledge of how the markers that are targeted by ADCs behave in cancerous cells, combined with methods to alter the behaviour of antibodies, indicates ways of generating antibodies with increased efficiency in delivering drugs to these cells. These approaches will be used to produce a new generation of ADCs that are expected to have superior properties over existing ADCs, and in particular, result in a reduction in unwanted side effects. Our proposed studies will combine the use of protein/antibody engineering with experiments to identify ADCs that have increased activity in killing tumour cells. Prior to testing in humans in clinical trials, it is essential to analyse the therapeutic effects of the ADCs in mouse models of cancer. Our specific objectives are: 1) To develop a new class of engineered ADCs that lead to increased levels of drug delivery. 2) To test the therapeutic effects of the ADCs in mouse tumour models. 3) To define the mechanisms that lead to improved therapeutic outcomes in mouse models.

	We plan to target breast and prostate cancers. However, our approaches are expected to be generally applicable to multiple other tumour types.
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 ADCs with improved activity that we propose to generate have the potential to provide curative treatments for cancer. The detailed mechanistic analyses that we plan to carry out are also expected to lead to new insight into this form of cancer therapy. In addition, we expect to be able to readily translate our studies in mice to the clinic.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 6060 mice over the 5-year course of the 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?	In our proposed experiments, mice will be injected with tumour cells under the skin or in the mammary fat. Mice will be carefully monitored and, typically, the tumours will not grow to a size where they interfere with the normal behaviour of the mice such as their ability to move. In the unlikely event that we observe effects of the tumour on the behaviour of the mice, or if the tumours ulcerate, the mice will be humanely killed. For studies investigating the behaviour of the ADCs in mice prior to therapy testing, the short-term nature of the experiments and regulated procedures that will be used are expected to primarily result in effects of mild severity, with a small percentage of mice showing moderate effects. Typically, therapeutic antibodies or ADCs are well tolerated and will be used at doses that are expected to be well below the so-called maximum tolerated dose, which is the highest dose that can be delivered without observing significant, undesirable side effects. For most of the protocols, mice with relatively small tumours will be used that are not expected to affect the normal behaviour of the mice. For the proposed therapy experiments (one of five protocols), the tumours will be allowed to grow for longer than for other types of experiments to allow us to determine therapeutic effects. Tumour-bearing mice in these experiments will be very carefully monitored for signs of abnormal behaviour such as abnormal gait, locomotion and/or hunching. Mice showing such effects, which are expected to be very low in number (5% or less of total), will be humanely killed. Consequently, in therapy experiments we do

	not expect the effects to be greater than the moderate severity level, with most effects being in the mild category. For anaesthesia, we will follow current, best practice methods and do not expect the mice to suffer adverse events. For non-invasive methods such as delivery of antibodies/ADCs by intravenous injection, mice will not be anaesthetised since these procedures result in only transient pain and/or distress. For harvesting of blood samples from mice, we will use volumes that are substantially lower than those likely to cause adverse effects such as anaemia. Mice will be bled using best practice methods by trained personnel. Based on many years of using similar protocols, we do not expect the effects to be greater than the moderate level, with most effects falling in the mild category. Wherever possible, we will use whole body counting of mice as an alternative to bleeding, since this procedure involves placing the mouse in a cylindrical body counter for around one minute, whilst still allowing some movement of the mouse. This procedure results in minimal disturbance to the welfare of the mouse. All mice will be humanely killed when the experimental goal or, if sooner, humane endpoint has been reached.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our goal is to replace animals with in vitro methods wherever possible. However, the complexity of the distribution of an antibody or ADC in the body, and its persistence in the blood circulation, cannot be modelled with in vitro systems. For example, possible toxicities due to unwanted distribution of an ADC to normal tissues or organs cannot be mimicked using in vitro cellular assays. In addition, investigation of the therapeutic effects of an ADC in reducing tumour growth, with all the complexities involved in tumour development in the body, cannot be modelled by in vitro systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use inbred strains of mice (i.e. with the same genetic makeup) for all of our experiments to reduce the variability that would typically be expected with outbred strains (that are not genetically the same). This results in a need for lower mouse numbers. Our aim is to use the minimum number of mice that we can to obtain statistically robust results that are reproducible across experiments. We will use both

	our prior experience in carrying out the proposed experiments, combined with power analyses, to determine the numbers of mice that we need for each experiment to draw reliable conclusions. In addition, we will carry out smaller, pilot experiments with low numbers of mice, to define doses, tumour growth rates etc. prior to expansion to larger experiments if we have not prior experience with the tumour model and/or therapeutic agent. Nevertheless, the use of tumour cell lines that are well validated in our studies or those of others will form the basis of many of our analyses, and this is expected to contribute to a need for lower numbers of mice.
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 broadly taken to be good models for the preclinical testing of cancer therapeutics such as ADCs. For example, for the ADCs that are clinically approved, preclinical analyses in mice was a crucial step in their development. A further advantage of using mice is the availability of genetically altered strains. To ensure high welfare standards, good animal husbandry, including environmental enrichment, is employed. We also expect our studies to result in few adverse events, given that antibody-based therapeutics are widely used in the clinic and are typically well tolerated. Generally, for this project licence application we expect that the severity of the procedures will be mild for a significant number of mice/procedures, but we have set the maximum limit as moderate. Nevertheless, the mice will be carefully monitored and if adverse events are observed, steps will be taken to alleviate them or the affected mice will be humanely killed. Death will not be used as an acceptable endpoint in any of our studies. Many of the tumour models that we plan to use are well characterised and humane endpoints have been identified before the mice exhibit signs of pain or distress. In many cases, we can assess the size of the tumour and use a limit of this as an endpoint. In addition, other measures of distress for the mice will be assessed, such as altered feeding, drinking or mobility, or poor condition that can be detected using other methods such as alterations in facial expression (e.g. see guidelines for this in https://www.nc3rs.org.uk/using-facial-expressions-pain-animals). Occasional ulceration of tumours can also occur, usually in outlier mice, and such outlier mice will be humanely

	culled. We also have onsite veterinary assistance to provide advice if and when necessary.

Project	**123. Epigenetic gene regulation in mammalian development and ageing**
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
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
What's the aim of this project?	The key objectives of this programme of work are (1) to understand how in embryos and germ cells information attached to the genome (epigenetic information) is removed so that embryo cells can develop into all tissues in a fetus and adult, (2) to understand how such epigenetic information is changed in cells during embryonic development and why this is important for healthy development, and (3) to obtain insights into how epigenetic information in cells changes during ageing potentially affecting lifespan.
Why is it important to undertake this work?	Our experimental work will give insights into the regulation of totipotency and pluripotency, transgenerational epigenetic inheritance, cell fate decisions during development, and epigenetic regulation

	of lifespan. This will benefit approaches to human regenerative medicine including improving the quality of embryonic stem cells and patient derived induced pluripotent stem cells (iPSCs). Several credible animal models of effects of fetal nutritional programming on metabolic alterations of future generations have been established, raising the prospect that developmental programming in humans might affect risk of metabolic disease in future generations. Our work will identify mechanisms and triggers of such transgenerational inheritance, potentially contributing to diagnostic or therapeutic strategies in the future. It will also identify epigenetic regulation underlying cell fate decisions in development, leading to better control over embryonic stem cell differentiation schedules for therapy. Finally our investigations into the epigenetic regulation of lifespan will contribute to a better understanding of human healthspan and ageing associated disorders and how this might be regulated and potentially reprogrammed.
What outputs do you think you will see at the end of this project?	We hope to see scientific progress in key areas of our enquiry, which would translate into peer reviewed publications, intellectual property together with its initial translation or licensing, and public engagement and dialogue about the new science we discover. We will also train new generations of students and postdocs in cutting edge biomedical science.
Who or what will benefit from these outputs, and how?	(i) Academic scientific community. Our research will contribute to future studies; a number of new technologies and datasets will be identified and made available to other users to advance future research. We will support and train visiting scientists in our emerging technologies, furthering scientific advancement nationally and internationally. (ii) Commercial sector, in particular biotech/pharma companies with interests in regenerative medicine, and epigenetic processes as drug targets or biomarkers. The key areas will be in ageing research by identifying novel pathways and biomarkers of healthy ageing, and in regenerative medicine on quality and potential of stem cells. (iii) Medical charity sector. Our research underpins efforts to understand the origins of the decline in health associated with developmental disorders and ageing, including identifying new disease drivers, new

	biomarkers and new treatment options. (iv) Staff and students supported by our lab, who will receive exemplary research training. Gaining technical skills and expertise to carry out research, in addition to other transferable skills, personal and professional development, will prepare them for careers in the academic or commercial research sectors or other related careers. (v) Funders. Our research underpins the delivery of strategic priorities for funders and provides excellent value for money. (vi) General public including, but not exclusive to, students, teachers, patients, the local, national and international community. Benefits to these groups will include increased knowledge, understanding and awareness of our research on epigenetics, including the impact of environmental and nutritional exposures during development and ageing and its potential social and economic relevance. Our research will contribute to new applications in healthcare and allow the public to make informed lifestyle choices for the benefit of their health. Our new knowledge and its translation will also drive the economy, through the creation of new jobs, products and services. (vii) Our research will equip policy makers with critical understanding of the impact and value of basic research particularly with regard to ageing, providing scientific rationale for the influence of diet and lifestyle on healthspan, reproductive biology and disease mechanisms.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We have many mechanisms in place to do this apart from the immediate academic one of publishing research papers and presentations at international conferences. This includes a well appointed and trained Knowledge Exchange and Commercialisation team, and a similarly advanced and engaged Public Engagement team. Recent examples include spinning out a company on the basis of discovering a mouse epigenetic clock, and public exhibitions of our science.

Explain why you are using these types of animals and your choice of life stages.	In this project, mice are used because they are the smallest mammal that is available for research in our studies of fertilisation, early development, and ageing. We need to use pregnant animals to study embryonic development in the uterus. We need to use ageing animals so that we can measure changes in the epigenetic information in cells with age which may underlie frailty and ageing-associated diseases. Mice are a well-established example used across the academic community, which is also important for historical and newly shared data sets for the study of development and ageing. Many genes have already been knocked out in mice so the available resources are superior to any other mammalian species. Juvenile stages produce larger numbers of zygotes and oocytes and therefore reduce the number required overall. There is sometimes however the necessity to use adult stages for the purposes of rederivation and cryopreservation if the juvenile stages are not available.
Typically, what will be done to an animal used in your project?	The most common procedure in this project is the breeding and maintenance to produce adult or pregnant mice that will be killed via Schedule 1 to supply tissue for the aims described in this project. We shall also be generating new genetically altered mouse strains using highly refined genetic modifications that will selectively affect gene expression in embryonic cells. Mice will be superovulated and then schedule 1 to provide embryos to establish the regulation of genes during early embryonic development. Smaller numbers of mice will have gene-modulating agents administered to help modify gene expression patterns in cells in vivo to trigger epigenetic pathways For mice that undergo surgery, mostly for transferring embryos, the duration of anaesthesia and surgery is short and the animals are expected to make a full and unremarkable recovery, although analgesia will be administered to mitigate short-lived pain.

What are the expected impacts and/or adverse effects for the animals during your project?	The general type of genetically altered mice produced under breeding and maintenance in this project will be the type that selectively affect gene expression in early embryonic cells using conditional knockouts and drug-inducible transgenics in genes rather than in the whole animal where constitutive ablation could have a severe phenotype, thereby avoiding adverse effects The Superovulation procedures are expected to result in no more than transient discomfort and no lasting harm. For ageing mice that will be Schedule 1 at various time points post 52 week of age, to measure changes in the epigenetic information, these animals are not expected to show abnormal phenotype but due to general ageing will be monitored using the “redacted”. For mice that are exposed to gene inducing agents the mice will be closely monitored but are expected to result in no more than transient discomfort and no lasting harm. For mice that undergo surgery, mostly for transferring embryos, the duration of anaesthesia and surgery is short and the animals are expected to make a full and unremarkable recovery, although analgesia will be administered to mitigate short-lived pain.
What are the expected severities and the proportion of animals in each category (per animal type)?	Overall, the expected severity of this project licence is Mild, with fewer than 5% of animals expected to experience a maximum severity of Moderate.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Animals remain necessary as it is not yet possible to recapitulate the process of fertilisation and early development in a purely cell-based system in a culture dish. Similarly there are no generally accepted cell based systems in a culture dish for fetal development nor for ageing.
Which non-animal alternatives did you consider for use in this	Embryonic stem cell based systems: • for the study of epigenetic information in early

project?	embryos • devising cells in a culture dish that resemble early embryo stages • To mimic development of early germ cells • To study development at and after embryo implantation Adult cell based systems: • For senescence and ageing
Why were they not suitable?	Embryonic stem cell based systems have yet to prove commensurate with many in vivo systems and are therefore not entirely suitable for pre- and postimplantation development and gastrulation. While senescence can be studied in vitro in cell based systems, ageing cannot at this point.
Enter the estimated number of animals of each type used in this project.	mice: 34830
How have you estimated the numbers of animals you will use?	The numbers are based on the number of transgenic and knockout strains we produce and use at any one time, the breeding strategies involved, which can involve putting multiple alleles together in a single animal, and the effect sizes of the measurements we are making, for which power calculations have been used.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	We have been able to reduce animals use through tremendous improvements in methods with which we can molecularly analyse properties of tissues in small numbers of cells, including in single cells. These improvements in methods have brought down cell numbers that numbered in their millions originally down to 50-100 cells and recently to single cells. This is resulting in substantial reductions in numbers of animals used.
What measures, apart from good experimental design, will you use to optimise the	• Use power calculations of optimized animal group sizes based on pilot experiments and advice from

number of animals you plan to use in your project?	the Institute statistician • Minimise inter-group variability using controls of matching age, sex and genetic background • Cryopreserve strains when no longer required • Use colony management software that helps avoid overproduction
Which animal models and methods will you use during this project?	Mice are used because many of the factors predicted to contribute to DNA methylation reprogramming have been described, so we are building on prior extensive published information and availability of the extensive genetic resource tools.
Why can't you use animals that are less sentient?	• Global epigenetic reprogramming has only been described in mammals • Important aspects of gastrulation and organ development are unique to mammals • A DNA methylation ageing clock has only been described in mammals and needs work across the whole ageing spectrum • We are interested to transfer our findings to human biology in order to contribute to diagnostic and therapeutic approaches in humans
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	• I have subscribed to the NC3Rs monthly updates • I will look out for 3R seminars in my institution and University • We will continue our work on cell systems that might replace animals
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	• Refined aseptic surgical technique • Animals approaching the limit of severity controls are checked more regularly and humanely killed (Schedule 1) if they are likely to exceed severity limit • Use of conditional gene ablation, which allows us to remove a gene specifically in target tissues rather

	than in whole animals where constitutive ablation would have a severe phenotype. • Effective colony management
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	Our animal usage guidelines are kept up to date with many aspects of refinement, such as substance volume and frequency limits and routes, advice against re-use of needles etc. This is done with reference to public resources such as the NC3Rs and procedures with care websites. Refinements developed in-house appear here and in individual Codes of Practise. The PREPARE guidelines go together with the ARRIVE ones and will help us plan experiments in a way that permits the results to be eventually published or reported using ARRIVE's guidelines.

	and prevent disease.
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 better understand the heritability of psychiatric disorders, scientists have been studying the genetic basis of disease heritability, but our understanding is still limited. We hope to identify both genes and their chemical modifications - so called epimutations - that alter disease risk across generations. We hope to learn how much different experiences can influence this. The genes and their chemical modifications uncovered by this project could help us better understand human disease susceptibility and it´s heredity. This will (A) facilitate the development of future research paths, to foster new treatments and therapies for psychiatric disorders and (B) help the design for novel strategies to prevent the inheritance of increased disease risk. In the long run our results may contribute to the minimization of the incidence of mental diseases and thereby mitigate the financial burden of the public health system.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the next 5 years, we will use up to 1000 mice (including genetically-modified) for breeding and up to 2000 mice for experimental procedures. 1000 of the 2000 mice used in experimental procedures stem from the mice generated in the breedings. Hence, we will approximately need a total of 2000 mice during the next 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?	We use a combination of environmental exposure and genetically modified animals to determine how changes in disease risk are inherited. To do so we expose wild type and genetically modified animals to one stressor, such as swimming or injection of stress hormones, and then breed them. At the same time we sacrifice some of these animals to obtain their sperm for in vitro fertilization. Then we do a range of behavioral and/ or metabolic tests on the resulting offspring and investigate whether a given stressor or genetic modification has heritable effects with implications for disease risk. Such tests typically include placing an animal in a box and observing where and how it moves around,

	placing an animal on an elevated plus maze with 2 closed and 2 open arms and again observe where and how it moves, allowing a mouse to swim and/or float in a tank of water, injecting an animal with insulin and taking small blood samples to measure glucose level just like in diabetic humans or injecting with glucose and taking small blood samples. We also try to track molecules, called RNA, that respond to the environment and that might be carrying information about the environment from one generation to the next. For this we also need to inject labelling substances into the body cavity of wild type and genetically modified animals during which animals are restrained by hand. The genetically modified mice are not expected to have any adverse effects. Many mice used in these experiments will experience enriched environments, for example when exposed to novel environments during behavioral studies, which could result in mild and transient anxiety when first experienced. Animals may be exposed to moderately stressful environments such as water, a restraint tube or substances with a slight sedative side effect for example a brightly lid field, but only for a short period of time. At the end of the studies, the mice will be killed using humane methods. Blood and tissues may be collected from them for measuring the body's response to the environment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mental disorders are reflected by a wide range of (mis)behaviors. Behaviour is a complex process, involving many different parts of the brain interacting with the rest of the body. It also requires sensory organs responding to a stimulating environment. Further, mammals undergo a very complex developmental program that, to the best of our knowledge, is designed to reset parental potentially disease predisposing changes. The environment in the womb during gestation and the maternal behavior in response to health-compromised males can also substantially influence disease risk heritability.

	We employ a thorough literature search and build on our knowledge gained in our previous work to identify potential alternatives to mouse experimentation. We have considered the use of plants, since they can inherit information about the environment from their ancestors, however they do not show any behavior, and can therefore not model diseases such as depression. Further, their way of producing offspring is substantially different to the one used in mammals and mechanisms found to transfer environmental information to the offspring in plants do not exist as such in mammals. *We have also considered the use of in vitro* technologies that use cells as a model. Only cells that can mature into sperm cells could adequately mimic the molecular processes necessary to encode environmental information and transmit it to the embryo. However, currently there is no cell model for growing mature sperm cells. Hence, current cell experiments cannot adequately reproduce the developmental complexity needed for the intended studies. Another possibility to animal studies is computer simulation. However, the current state of this technology is not advanced enough and does not comprise enough data yet to adequately predict heritable changes of disease risk induced by the environment. Therefore it is necessary to use live animals for studying the impact of environmental factors on offspring behavior and disease risk. Despite efforts within our laboratory to undertake such studies in worms called C. elegans, a less developed animal, it has proven insufficient to model the complexity of human behavioral conditions and in recapitulating mammalian inheritance of environmental information due to them not reproducing wholly by sex between males and females. Worms can be male and female at the same time within the same animal and therefore can produce offspring without mating. Therefore the biological processes taking place during their

	reproduction do not reflect those happening in mammals. Nevertheless our studies in worms have gained valuable knowledge about certain genes to be involved in the transmission of environmental information, that are similar between worms, mice and humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We review the literature and analyze data from tissue collected from dead animals to identify those genes most relevant to study. Whenever these genes have similar relatives in worms we conduct preliminary studies in worms. Experiments will be well-planned and detailed protocols written before the start. The scientists conducting the mouse experiments will not know what treatment the mouse has had before so to ensure unbiased data collection. Another scientist will assign the order of animals to be tested, taking care that an equal number of treated animals and control animals is used each time. We use statistics to estimate how many animals are needed to achieve our research goal. These mathematical calculations use existing data to determine how many mice are likely to obtain a meaningful answer to our question on the environmental impact on disease heritability. When no such preexisting data are available we base our estimations on our extensive experience with mouse behavioral and metabolic studies. The establishment employs specialized statisticians that can be consulted to ensure the accuracy of the estimations. This enables us to use the minimal number of mice necessary for each experiment. After each experiment data are analyzed using state of the art mathematical methods. To this end specialists can be consulted. Whenever possible we intend to use existing mouse colonies from national and international repositories rather than creating them ourselves. When different colonies are needed, we intend to use a service at our establishment that has specialist expertise to help reduce the number of animals used for generating these different mice. New mouse lines and our data

	will be made available to other researchers around the world.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse has been selected for this work since, mice have similar brains and sensory systems to humans and display many of the same behaviours. Mice also have very similar genomes to humans, so findings about heritable disease risk factors can be translated to humans. Due to their long history as a model organism the best tools are available to study their genes and the biological processes of their inheritance. Mice also breed very quickly, so they are an ideal model for studying behaviour across generations in a reasonable amount of time. We will use genetically modified mice that are produced by a team of highly specialized individuals. They are very experienced and have refined their working methods to obtain genetically modified animals. Our selection of genes to study is based on a thorough literature search and our data collected in preliminary studies in worms. Many of the procedures employed in this project license are enriching for animals (such as providing additional physical exercise). If procedures cause transient harm, the discomfort will be kept to the shortest period possible, as to still obtain valuable data. Appropriate anesthesia and painkillers will be applied for any surgical procedure adhering to best practice guidelines from LASA to minimize animal suffering. If any animals have to undergo multiple tests the mildest tests will be done first, and the most invasive will be done at the end. We preset decision points, so that when data from milder procedures have been collected decisions can be made quickly on the continuation so to prevent unnecessary more invasive procedures. Whenever substances have to be injected we use standard operating procedures adhering to best practice recommendations. We track all procedures carried out on a given mouse via a state of the art database. Decisions on mouse welfare can be made

	promptly, with access to the appropriate data for the mouse. We are continuously monitoring the literature to evaluate whether in vitro models become available to, at least in part, substitute our animal experiments.

Project	**124. Epigenetic regulation of murine embryo and placenta development**	
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)		
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 are a better understanding of three genetic disorders such as Rett, CDKL5 and Pelger-Huet syndromes. Furthermore we will have a better understand of placenta development and the role of genomic repeats (i.e. DNA fragments that re present in our DNA in multiple copies) ERVs in X	

	chromosome inactivation and miscarriages.
What species and approximate numbers of animals do you expect to use over what period of time?	Wild Type (WT) and Genetically Modified animals: 1600 over the 5 years 1600 over the 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?	All animals used in this proposal are healthy or expected to be healthy. The severity expected is mild and animals will be killed in a humane way, with no sufferance for the animal. We will do our best to ensure the optimal conditions for the animal welfare.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will do most of the work in cell lines . Validation in tissues from animals is still the golden standard for proceeding in clinical trials. Therefore, only a very limited animal work will be done for these projects.We need to use animals because neurons do no mature properly in the petri dish and they cannot recapitulate neurons from autism spectrum disorders. Also placenta models are very primitive and not informative for the type of work we aim to perform. We cannot use chicken or drosophila because they do not have a placenta and they have different sex-specific gene regulation programs compared to mammals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Mice will be subjected to super-ovulation procedure, therefore producing more animals for breeding pairs. This will increase the number of pups and reduce the number of breeding pairs. Statistical testing will be used at all stages in order to reduce the number of animals.
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.	Housing and caging will be adapted for the non-standards types of laboratory mice, such as hiding places and excess of nesting materials.

Project	**125. Epigenetics, growth and energy metabolism**	
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)	Growth in the womb is controlled by our genes and also the environment provided by the mother's body. Unlike most animals, the early growth of mammals takes place in the womb. The young are entirely dependent on their mother for food, which means that her diet is important for their growth. Good growth in the womb is important for survival at birth and for good metabolic health throughout life. Metabolic health includes the balance between muscle and fat in the body and the ability to process sugars properly. Poor metabolic health conditions such as obesity and diabetes are amongst our most common debilitating health problems and greatly increase the risk other killer conditions, notably heart disease and	

	cancer. Our work concerns genes we have identified that control growth in the womb and also influence metabolism in later life. We aim to test whether these genes do indeed form important links between early growth and life-long health. By changing the mother's diet, we will ask how the genes interact with environment during pregnancy to influence size at birth. The same young will then be monitored into adult life using a host of measurements, including the accumulation of fat and ability to process sugars (relating to obesity and diabetes, respectively). Some individuals will be challenged with a high fat diet and some will be kept into old age to assess their ability to live a long and healthy life. A second aim is to discover how these genes influences these processes. We will ask whether, in addition to altering the size of the body at birth, they fundamentally change body proportions by altering the formation of lean and fat during early life. Alternatively, or in addition, we will ask if they alter the way that fat accumulates in later life. Another aim is to establish how the identified genes might work together. One of the genes promotes growth and limits the accumulation of fat in the body, another gene limits growth and promotes fat accumulation. We propose that the opposing actions of the two genes are part of a common system that allows fine-tuning of growth and body fat content, including according to the nutrients available from the mother's diet. To uncover how this regulation works we need to identify other genes that are part of the same system. The fine tuning process likely involves turning up the activity of one gene and turning down activity of the other. A common feature of both genes is that they belong to a small but important sub-set of gene that are highly susceptible to 'epigenetic' control. It is this layer of control that probably allows the environment, including maternal diet during pregnancy, to influence gene activity in a manner that can have lasting effects on the body. We need to know more about how this type of gene regulation works if we are to understand the links between growth during early life and health outcomes in later life.

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)?	We expect to contribute toward the understanding of major health issues relating to growth and metabolism. Obesity alone is a major global health threat with around 60% of people worldwide classed as obese or overweight. In developed and developing nations prevalence is increasing, notably in children, and this trend is projected to continue. Direct health care costs for overweight and obesity are estimated at >25% of total EU healthcare spending (the wider costs to patients and carers are much greater). Obesity is a complex condition due to the involvement of many genes as well as environmental and lifestyle factors. Our studies of specific genes that affect growth in early life and how they are influenced by factors such as maternal diet could lead to significant contributions to understanding this burgeoning heath problem, as well as other prevalent disorders, including diabetes, heart disease and cancer. Knowledge of the underlying causes may help identify those at risk and pave the way for new interventions (potentially including dietary advice or supplements during pregnancy) and treatments. New information about the interactions between mother and child might provide insight into how and why these health problems increasingly affect young people and may be reinforced from one generation to the next.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 3,600 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 our work concerns the breeding of mice carrying defined genetic mutations that affect growth, lean to adipose body proportions and energy metabolism. Most will experience only mild adverse effects as a consequence of their genetic alterations, for example, they may be leaner or fatter than normal. This may have adverse health consequences that might include poor handling of sugars (akin to mild diabetes), higher susceptibility to cancers and a shortened life-span, which are the health problems under investigation. Some mice will undergo tests for their ability to store and use glucose, or to respond to insulin, which involves injections and collection of blood samples, usually after an overnight fasting period. Mice

	may need to be placed in a restraining device for a few minutes while taking blood samples, blood pressure readings or for body scans (e.g. NMR or micro-CT scans). Animals will be anaesthetised for some types of scan where they must be still. Mice may also need to be housed alone for several days in order to measure their activity, food and water intake or energy expenditure. Most of the outlined procedures cause at most, transient pain or distress, which is considered mild harm. Some animals may be subject to repeated tests of this sort at different ages as this allows collection of data connecting early growth and environment with changes in health as the body ages. This will entail keeping some mice into old age, however, animals that become ill at any time will be humanely killed , including any that develop overt symptoms of illness, such as unexpected weight changes or the appearance of lumps that could be tumours. At the end of the experiment mice will be humanely killed and usually a range of tissues taken for analyses that are part of our work. Some animals will be bred with genetic defects that cab severely affect growth in the womb, such that they are not expected to survive beyond the first few hours after being born. These animals will be humanely killed for tissue analysis at fetal stages or as soon as possible post-partum on the day of birth.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We aim to understand growth during early life and subsequent effects on adiposity, metabolism and longevity. We do use cell culture where possible, for instance to study in detail the changes occurring at the cell and molecular level. However, ultimately there is no substitute for the whole animal because we study gene function at the level of tissues, organs and the whole organism and developmental processes that rely on interactions between mother and offspring which are unique to mammals (those mediated by the placenta and mammary gland).

2. Reduction Explain how you will assure the use of minimum numbers of animals	We minimise numbers using the following measures: Keep up with developments in the field to avoid duplication of experiments and use knowledge derived from complementary technologies (including our own in vitro studies). Apply statistical analysis routinely to estimate minimum numbers of animals required for valid comparisons. Power calculations are used to project numbers, and are required by all major funding bodies. Plan genetic crosses efficiently to generate animals of the desired genotypes, including appropriate controls (typically littermates matched for age, sex, genetic background and environment, including any influences of the mother). Collect multiple samples from the same animals, where possible in longitudinal studies. For example, sophisticated body scanners have replaced the need to kill animals at different ages to track changes in body composition. Multiple tissue samples are typically taken for several different analyses after animals have been humanely killed at the end of each experiment. Pool data from different time-points (when appropriate) for statistical analysis. Use statistics, modelling and network analyses for optimal use of the data we obtain.
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 work is specific to mammals, including understanding of the critical early-life growth periods involving interactions between mother and offspring and how these impact on physiology and behaviour of the whole animal. Therefore, the work cannot be carried out in non-mammalian animals or purely in vitro. Among mammals, mice provide the optimal system to combine prior knowledge of genetics growth and physiology, with the ability to

	manipulate genes and determine their function. We always seek to minimise exposure to stress, harm or pain, e.g: minimising periods of isolation and fasting, without compromising experimental validity and by appropriate administration of anaesthesia and pain relief. Careful monitoring is employed to detect harmful defects (such as cancers) and alleviate suffering at the earliest opportunity.

Project	**126. Establishing PKC-superfamily members as targets for new cancer 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)	Amongst the key changes in cancers are those associated with a family of cellular regulators, referred to as protein kinases. Here we address the cancer-associated actions, interventions and physiological liabilities of members of a subfamily of protein kinases, termed the protein kinase C family. We will determine if inhibition of aPKCi impacts tumour growth. We will determine if PKCe inhibition/loss is associated with reduced tumour formation. We will assess the phenotype of PKN family loss and assess	
	impact on tumour incidence.	

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)?	We are seeking to improve cancer patient treatment options through validating and enabling drug development programmes directed at specific molecular targets. During the course of this licence we will test the effects and potential liabilities of genetic removal of PKN subfamily members in the context of a prostate cancer model (PKN3) and a colorectal cancer model (PKN1-3). We will test directly the consequences on aPKCi inhibition in the context of mutant Ras driven lung cancer in a series of different genetic models of disease. We will test the validity of PKCe as a target for intervention in p53 mutant tumours both through genetic ablation and also through inhibition. All studies will be written up and disseminated to the wider research community and we anticipate that one or more of these studies will lead to progression of drugs into Phase 1 trials in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	It is expected that we will use 10000 mice during the course of these studies. These will include embryos, but most mice will be used as 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?	The adverse effects will relate to tumour formation and any stress associated with possible heart hypertrophy (for one subfamily of genes). For the majority of the tumour formation studies, we are breeding tumour prone genetically modified strains where we have knowledge of the timing of tumour formation and hence can actively monitor animals at the appropriate time to ensure that animals do not suffer unexpected effects. The crosses and treatments we propose to perform are based on ex vivo experiments where we have observed suppressive effects of interventions and hence the expectation is that we will suppress tumour development or progression and hence reduce the tumour-associated adverse effects seen in these strains. In the case of the PKN2 knockout, in the context of PKN1/3 loss, we are seeking to induce colorectal cancer with a well-tested promotion protocol. There is the possibility of inflammation associated with this

	protocol, however careful monitoring will be applied to ensure that if this occurs then any associated adverse effect is limited. In respect of this genetic manipulation, the expectation is that there will be a reduced tumour burden with loss of this gene subfamily and hence no additional adverse effects. A related colorectal induction protocol is also to be used where we wish to test directly a drug intervention against another member of this gene family (PKCe). Here a less inflammatory protocol is to be employed which works in the particular genetic background; there is little likelihood of an inflammatory response here. As above, these manipulations are expected to reduce tumour burden and hence reduce adverse effects. All animals will be humanely euthanised at the end of the experiments or once the humane endpoint is met
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Extensive *ex vivo* studies have been carried out on the genes under investigation here, leading to specific predictions regarding reliance of certain tumour types on the action of a subset of these genes. However, these predictions are not sufficient to define them as viable drug targets in human cancer, as they rely upon monocultures of tumour cells not accounting for the complexity of the *in vivo* environment; it is noted also that these genes are widely expressed in normal tissues. Hence to understand whether the dependencies observed *ex vivo* contribute to *in vivo* tumour formation and progression, and whether targeting these genes offers a therapeutic opportunity, demands data from a suitable *in vivo* model organism. The gene family under investigation is comprised of 12 genes (PKC superfamily) and is uniquely present in mammals with a limited repertoire present in other model organisms, ranging from 1 in yeast to 5 in drosophila. For those genes specifically under investigation here, non-mammalian model organisms do not provide either the complexity of the mammalian family (not represented or only in part) nor the context (e.g. stroma, vasculature, genetically engineered

	tumour models) in which to assess roles in tumour behaviour and the potential liabilities for patient intervention.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The fundamental principles of breeding mice will be observed. As a general principle experiments will be designed to employ the least number of mice in order to derive a statistically sound answer. Specific advice on design and analysis has come from individuals who have extensive experience of work with the tumour models we intend to assess.
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 complexity of the mammalian gene family is represented in mice alongside the tumour models, making mice currently the only choice for these *in vivo* target validation studies. We will employ good husbandry practices including regular monitoring of all animals. Through this good practice we will closely monitor the welfare of our animals and look for any reaction to experimental procedures. We will increase monitoring following any acute procedure. We will utilise humane endpoints to minimise suffering and especially in any cases of unexpected / additional suffering. The use of humane endpoints will be considered carefully at the stage of experimental design, and reviewed throughout experiments in light of our observations.

Project	**127. Establishing the mode of action of novel plant extracts in poultry feed**	
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)	Broiler chickens, reared for meat, are the most numerous farm animal globally. Globally, around 60 billion broilers are produced in this rapidly expanding food sector. Because of intensive production methods (e.g. high stocking density), broilers are prone to bacterial diseases, especially relating to gut health. To combat this, in the past broilers were routinely fed antibiotics, but because of fears that this was contributing to antimicrobial resistance, this practice has now been banned in many parts of the world, including the EU. To improve broiler health, welfare and production, there is an	

	urgent need to find effective and safe alternative antimicrobial compounds that can be fed routinely. Various plant extracts have been shown to have these properties, but their mode of action is not clear. The aim of this project is to use a range of approaches to improve our understanding of the effectiveness of candidate plant extracts (citrus and cucumber extracts) and characterise the physiological pathways they influence. To do this, we will identify novel biomarkers and of immune and gut function in broilers and quantify how they are affected by diet formulations containing the plant extracts. We will use a range of approaches (transcriptomics, microbiomics, proteomics and metabolomics) to investigate this process from multiple perspectives. These techniques allow the characterisation and quantification of a range of biological molecules that relate to the structure, function, and dynamics of an organism.
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 allow us to determine the effects of plant extracts with promising antimicrobial properties on the health and growth of broiler chickens in commercially relevant conditions. The OMICs approaches we will employ provide the tools to better understand the multiple pathways involved. This validation of novel therapeutic interventions in feed is likely to result in significant bird health and welfare benefits for large numbers of animals, and production gains which may have implications for food security. Further, common disease causing bacteria in poultry such as certain strains E.coli are potentially harmful to people, so improved control of outbreaks in broiler flocks could also protect public health.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 1200 chickens over the five year course of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at	The project has moderate severity because we will carry out immune challenge experiments on broilers, to mimic natural bacterial disease. This involves a single injection with an agent that causes short-lived inflammatory response associated with loss of appetite, malaise and

the end?	increased body temperature. The effects of this immune challenge will resolve within 48 hours and we will closely monitor the birds during this time. To determine the effects of diets containing plant extracts (and allow comparison with control diets or diets containing established antimicrobials), birds will be blood sampled at intervals of 6 to 24 hours and some will be humanely killed to allow us to harvest tissue for analysis. At the end of the experiments the birds will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Replacement of chickens with a non-sentient alternative is not possible as we aim to characterise the effects and mode of action of novel potentially beneficial diet ingredients in the context of responses to immune challenge, which can't be replicated in artificial models. We have fully considered alternatives but to assess the value of novel anti-microbials on growth and intestinal health requires whole animal model in the relevant species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully calculated the minimum meaningful numbers of animals for each experiment, based on previous studies of responses to immune challenge in broilers. Where appropriate, we will employ a factorial statistical designs to maximise statistical power and allow identification of interactions between our measures and causal factors, minimising animal numbers. We will randomly assign animals to experimental groups and to reduce variation, we will use chicks of the same broiler strain from the same hatchery reared with uniform housing and husbandry.
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 work on broiler chickens as we are aiming to address health and welfare issues in this farmed species, which is the most numerous globally. We will feed novel plant extracts and control compounds in fully nutritionally balanced diets. We will regularly weigh the birds to monitor normal weight gain and we will stop using any diet associated with reduced growth. We will habituate the birds to

	handling, reducing stress associated with subsequent procedures. The immune challenge model we have chosen to use is well established in chickens and reliably simulates a measurable immune response. This approach is a refinement compared to deliberately infecting birds with bacterial diseases. To limit adverse effects, we will use the lowest effective dose to achieve our results, based on previous published work.

Project	**128. Establishment and healthy maintenance of the blood system**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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)	During adult life, blood cells are continuously produced by a population of unique blood cells that is generated during embryonic life. We know very little about the formation of these unique blood cells, termed stem cells. In the first part of the project, we will define the role of novel regulators of embryonic and adult blood stem cell formation. Many blood cell diseases can be cured by injection of these unique blood stem cells in sick people. Sadly, the availability of these cells is often limited because these cells can only come	

	from donation from healthy people. In the second part of the project, we will test for novel sources of these stem cells that do not depend on donation.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work is interesting in its own right, and it will help efforts to understand haematopoietic development and the generation of blood stem cells. Blood stem cell transfer has been used in the clinic for many years now to treat multiple forms of blood cell diseases. Unfortunately, a severe limitation in treatment with blood stem cell remains the restricted availability of compatible donors. The efficient generation of blood stem cells would represent a remarkable progress, providing access to limitless sources for use in the clinic.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used in this project. We anticipate using around 4500 animals during the 5-year period of this 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?	The majority of the animals will undergo procedures to study blood cells; this includes blood withdrawal, immunization, irradiation and bone marrow transfer, injection of drugs affecting blood cell biology, genetic modification of regulators of blood cells. Aseptic techniques will be used to minimise potential infection and anaesthesia will be used when necessary to minimise the pain and discomfort from the procedure. The level of severity will be moderate for all these experiments. Adverse effects might include stress and mild pain from treatments such as injection and irradiation. All animals in these experiments will be closely observed for sign of poor health and behaviour. Supportive therapy such antibiotics and analgesics will used to mitigate pain and distress or possible complications from the procedures. At the end of all experiments, animals will be humanely killed by an approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals	This programme of research is based on many experiments using cells *in vitro*. A large amount of pilot experiments and optimization are

and why you cannot use non-animal alternatives	performed using these assays. As a result, this decreases greatly the number of experiments performed with animals. Unfortunately, no assays in a dish or a test tube are good enough to test blood stem cells. The regulation of blood cell production can also only be studied in animal and the specific structure of the body where blood cells develop cannot be replicated in a dish or a test tube.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ask for help from our statistician on a regular basis and particularly before starting new experiments. This will allow predicting the length and strength of treatments and how many animals are needed to answer the questions we are studying. We will mostly use experimental procedures that are well established. We will regularly discuss with people, at the university and in the larger community, who are experts in experimental procedures and animal well-being. We will use hygienic techniques and clean, disease-free, animals to help limit the numbers of animals required. The use of new technologies such as imaging blood cells in live animals under anaesthesia will also contribute to reducing the number of animal used.
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 have been chosen for the study because they represent the least sentient species from which useful experimental data can be generated Mice have considerable genetic and biological similarities to human with regard to blood cell formation. They are the best characterized specie to study the function of blood stem cells. Experimental protocols are particularly well established for this animal model. Data acquired over time using mouse models have translated well into clinical trials.

Project	**129. Establishment of early pregnancy**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand the physiological mechanisms associated with superovulation and the establishment of pregnancy. Given that these are conserved across species, the results of this project will be of both clinical and veterinary value. Since the establishment of pregnancy is a rate limiting step associated with many experimental procedures (e.g. the breeding of specific experimental models of human disease), this project will also allow us to improve the methods used to achieve pregnancy and reduce both the numbers of animals used overall but also the severity of the procedures that they are subjected to.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are several potential benefits to this research: (i) For humans: this will highlight the mechanisms associated with superovulation and the establishment of early pregnancy and highlight novel potential interventions for the management of infertility, recurrent miscarriage and preterm labour. (ii) For animals: the methods developed and optimised as part of this project aim to streamline many laboratory protocols with a view to replacing the use of vasectomised males and reducing the number of females overall in research programmes which use rodents as models (mice and rats). Moreover, it is expected that those that are still used will have refined, milder and less-invasive measures used for establishing pregnancy. This could benefit hundreds of thousands of animals worldwide per year as well as potentially having translational benefits for the farming industry in the longer term.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: 5,300 animals. Rats: 900 animals. 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 adverse effects to these well-established procedures are minimal and the novel ones being developed will present a reduction in severity. The animals will be humanely euthanized at the end of each experiment. Pups generated from experimental females will be used in further experiments wherever possible in order to minimise the numbers of animals used overall.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mechanisms associated with the establishment of pregnancy are highly complex and systemic such that they cannot be studied in alternatives (e.g. cells/organs).
2. Reduction Explain how you will assure the use of minimum numbers of	The number of animals will be reduced in two ways: (i) by careful experimental planning with support from a statistician in order to minimise wastage and (ii) by improving existing

animals	experimental protocols used for achieving a pregnancy after embryo transfer.
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 used as a model for other rodents as it is the least sentient species, yet remains a representative and useful model. Welfare costs will be reduced by using less invasive procedures (e.g. pessaries instead on injections) as well as ensuring that any animal used in an experimental procedure (none of which are more than moderate severity) is regularly monitored and receives pain control if appropriate. Animals will also be housed in social groups where they can exhibit normal behaviour.

Project	**130. Evaluation of developmental and therapeutic potential for preimplantation embryos and embryonic stem cells**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	**Aim: understanding of how early embryos develop and implant in the uterus.** We use the mouse as a model as it is a mammal like humans with a similar reproductive process and its genetics are well established. We apply our findings to human reproduction and development e.g. to determine if critical components are present in human embryos (obtained after informed consent from the IVF lab) or whether experimental interventions alter key implantation stages.	

	Aim to understand normal and abnormal human tissue development We use human stem cells to model cell specialisation and test if findings from mouse are relevant to human development. We make tissues from human stem cells made from patients with genetic disease. Because we can make specialised tissues from these stem cells we can test what goes wrong in the development of these diseased human tissues. We aim to test these in animals since the tissues do not develop fully in the dish, they need the body environment. We make human stem cells become a variety of tissues of the body in order to compare regular development with that from cells with genetic mutations. We are studying diseases of cartilage and bone as well as of the kidney, nervous system and vasculature
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By understanding the process of early development and implantation we hope to develop therapies to overcome some rare incurable human diseases. We do this by developing mini tissues in a dish from stem cells generated from patients who are healthy or have mutations found in patients with such rare diseases. We have developed such tissues for kidney the vasculature, cartilage and bone. By studying what goes wrong in the disease tissue we identify targets for drugs and can test these. However the tissues only fully develop in the body so we need to test them by implanting in an animal. We can generate cartilage cells from human stem cells and implant them into small defects in the rat knee joint to show they can repair these defects. Thus we produce preclinical data to help us to develop a new safe and efficacious cell therapy for use in humans who have e.g. Osteoarthritis or sports injury. We aim to improve ongoing pregnancy rates in IVF/ART and determine the long term effects of these procedures by optimising embryo-maternal communication. This includes identifying druggable pathways that enhance or accelerate embryo implantation, or stressors that should be avoided in the IVF laboratory.
What species and approximate numbers of animals do you	780 mice over 5 years 200 rats over 5 years

expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will give natural reproductive hormones by injection with little adverse effect except soreness at injection site. Embryos will be collected from animals after humane killing. To assess embryo implantation, embryos will be transferred to non-pregnant recipient animals by injection into the uterus during anaesthetised surgery. Some discomfort will follow surgery which will be managed with analgesics, and infection is very rare. Implantation of cells to test if they can form a benign tumour containing a variety of tissues tells us if these cells are able to make specialised cells. This will give some discomfort following surgery (minimised by use of a syringe for application where possible) which will be mitigated by analgesics, but infection is very rare. Animals will develop the benign tumours which do not invade and spread and are small, but if cysts develop and cause discomfort then animals will be humanely killed. Similar surgery will test if stem derived tissues can form mature cells of different tissues when implanted under the skin. There is little discomfort but animals will be monitored as for benign tumours. Cartilage forming cells will be put in a small abrasion in a hind limb joint of a rat to test potential for joint repair. This will give some discomfort following surgery mitigated by analgesics, but infection is very rare The rats may have a stiff joint the next day following surgery. All animal will be killed humanely at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We cannot study the process of embryo-implantation into the uterus in the human for ethical reasons. We use the mouse because a mammal is essential for investigation of embryo implantation into the uterus; it provides a model similar to human. The mouse is considered to be the least pain-sensitive mammal. There are no entirely satisfactory models in a dish for embryo implantation, so a combined approach using research outside the body (in vitro) and with animals is essential. Data on implantation obtained from mouse embryos in a dish (our main

	experimental model) must be checked in animals. We use embryonic stem cells (hESCs) to study how embryonic cells become specialised, avoiding animal use and comparing to human and murine preimplantation embryos in vitro. In vivo protocols are only used when there is no alternative, such as to validate data obtained in vitro. This is essential because we can only examine the earliest stages of tissue development/implantation in the dish. We use the gold standard method for testing human embryonic or similar stem cells for potential to form specialised cells and tissue formation. This is the benign tumour assay in mice, which requires implantation into a mouse because the cells cannot reproducibly produce tissues in a dish, or in alternative models (e.g. zebra fish as an alternative is unsuccessful). For preclinical research on hESC-derived chondrocytes it is essential to test the cells for ability to make true knee-type cartilage in the joint. There is no reproducible model in a dish to test this. Thus we need to use the rat cartilage repair model, the rat being a mammal with sufficient body weight and depth of cartilage to provide a valid initial test system (not the case for mice). We cannot test human cartilage cells in non-mammals to give meaningful data on joint repair.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Cartilage cells: where possible experiments are undertaken with stem cells thus avoiding mouse embryos. This is not possible for embryo implantation. Here we carry out most studies with very early embryos: a ball of < 100 cells together with cell lines or cells from the lining of the human uterus, in a dish. Only validation experiments will be done using mice. Embryos: We will use the minimum number of female mice to generate the minimum number of embryos needed for statistical significance in implantation studies in culture. Benign tumours: are produced from stem cells by 4/ 5 mice injected. Therefore a minimum to assure tumour formation of 4-6 animals are used for each human stem cell lines tested. Whole animal non-invasive imaging for teratomas will increase the information/animal and reduce animals used in

	future. Cartilage repair; Cartilage repair from our cells is seen in around 66% of joints. Therefore we need a minimum of 5 animals at each time point to monitor the process of repair. There is no reliable in vitro assay to assess cartilage repair. We have also introduced various whole animal imaging steps to increase the information obtained from each animal and reduce animals further. We do extensive monitoring of all cells and embryos in a dish before use in animal models to ensure reproducibility and avoid animal wastage. We use our extensive experience to determine the number of animals we use is kept to a minimum. We will evaluate all experiments immediately on completion so that we do not use more animals then 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.	We use the mouse for studies on reproduction because a mammal is essential for investigation of embryo implantation and this provides a model similar to human. The mouse is considered to be the most tolerant, least pain-sensitive mammal, with the best understood genetics. For surgical procedures, we use general anaesthesia in purpose built operating theatres with best-practice operating techniques to avoid infection and surgical complications. We apply pain killers to minimize postoperative discomfort. The cartilage repair experiments are done in rats since the rat is considerably more weight bearing than mice and the joint in the mouse is too small to reliably confirm joint repair especially as this needs to scale-up for human joint defects which can be up to 8mm across. The rat work is designed to be a necessary step before larger animal models (e.g.minipigs) and phase 1 human clinical trials in future work. We are developing imaging techniques to monitor cartilage repair in situ which will allow us to i) visualize repair in animals harmlessly; ii) monitor the time course of repair; iii) use fewer animals since animals can be viewed at different times and killed humanely after a final scan.

Project	**131. Evaluation of innovative, small medical devices for improving current diagnostic and interventional medical procedures**	
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 evaluate feasibility and safety of innovative medical devices for inspection of the digestive tract (gastrointestinal endoscopy) or for abdominal surgery. The following will be answered: *Is the concept feasible?What is the most efficacious design?Is the concept safe and suitable for transitioning to redesign towards clinical use?*	

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 novel devices developed in this project are related to high impact, major clinical needs with millions of individuals potentially benefiting from the successful implementation of the technologies. For example, bowel cancer survival rates could be drastically improved with the adoption of our robotic system which could provide an efficient, easy-to-use, painless alternative to the conventional procedure. Wider groups (e.g. the NHS and the UK as a whole) could also benefit from significant economic savings, particularly with the successful adoption of low-cost concepts. The work will be disseminated in peer reviewed journals and conferences, with the publications being relevant to actively researched areas in medicine and robotics. This will provide valuable knowledge to the wider research community. The work focuses on high impact, clinically relevant areas where there is still significant scope for innovation. Therefore, the technologies developed have a tangible and significant need, ensuring that support is readily available to push the innovations to clinical use. The extensive facilities, technical expertise and previous research track record of the group will ensure maximum success rate.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 60 adult pigs over 5 years. This is the minimum number required to ensure the effective, safe and ethical development of our technologies.
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 devices/technologies developed in engineering laboratories will be extensively tested on benchtop and/or in human and animal tissues before being considered suitable for animal trials. Animal experiments proposed in the applications will be carried out under general anaesthesia. Small moveable devices will be inserted either in colon, stomach and small intestine or in the peritoneal cavity and manipulated by hand or by external sources. After the testing period, animal will be humanely killed without regaining consciousness. Animals will be at risk of the usual complications of general anaesthesia. Balanced anaesthesia will be induced and maintained by a veterinary surgeon. Any animal showing anaesthetic complications that jeopardise animal welfare or

	scientific aims will be killed immediately by schedule 1 method. In our experience, no adverse effects are expected by the manipulation of devices inserted in the digestive tract or peritoneal cavity by using our minimally invasive technology in anaesthetised animal. The entire procedure may take up to 6 hours during which fluids will be administered intravenously as a routine routinely and temperature maintained via a warming pad. Minimally invasive surgical procedures will be carried out under sterile conditions and every effort made to avoid and control adverse events. As all procedures will be carried out under terminal anaesthesia, we do not expect any adverse effects during manipulation of small medical devices by non-invasive or minimal invasive techniques. At the end of experiment, animal will be killed by a schedule 1 method (overdose of anaesthetic).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to design appropriate medical technologies and evaluate their safety, an environment as close to that of the living human is required. These conditions are necessary to fully assess safety and functionality of the device, including those impossible to recreate synthetically on the bench-top. Testing in an animal model facilitates the refinement of the device design, and specifically the reduction/mitigation of risks associated with its use in humans. This is ethically crucial before investing resources in redesigning a device/technology towards clinical trials. To-date, no synthetic alternatives are available that can completely recreate conditions found in a human because of the immense complexity. The devices in this project will be developed first in the laboratory using synthetic environments (e.g. silicone) and where possible, in simulation. However, the devices are designed to interact with the living soft tissues and hence their properties are crucial. Therefore, a realistic model is required to ensure the device functions properly before in-human use. Pigs are often used because of their similar size and tissue properties to humans.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Synthetic test environments and/or human cadavers will always be used extensively first and where possible, work with animals will be avoided completely. Benchtop work will be used to answer fundamental questions and to refine the concepts as much as possible. Once operating with satisfactory performance and achieving full functionality, animal models will be used only where is completely necessary for the assessment of device safety and efficacy. If technically feasible, multiple devices will be tested on the same, single animal and thus make most efficient use of the available resources. If we don't have at least 2 devices/technologies ready to be tested in a single animal, the trial will be cancelled and tests will be postponed to the next planned experiment. This project is aimed at pilot feasibility trials and therefore, data analysis will be descriptive and graphical. No formal statistical comparison will be made. This, combined with the extensive testing on each device that is planned before the animal experiment (i.e. benchtop and human cadavers), results in the minimum number of animals required to test each device. Based upon our extensive experience, three consecutive successful animal trials will be sufficient to establish whether the device/technology is good enough to pass to the next phase (i.e. redesign and specific pre- clinical testing towards pilot human trials).
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 pig model is widely accepted as a suitable replacement for the living human model for abdominal surgery and interventions relating to the digestive tract. This is because of its comparable size, tissue properties and - with the exception of the large bowel - anatomy. All devices will be tested extensively on benchtop and human cadavers prior to animal work and will only be tested in animals if completely necessary. This will ensure the work done in animals is carried out with minimal risk/harm to both the operators and animals. Experience gained from the animal trials will be used to not only refine the device, but also the procedure itself, ensuring all are performed efficiently and with minimal harm to those involved. All testing will be performed under terminally anaesthesia under non-recovery conditions.

Project	**132. Evaluation of pharmacokinetic properties of novel drug candidates**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to evaluate the pharmacokinetic (PK) properties (absorption, distribution, metabolism and excretion) of novel compounds in mice and rats and assess whether they support further development of the compounds as potential drugs, targeting areas of unmet clinical need. This *in vivo* evaluation of drug candidates is an essential part of the drug discovery process. *In vitro* data is utilised in the selection of compounds for animal testing, however, no *in vitro* techniques can fully predict the *in vivo* behaviour of all compounds. By obtaining appropriate *in vivo* PK data early in the	

	programme of work, the most suitable compounds can be selected for progression into pre-clinical and then clinical efficacy studies, with a much greater chance of success. The majority of studies involve the administration of a single low dose of the compound by a selected route and taking timed blood samples for the assessment of compound levels. Standard PK parameters will be determined from these data (e.g. half life, apparent volume of distribution, clearance). Results from studies carried out under this licence can be used to optimise dosing regimes prior to testing compounds in animal models of disease. Data produced can also be used to improve the design of future compounds.
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 long-term benefit of this project will be the selection of novel compounds for drug development with a higher probability of success in the clinic, so improving the availability of new medicines in areas of unmet clinical need. In the short term evaluation of PK properties of compounds will filter out those with unfavourable characteristics so that only those most likely to be efficacious will be tested in future animal models of disease. The data generated will enable clear decisions to be made and will be used to improve the design of future compounds.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice are requested on this licence. It is anticipated that no more than six hundred rats and five hundred and fifty mice will be required over the course of this licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All staff involved in conducting proposed studies are highly experienced, so adverse effects from routine procedures such as dosing (by various routes of administration), sampling and use of anaesthesia, are infrequent. Compounds will be selected on the basis of pre-determined in vitro properties. Doses will be kept as low as possible and all animals on study will be closely monitored. The severity level for Protocol 1 is moderate and Protocol 2 is Non-recovery. All animals will be humanely killed at the end of all protocols.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Computer modelling and *in vitro* screening provides extremely useful information on candidate compounds. These studies, however, are not sufficiently accurate at predicting the behaviour of compounds *in vivo,* as PK properties are the result of complex interactions within the body that cannot be replicated *in vitro*. Therefore animal testing is essential to achieve the objectives of this licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Cutting edge technology and computer modelling are used to design drugs that target selected therapeutic areas. Extensive *in vitro* testing is undertaken, which filters out the compounds with the most favourable characteristics for *in vivo* testing. For mouse studies, increased bio-analytical sensitivity means that smaller samples can be used to give reproducible meaningful data. This means that multiple samples can be taken from one animal, so reducing the numbers required for each study. The smallest group sizes are used to allow for statistical analysis (e.g. 3 rats for a full PK study).
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 are used as they are the lowest suitable species where data can be extrapolated to humans. The project seeks to aid compound selection for use in animal models of disease. Some of these models are performed in mice, others in rats, hence the requirement for both species. Competent personnel will perform all studies covered by the project licence. Guidelines limiting the volume and frequency of substance administration and sampling will be strictly adhered to. Animals will be closely monitored over the course of the studies. Humane endpoints have been discussed with the named vet.

Project	**133. Examining the role of mitochondria in neurodegeneration and vascular disorders**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	A common factor in neurodegenerative and cardiovascular diseases is the dysfunction of mitochondria - small components of all cells that are vital for providing energy, detoxifying potentially-harmful chemicals and influencing biological signals such as calcium waves. Mitochondria move within cells to sites of need (particularly important for the energy-demanding cells of the brain), but they can be damaged by toxic or stressful events and then contribute to cell death. Calcium waves are one of the primary forms of signal that cells use to both control internal processes and to co-ordinate organ-	

	wide responses. Precise control of these calcium signals is still not well-understood, particularly in chronic, complexes diseases such as diabetes, high blood pressure or Alzheimer's disease. One potential cause of stress to both the brain and blood vessels is a component of the blood that transports fats and cholesterol, called Apolipoprotein-E. ApoE circulates in the blood and is also present in the brain. Dysfunctional ApoE can impair mitochondria in both brain cells and blood vessels, potentially contributing to the development of dementia. ApoE is the main genetic risk factor for Alzheimer's disease. Of the three human versions of Apo-E (2, 3 or 4) inheriting only Apo-E4 increases the risk of Alzheimer's disease 20-fold. Apo-E4 also increases the risk of blood vessel dysfunction, before dementia starts, however it remains unclear whether the primary, disease-causing deficits occur in the blood vessels of the brain, or the brain cells that they supply. **Aims** This project will use rat models of Alzheimer's and cardiovascular diseases, allowing the animals to age in order to determine the critical steps in disease development. Alterations in the function of cells, their mitochondria and their calcium signals will be examined in the various cells in the brain and blood vessels, to better understand how treatments and preventative strategies should be focussed. Dietary modifications that exacerbate blood vessel dysfunction (high-fat plus high-carbohydrate) will be contrasted with those that are reportedly neuroprotective (high-fat but low-carbohydrate). Exercise is reported to provide neuroprotection via the mitochondria and as such will be examined for its influence on mitochondria, brain cells (including blood vessels in the brain) and disease progression.
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 examines instigating events that lead to impairments in Alzheimer's disease and in vascular conditions including diabetes and hypertension. In the short-term, the primary benefits will be increased understanding within the scientific community as to the nature of these disease-preceeding alterations within individual cells and

	tissues. This understanding will inform and direct further research, establishing experimental platforms for the evaluation of new treatment and prevention strategies. This project will also drive a better appreciation of the complex nature of vascular and cerebral responses and adaptations in disease. The intact-blood vessel technologies developed for, and utilised in, this project will expand both the scientific and clinical understanding of how complex organs such as blood vessels function in health, in disease and in response to drug treatments. In the longer term, the project has the potential to inform patient advice on how alterations in diet or exercise could be used as strategies to treat, delay or even prevent Alzheimer's disease-like dementias. Novel pharmacological agents to protect brain cells in the early stages of dementia may also be developed as a result of this project. Additionally, this project aims to improve the management of vascular diseases by enabling a more-informed choice of pharmacological therapies and potentially uncovering novel pharmacological targets.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use wild-type, ApoE knock-out and ApoE4 knock-in Sprague Dawley rats, the latter of which display features of Alzheimer's disease from 3 months (9 breeding pairs generating litters and aged adults). This project will also use the Spontaneously Hypertensive Rat (SHR) strain that develops high blood pressure without the need for invasive drugs or surgery that other models require. This model develops very similarly to human chronic high blood pressure, thus providing a useful, informative model. The project will also investigate obesity-induced alterations in blood vessel function using the Zucker Obese rat strain. Additionally, two genetically-altered strains of mice will be used in this project: the GCaMP-GR transgenic mouse and the db/db diabetic mouse. The GCaMP mouse allows us to see signals in specific cells of the blood vessels, enabling discrimination of where signals start and how they are transmitted to control blood vessel responses (and thus how this might be compromised in blood vessel disease). The db/db mouse develops diabetes with a gradual onset and worsening of conditions, however we will impose a restriction of keeping these mice to no older than 6 months. A maximum of 5,600 animals will be used
	over the total 5-years 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?	Rats will be allowed to age for up to 2 years in this project, with some given optional access to an exercise wheel and some given variations in their diet (altered fat/carbohydrate content). During this time the animals' cognitive and cardiovascular functions may be tested (by maze/recognition tests and ultrasound of the heart respectively). Finally, the animals will be humanely euthanised and tissue removed to allow age-associated changes to be examined both in brain tissue and intact blood vessels. The severity limit for the majority of project is Mild (the exception being the diabetic mouse model which is Moderate), with the actual severity expected to be sub-threshold for the majority of animals. Expected adverse effects of this project are: - ApoE knockout rats can have increased fatty deposits in their blood vessels, leading to an increased risk of stroke. Cardiovascular function will be monitored to restrict severity of adverse effects to Mild. - ApoE4 knock-in rats are expected to develop cognitive deficits akin to human dementia, including decreases in brain volume and working memory and potentially increased aggression. It is anticipated that such deficits should not impair normal function. - The ApoE and wildtype control animals will also be maintained for up to 2 years to allow age-associated deficits to develop. Again, the maximal severity is Mild, additional animal husbandry care will be provided to increase comfort where necessary. - SHR rats have elevated blood pressure and are therefore more prone to adverse cardiovascular events. They can also display insulin resistance and metabolic disturbances. -Obese Zucker rats gain considerable weight from 3 weeks of age with associated increases in blood cholesterol and fats as well as development of extensive fat stores. We will only maintain these animals for ~4 months, to avoid the more severe adverse cardiovascular effects this altered metabolism can eventually lead to. - db/db mice develop spontaneous diabetes, leading eventually to morbid obesity and metabolic disturbances. Obesity starts at 3 to 4 weeks of age, alongside an uncontrolled rise in blood sugar, depletion of insulin-producing pancreatic cells, peripheral neuropathy, myocardial disease and death by 10 months of age. Wound healing is delayed, and metabolic efficiency is increased. We will only keep these mice to a

	maximum of 6 months of age to restrict severity to Moderate. This restriction will ensure that their symptoms, at worst, are along the lines of obesity with associated potential discomforts of altered blood pressure, heart rate, mobility or grooming behaviour. - GCaMP-GR transgenic mice are not reported to have adverse effects. - Modifications to the animals' diets may result in changes in weight, with the potential for obesity and also for decreased palatability. As the ApoE knockout rats already have elevated blood cholesterol, any alteration in the diet of these rats will be started as a pilot trial, with animals monitored daily for visual signs of adverse effects and weekly for blood sugar/lipid alterations, ensuring that severity does not exceed Mild. - Cardiovascular function will be tested by echocardiogram (heart ultrasound), carried out under general gaseous anaesthesia. This has the potential to cause cardiorespiratory depression and decreased blood pressure, if any such signs are observed recovery from anaesthesia will be rapidly allowed. - Blood sampling may result in temporary Mild discomfort, which will be minimised by the use of topical analgesics. The animals will be humanely-killed at the end of the project, either by Schedule 1 methods or under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal tissue is necessary to provide the complex mixture of cells that are all involved in the development of Alzheimer's Disease. This includes the interplay between the microscopic blood vessels in the brain and the surrounding nerve cells and helper cells. This project will also examine how lifestyle alterations such as modifications to diet and exercise, affect the onset of Alzheimer's Disease. Such systemic changes cannot accurately be studied using non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Minimal animal numbers will be used by taking samples of intact tissue (both brain tissue and blood vessels) and maintaining them in the laboratory. This will allow for informative, longitudinal studies and will maximise the experimental output from each animal. The statistical power calculations tool G*Power and the NC3Rs' Experimental Design

	Assistant have been used to calculate the experimental group sizes that are necessary to produce statistically-significant results with the least number of animals possible.
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.	This project will use rat models of cardiovascular and Alzheimer's Diseases. These are refined mouse and rat models designed to closely resemble human disease progression, which will allow imaging of changes to cell function during the onset, progression and potential offset of the disease by dietary or exercise modifications. Animal suffering will be minimised throughout wherever possible, for example by use of local analgesics. None of the protocols in this project exceed Mild severity.

Project	**134. Exploring the role of the Coxsackie and Adenovirus receptor (CAR) in respiratory inflammation 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)	In the lungs, there is a single layer of cells that defends the body against environmental insults. This layer of cells has a dual role: it is a physical protective barrier and it also communicates with the cells of the immune system when any kind of insult/pathogen enters the body through the lungs. The integrity of this epithelial layer is therefore particularly critical, and when it fails, respiratory diseases such as asthma or Chronic Pulmonary Disease (COPD) can occur. Our previous research has identified a protein	

	called Coxsackie and Adenovirus Receptor (CAR) as an important protein in the junctions between the cells in the lungs and we now believe that by understanding how this protein works in whole organisms, we may be able to develop new drugs to treat lung disease in the future. Our previous work using cells grown in the lab has shown that CAR does keep lung cells more tightly packed together but it also encourages inflammatory cells to arrive to the place where the insult has happened, and we believe this may cause problems in patients with lung diseases. As the lab is an artificial environment, and does not contain other important physiological aspects we see in lungs, we now want to expand on our research to study the role of CAR using models of lung inflammation in live organisms. We will use house dust mite as a trigger for disease (HDM), because a lot of people with asthma or other respiratory problems are allergic to dust. We will also use Rhinovirus infection as an additional model, as this also triggers many respiratory problems including in asthmatic patients. We will use mice in which we are able to remove CAR from the lungs. Control mice (with CAR in the lungs) and knockout mice (without CAR in the lungs) will be treated with HDM or Rhinovirus, and we will study (a) immune cell infiltration (the cells from the immune system that go to the lungs) (b) lung tissue remodelling (the lung structure) which is very important because after continuous challenge, the lung starts producing fibrotic tissue and lungs get stiffer, which can reduce the ability to breathe properly. Through our discoveries that CAR can control lung cell behaviour and immune cell influx, we will also use animal models to determine whether CAR can also promote lung cancer formation and growth. Sadly, lung cancer is the most common cause of cancer-related mortality worldwide, and a poor 5-year survival rate, which remains stable at 15%. We know immune cells can play a key role in allowing tumours to grow and reach to other tissues (form metastasis). Our experiments will determine whether removing CAR or blocking its function can reduce tumour cell formation and invasion. The combination of

	these different experiments will allow us to understand if using drugs to block CAR might help patients with disease such as asthma COPD as well as cancer.
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 primary potential benefits relate to the generation of new knowledge about the initiation and control of lung inflammation and tumour progression. Our previous data has shown that maintaining the integrity of the layer of cells in the lungs is essential to control normal homeostasis. When this layer is disrupted (for example after inhalation of dust or after the entrance of a pathogen in the lungs), this can lead to inflammation, that results in a lot of immune cells in the lungs and tissue remodelling (the lungs change and get stiffer). If the inflammation persists, that can produce the appearance of a tumour. As we know that CAR is involved in maintaining the integrity of the cells, we are removing CAR from the mice lungs, with the aim of identify key genes and proteins that can targeted at all stages of the inflammatory and cancerous process, from the initiation to eventual disease progression in our related models.
What species and approximate numbers of animals do you expect to use over what period of time?	Approx. numbers of 2000 animals 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 I have previously mentioned before, we are going to remove CAR in the lungs. We have done these experiments before and we know that the mouse lungs are perfectly normal. To remove CAR, I will need to administer tamoxifen to the mice. The tamoxifen can be toxic, but its toxicity has been very well studied and there are stablished volumes that can be administered to the mice depending on the animal weight. Of course, we will weight the mouse before administering the tamoxifen and we will adjust the dose depending on the mouse weight. After the administration, we will monitor and weight the mouse every day. In my previous experience, only 2 mice (out of around 100) have suffered any kind of distress and they both recovered. In the case of induce lung injury (for example, after making them inhale house dust

	mite or infect them with Rhinovirus), mice will be monitor very carefully and if there are any signs of pain or distress the animals will be humanely killed. Adverse effects could be also related to tumour growth, particularly in subcutaneous tumour implantation models upon reaching a certain size. We will monitor mice that receive tumour cells very carefully and if there are any signs of pain or distress (before the tumour reaches pre-defined size limits) the animals will be humanely killed. Within this body of work there are no protocols more severe than 'moderate' level and animals will be humanely killed by Schedule 1 method after experimentation
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we utilise *in vitro* and/or *ex vivo* techniques as much as possible, the field of tumoral and inflammatory microenvironmental investigation requires *in vivo* models because we want to study the interaction between different kinds of cells in the body. This kind of "co-culture" experiments (when we try to culture in plates more than one cell type) are difficult to perform in vitro in the lab, and they don't reproduce the exact microenvironment or crosstalk between different kinds of cells. Also, related to study tumours, mice are necessary to adequately recapitulate live body variables, such as tumour oxygen levels, blood supply, and the stiffness of surrounding supportive tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments have been designed with input from the Statistics department in order to ensure minimal animal numbers in experiments whilst ensuring the experiments are adequately powered. I have also familiarised with the software "PowerG", that allows you to make power calculation to better plan the *in vivo* experiments. Non-invasive imaging techniques will also drive down numbers as there will be less of a need for routine sacrifice to assess tumour progression.

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 species of lowest neurophysiological sensitivity that provide the necessary size to allow us to study lung injury models with adequate resolution. We will try to reduce suffering as much as possible during all invasive procedures. Inhalational anaesthesia will be used to minimise transient pain and distress, where possible, during tumour induction, sampling, measurement and combined with imaging procedures. Animals will also have full recovery between periods of anaesthesia, rehydration during long imaging sessions, monitoring of respiration/cardiac function and maintenance of body temperature during imaging. Welfare score sheets and frequent monitoring will be used to assess progression of tumour burden, animal health and behaviour so humane end-points are reached well before onset of clinical adverse effects.

Project	**135. Exploring the tumour microenvironment to find better therapies for 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)	Our main objectives are (a) to understand how non-cancerous stroma cells surrounding the tumour cells contribute to cancer disease progression and (b) to investigate whether disrupting the cross-talk of stroma cells with cancer cells has implication on cancer progression and its response to therapy. Our studies have a translational focus on pancreatic and breast cancer. Both cancers are aggressive metastatic disease and the treatment of metastatic tumours is an unmet clinical need. During cancer progression, cells from our immune system and organ resident cells progressively accumulate at the primary tumour site. Although	

	these cells are initially recruited to inhibit and/or kill malignant cancer cells, recent studies have shown that in fact the opposite can be true and that our immune cells and other stroma cells contribute to the establishment of a tumour promoting microenvironment at the primary and at the metastatic site.
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 primary potential benefit relates to the new knowledge about how stroma cells promotes cancer disease progression and metastasis, with a translational focus on pancreatic and breast cancer. This will be of interest to pre-clinical scientists interested in pancreatic or breast cancer biology, or cancer progression and metastasis in general. We aim to publish our findings in peer reviewed academic journals. The secondary potential benefit relates to the possible identification of new molecular targets which may lead to improved diagnosis and/or treatment of cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	All animal experiments will be performed in established mouse models over a period of 5 years. The number of animals used for each treatment will be kept to the minimum required, this being determined by statistical analysis of the results of multiple previous experiments performed myself or by other laboratories for each of the proposed protocols.
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 this project we will test in well-established murine pancreatic and breast cancer models whether inhibiting or modulating of specific stroma cell functions affects susceptibility to cancer progression and it's response to standard chemotherapy or immunotherapy. At the end, all animals will be humanely euthanized by a Schedule 1 method. Adverse effects will be kept to a minimum to achieve the over-riding clinical scientific objectives described and the majority of studies are expected to be in a mild/moderate severity level.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use	Our animal experiments will be complemented by in vitro experiments using cell lines and short term primary cultures of stroma cells and cancer cells. However, this complex interaction between stromal

non-animal alternatives	cells and malignant cancer cells in vivo mean that all relevant experiments cannot currently be performed in vitro. We will also study fresh and preserved tissue samples obtained from biopsies from cancer patients , but for ethical reasons it is not possible to manipulate gene expression in human subjects.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used for each treatment will be kept to the minimum required, this being determined by statistical analysis of the results of multiple previous experiments performed myself or by other laboratories for each of the proposed protocols.
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 protocols and procedures used in animals will be the mildest possible affecting the minimum possible number of animals to achieve the over-riding clinical scientific objectives described.

Project	**136. Factors affecting fish populations**	
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
	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)	This project is designed to generate reliable and objective scientific evidence on the impact of selected environmental factors affecting fish populations in support of specialist advice provided to stakeholders, national and international governments and other international organisations on the conservation and management of fish stocks. In particular, the project will examine how man-made activities within the aquatic environment and modifications to the freshwater and marine environment may affect the ability of migratory fish to move between their feeding and spawning grounds and to provide advice to government departments on mitigation	

	measures. Specifically, the project will focus on renewable hydropower schemes and construction activity within rivers, estuaries and coastal waters. The project will use an integrated scientific approach with field based studies and physiological measurements to examine how changes to the environment affect wild fish at both the individual and population levels.
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)?	Information on all aspects of fish biology and ecology will permit better advice to Defra Policy customers, and to the Environment Agency (EA), International Council for the Exploration of the Sea (ICES) and North Atlantic Salmon Conservation Organisation (NASCO) on the conservation and management of fish stocks. The work will also support the Water Framework Directive, the Salmon and Freshwater Fisheries Act 1975, the Eel Regulations 2009 and the Habitats and Birds Directives of the European Union, applied through the Conservation of Habitats and Species Regulations 2010 (SI No. 2010/490), commonly known as the Habitats Regulations.
What species and approximate numbers of animals do you expect to use over what period of time?	There is no alternative to the use of living animals, as the principal aim of the work is to describe the behaviour of fish in relation to changes in their natural aquatic environment in order to conserve and manage populations. Consequently, a range of fish species (including salmon, sea trout, European eels, coarse fish, lamprey, shad and smelt) will be studied over the course of 5 years in order to provide relevant and meaningful data on which decisions are made. In developing the project, advice has been obtained from a statistician experienced in animal research, regarding animal numbers and design. The numbers used will range from 1000 to 50 000, depending on the procedure utilised. For example, large number of fish will be used solely for sampling and tagging procedures, with mild and moderate levels of severity.
In the context of what you propose to do to the animals, what are the expected adverse effects and the	The behaviour of fish in the wild will be studied using a range of telemetry techniques which involve the use of electronic transmitters and

likely/expected level of severity? What will happen to the animals at the end?	remote sensing devices. Most procedures will involve mild and moderate levels of severity, apart from terminal sampling. However, it will be ensured that smaller numbers of fish are used for those procedures when possible. At the end of each procedure, before fish are released to the wild, they will be assessed by appropriately trained and qualified individuals to ensure that the fish are fit and their welfare is protected. Alternatively, they will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The principal aim of the work is to describe the behaviour of fish in relation to changes in their natural aquatic environment in order to conserve and manage populations. Therefore, there is no alternative to the use of living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experimental work will utilise the published literature and previous experience by the Project Licence holder and colleagues who undertake similar work to ensure that the minimum number of animals are used that will permit a robust statistical and meaningful analyses of the results. All experimental work will be discussed and agreed with a professional Statistician, who will provide statistical support to all aspects of the research, from designing the experimental approach to conducting and reporting the analyses
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 purpose of the work is to provide advice on the conservation and management of fish stocks. Therefore, a range of fish species need to be studied to produce adequate data on the impacts on fish populations from a wide range of environmental and man-made changes to the aquatic environment. The methods chosen are based on previous experience and research and will provide evidence that will form the basis of suitable advice to Government on the factors affecting fish populations and recommendations for suitable mitigation. Where fish undergo a procedure and recovery, they will be monitored for a suitable period of time in order to assess any adverse impacts and ensure a minimum of
	suffering.

Project	**137. Factors controlling macrophage function 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
		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?	We want to understand what factors control the function of immune cells called macrophages, in order to be able understand how we can therapeutically manipulate their function during disease.	
Why is it important to undertake this work?	All tissues are populated with immune cells called macrophages that help protect the body from invasion by microbes, repair tissues following injury and contribute to normal day to day running of the tissue. Part of the function of macrophages is to capture and destroy microbial intruders, but also to clear dead, dying and damaged tissue to prevent overt inflammatory responses to injury and enable repair. However, in some individuals, these functions of macrophages can become uncontrolled or defective, leading to susceptibility to infection, excessive inflammation, and	

	organ dysfunction. Importantly, macrophages are thought to be very flexible cells whose function is largely controlled by their environment. First, we want to understand what tissue-derived signals dictate macrophage behaviour in normal healthy tissues and during successful tissue repair/inflammation resolution to allow us to promote or target these signals when macrophages behave aberrantly during disease. However, emerging evidence suggests that the flexibility of macrophages may be limited by their previous experiences. In other words, the life history of a macrophage will determine how it behaves during subsequent stress responses. Hence, our second aim is to understand how macrophages learn to respond differently to inflammation and injury and if education of macrophages in this way fine tunes the balance between tissue repair vs destruction, immunity vs infection, and tolerance vs autoimmunity.
What outputs do you think you will see at the end of this project?	This project will address fundamental principles in macrophage biology and is therefore likely to have far reaching implications for many disease processes. As such, the primary output will be generation of knowledge that will be largely disseminated by publication in peer reviewed journals and presentation at conferences, seminars and workshops. I will also continue active participation in public engagement events, media interviews and our institutes Public Engagement and Communications Committee to increase the public's awareness of our work and its implications for health. Certain elements of the research may also generate exploitable intellectual property that could lead to patents, and in the longer term to development of spin-out companies to generate commercially exploitable immunological therapies, such as therapeutics that could be used to boost or alter the function of macrophages during disease.
Who or what will benefit from these outputs, and how?	The 'discovery research' nature of this project means that the short-term beneficiaries will primarily be the academic community. Although only a restricted number of disease models and tissues will be investigated under this license, the broad range of diseases in which

	macrophages play a role and the fundamental principles of macrophage biology that will be examined in this project means that the knowledge produced is likely to affect a broad section of the academic community (eg immunologists, clinicians, physiologists, and those interested in aging). The knowledge generated will also be relevant to the pathophysiology of the modelled human diseases and therefore will be of interest to clinicians who specialise in these fields. The outcomes of this project could also steer future research towards new macrophage-based therapeutic strategies and hence will be of long-term benefit to the pharmaceutical industry and charitable organisations that fund medical research and ultimately to human health. The timeframe for improvements to human health would be expected in the decades, while increased investment in Research and Development in these areas could occur within 3-5 years.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	Findings from this project will primarily be communicated and disseminated through publication in widely-read peer-reviewed journals, but also presentation at local, national and international congresses and individual institute seminars. To ensure maximum dissemination, only journals with green or gold open access options will be considered. Furthermore, to expedite dissemination of knowledge, data will be published on an open access preprint repository such as bioRxiv. To ensure dissemination of all new knowledge and prevent unknowing and unnecessary repetition of experiments by others, I will seek to publish all data generated under this project including negative results, again utilizing preprint repositories such as bioRxiv if necessary. To rapidly translate our findings to the human arena I will exploit collaborations with local clinicians to access clinical samples from patients with liver disease and peritoneal pathologies and, where possible ostensibly healthy individuals (eg livers rejected from transplants, peritoneal cells from patients undergoing hernia operations or elective laparotomy). For this purpose, the medical school environment at our institute is ideal for developing collaborations to assess the relevance of findings in human disease and to begin translational

	research. Research at our institute is also ideally placed for exploitation, with a dedicated wholly owned subsidiary in place to offer management of technology transfer, company formation and incubation facilities. Hence, this will maximise commercial outputs should the proposal yield potentially exploitable opportunities beyond academia. As an alternative strategy, pharmaceutical and biotech companies could be engaged through presentation at national and international forums at which representatives are often present, or directly via channels in place at our technology transfer subsidiary.
Explain why you are using these types of animals and your choice of life stages.	We study mice because their immune system, tissue physiology and development are very similar to that of humans and because scientists have created many genetically altered mouse lines that allow us to dissect in fine detail what happens during immune responses or following tissue injury. For example, we can now determine the precise function of a molecule, often in a specific cell type and even at a precise time in development or point during infection, injury or inflammation, and we can follow the fate of individual immune cells and determine how their function changes over an entire disease course to determine how they enable or hinder resolution of disease. As we wish to understand how immune cells are programmed during normal development and whether this depends on if they come from the bone marrow in adult animals or were established in tissues right at beginning of development in the embryo, we need to study these cells over all development stages of an animal. Specifically, pregnant dams are needed so we can label and track cells that develop in embryos through to adulthood; we need to study neonates and juveniles to determine what happens in tissues following birth that prevents new immune cells from entering from the blood, and we need to study adults to determine how these different types of immune cells function when tissues become infected or damaged.
Typically, what will be done to an animal used in your	Invariably most experiments start with a step to track specific types of immune cells, chiefly macrophages.

project?	This will include in embryos in utero by administration of substances to pregnant dams, or by tracking development of cells in neonates, juveniles or adults by administration of substances or cells at these different life stages. For this purpose, some mice may be exposed to radiation to allow us to identify and study cells that come from the bone marrow. We will then assess the fate and function of these immune cells over the course of development in healthy tissue, providing us with a baseline from which to understand what are 'normal' and 'healthy' functions. To understand what signals dictate this normal healthy function and how these change with disease, we will then manipulate the tissue environment by depleting specific types of tissue/immune cells, blocking signalling pathways by giving drugs, or manipulating expression of genes in specific cell types using genetically altered animals, or by inducing acute or chronic chemical injury in the liver or physical or inflammatory injury in the peritoneal cavity by surgery or administration of inflammatory compounds intraperitoneal injection. As we are particularly interested in how the sex of animal effects the function of macrophages, some animals will have sex organs removed surgically. To establish the long term effect of altering the tissue environment or of inflammation or injury on the normal protective function of tissue macrophages, some animals that have received modifications to their tissue environments or an initial episode of inflammation or injury, will be allowed to recover for a period of months before receiving a subsequent bacterial infection or repeat episode of tissue injury or inflammation. When performing so called rescue-of-function experiments where we set out to prove that a cell type that we have first shown is required for normal function of our immune cells is needed because it produces a factor X, we will both deplete a cell type and then "rescue" the effect of cell loss by administering mice with factor X in drug form, before subsequently infecting or causing tissue injury. In some experiments, small volumes of blood may be taken usually from the tail vein, for example to screen for efficacy of cell labelling or depletion techniques. Finally, some experiments may end with animals being killed under terminal anaesthesia to enable removal of large volumes of blood or allow fixation of tissues.

What are the expected impacts and/or adverse effects for the animals during your project?	Acute liver injury can lead to a short period (6-12 hrs) of moderate suffering and discomfort evidenced by reduced activity and reduced food and drink consumption but from which animals quickly recover. Chronic liver injury is generally well tolerated as lower doses of chemical toxins are used. Each bi-weekly injection of toxin can induce periods of transient (<30mins) lack of activity indicative of general discomfort while chronic injury can lead to sustained mild (<10%) to moderate (<20%) weight loss. A small number of animals may exhibit >20% weight loss, hunched posture, pale skin, and inactivity, indicative of the upper limit of the moderate pain and discomfort threshold and if so, will be removed from the study and killed. Sterile inflammation in the body cavities is generally well tolerated and does not result in clinical signs of discomfort or pain. However, injection of some bacterial components that induce inflammation can lead to transient loss of body temperature (12-24hrs), which will be counteracted by housing animals on heat mats. The time course of intravenous infection with bacteria will be limited to 30 mins to ensure animals are killed before they experience more than mild discomfort. Surgical removal of ovaries or testes is performed under general anaesthesia and is accompanied by delivery of appropriate post-surgical analgesia for pain relief to ensure animals do not experience more than a moderate degree of pain and discomfort. Most animals exposed to radiation will receive body protection and therefore will experience only mild adverse effects associated with general anaesthesia rather than exhibiting outward clinical signs. Exposure to lethal whole-body levels of ionising radiation leads to elevated risk of bacterial infection, diarrhoea, and signs of general discomfort for a period of several days. These impacts are controlled by keeping animals on water containing antibiotics for 1 month post irradiation and supplementing their diet with easy-to-eat food.
	Delivery of most substances that interfere with cell signalling, cause deletion or upregulation of gene expression or depletion of cells does not in general lead to more than transient distress associated with route of delivery, or mild irritation at the injection site.

What are the expected severities and the proportion of animals in each category (per animal type)?	Approximately 40% of animals will be used for establishment and maintenance genetically altered animal breeding programmes or for production of genetically altered mice that will simply be used for harvesting tissues. These animals will fall will into a subthreshold limit of severity. Of the remainder, approximately half of animals will experience degrees of suffering that fall within a moderate severity category, including induction of sterile and bacterial peritonitis/pleuritis, acute liver injury, chronic liver fibrosis, and exposure to whole body irradiation, while the remainder will experience degrees of suffering that fall within mild severity including induction of short-term (<30min) intravenous bacterial infection, adoptive transfer of cells, substance-mediated induction or deletion of gene expression and cell depletion strategies, and irradiation with body protection. Animals killed under terminal anaesthesia for extraction of blood or fixation of tissues but not experiencing any other procedure while alive will be classed as non-recovery but will be relatively few in number.
What will happen to animals at the end of this project?	used-in-other-projects
Why do you need to use animals to achieve the aim of your project?	The immune system and most disease processes rely upon a complex series of spatially and temporally segregated interactions between cells of diverse function and origin. To understand such complexity, it is essential to undertake research in vivo, since this cannot yet be modelled meaningfully in vitro.
Which non-animal alternatives did you consider for use in this project?	1) Analysis of human biopsies. 2) Use of cell lines.
Why were they not suitable?	The types of analysis required to track cells in the body to determine how the life history of cell determines its

	function are not possible by simple analysis of human tissue biopsies. Although a limited assessment of whether immune cells are generated from the tissue or bone marrow can be made from biopsies of patients receiving sex-mismatched tissue or BM transplants, access to these kinds of samples is difficult due to scarcity and ethics prevent removal of cells or biopsies without good clinical reason or in sufficient quantities to allow in vitro analysis of cell function. In addition, a main objective of this project is to understand how and why macrophages behave a certain way in healthy tissues, as this will provide a baseline from which we can fully understand pathological processes that occur in diseased tissues and determine what features need to be reinstated to enable tissues to return to a resting healthy state. Again, ethical considerations largely prevent the removal of healthy tissues from humans for this purpose. Hence, our analysis of human tissue will largely be restricted to the context of disease or following disease resolution. Many functions of immune cells are imprinted by their immediate tissue environment and these are lost rapidly after they are removed from the tissue. This largely precludes the use of cell lines for our work. Hence, while studies of the micro-environmental factors that control and imprint tissue-specific macrophage features will be performed in part using mixed cell cultures in vitro, this methodology will still require primary cells from animals, culture supernatants of primary cells, or slices of primary tissues, together with validation of results using in vivo methodology.
Enter the estimated number of animals of each type used in this project.	mice: 7600
How have you estimated the numbers of animals you will use?	The number of animals has been estimated based on experience gained under my previous Home Office license. In this regard, the work plan and nature of experiments covered by this project are similar in design to those covered under my previous license. Hence, I have based estimates on successfully obtaining funding for two 5-yr post-doctoral positions (Wellcome Trust

	Investigator Award) and two 3 yr post-doctoral position (MRC project grants) to execute the work detailed in this license, and the average yearly animal use per group member returned under my current license. This experience-based estimation has reduced the predicted animal use by almost 40% compared with my previous license.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	For all of our experiments in-bred mice are used to reduce how the different the individual animals. We also generally use a randomized block design for experiments to further reduce factors that could cause differences between animals that are unrelated to factors being tested in experiments (eg cage to cage variation). By reducing natural differences between animals in this way, we reduce the number of animals needed to identify differences caused our experimental test. In cases where we cannot use randomized blocking to reduce cage effects, we will still ensure that animals are littermate-controlled to reduce variations that can arise between litters. Our experiments are also designed to reduce the number of variables to as few as possible and thereby reduce the number of control groups required. In this respect, we will always consider carefully whether it is important to include 'naïve' as well as 'vehicle' control groups in experiments, or if the latter alone is sufficient for interpretation of results. To ensure best practice in statistical analysis and experimental design all new staff members working under this license will attend the in-house 'Experimental Design Course'. Planned experiments are discussed regularly within group meetings to ensure all are correctly controlled and to facilitate sharing of tissues/data for the most effective use of animals.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	My group routinely perform pilot experiments to determine sample sizes required by power calculations. We standardly base power calculations on the ability to detect at least a 2-fold difference between groups, which we regard as an acceptable cut-off for identifying important biological effects with the benefit of reducing the group sizes required. Experiments are then performed on a minimum of two separate occasions to
	ensure reproducibility, following which meta-analysis of data from pooled experiments is used to reveal less pronounced effects but without increasing overall animal use.

Which animal models and methods will you use during this project?	Mice represent the most tractable model for the in vivo study of macrophage biology given the availability of transgenic strains and commercial reagents required to effectively determine cell origins, lifespan and the function of individual genes within defined cell subsets. Importantly, the use of mice is a refinement compared to other higher mammalian organisms as they have a lower degree of neurophysiological sensitivity. Furthermore, mice are an appropriate model since they reproduce many features of the human immune system relevant to this project. I am committed to ensuring the most refined protocols are used in our studies. To this end, my group has pioneered the use of a method to identify and track bone marrow-derived cells and determine the lifespan of tissue resident macrophages that involves irradiation but with lead shielding to protect the large proportion of animal tissues. This represents a significant refinement to studies that use whole body irradiation or parabiosis. While we still employ whole body irradiation without shielding in some circumstances, this is atypical and we ensure that the irradiation is delivered in split doses as this is better tolerated by animals. In a further refinement, we have developed a system to directly transfer and track mature immune cells thereby by-passing the need entirely for irradiation in a significant proportion of our work. To study the long-term effects of local inflammation on function of peritoneal macrophages, our first approach will be to use sterile models of inflammation since these are accompanied by very limited clinical signs. While we will also determine the effect of surgical injury on long term function of peritoneal macrophages, we will dissect the mechanisms underlying common effects of surgery and sterile inflammation using the less harmful sterile models. Blood and local bacterial infections will be used to determine the effect of tissue factors, sex or previous inflammatory events on the function of tissue macrophages in local immune defence. We will primarily use infection models that are well tolerated (eg attenuated Salmonella strains), but will also use more virulent infection models but purposefully choose early endpoints to assess the ability of animals to contain

	infection but before development of severe levels of harm. Models of acute and chronic liver injury will also be used. Acute injury is generally well tolerated in mice and induces only transient suffering at the low doses used by my group. The model of chronic liver injury that we will use is more controllable and predictable than other such models and is also reversible, meaning animals quickly recover following cessation of injury. Although this chronic injury model is commonly studied over a period of 12 weeks, we have found that even only 4 weeks of injury leads to a long-term effects on liver macrophage function that persist following recovery. For this reason, in most experiments we will purposefully use this shorter time frame to reduce suffering. To determine plasticity of resident macrophages, we will adoptively transfer cells into the lung by intratracheal injection. The lung site was chosen specifically as intratracheal injection causes less suffering than transfer into other tissues as it is minimally invasive and is performed under inhalational anaesthetic.
Why can't you use animals that are less sentient?	A mammalian model is required to study an immune system with a high degree of functional, anatomical and developmental similarity and mice have a lower degree of neurophysiological sensitivity than other mammalian model organisms. The duration of the homeostatic and pathological processes to be studied prevent using mice under terminal anaesthetic.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Our institute employs a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. My group consult closely with this team and take full advantage of the extensive resources provided on their website to ensure we are following current best practices. These resources include comprehensive guidelines and standard operating procedures for most common rodent procedures that are continually being updated. Our university is also in the process of adopting the improved rodent handling methods that reduce animal stress (detailed by Hurst et al. Nat Methods 2010) and our animal facilities now provide environment enrichment as standard. My group will adopt these methods alongside the staff in our animal facilities. We will also take full advantage of the annual 3R's seminar day organized by the University's Animal Welfare Committee to find out about pioneering developments in best practice.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	In line with BVS policy, we will adopt the latest techniques in animal handling (eg cupping) to significantly reduce the stress associated with procedures. Furthermore, where possible, the least invasive methods for dosing and sampling will be applied. Anaesthesia and analgesia will be provided where suitable (eg for humane restraint, during and recovery from surgery). To reduce infection risk, the best aseptic technique will be used during surgery (eg sterilization of instruments between animals, full surgical drapes) and immunocompromised mice will be housed in IVC cages. Infection experiments with either utilize attenuated organisms, or early endpoints will be used that prevent animals experiencing more severe harms.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	Our institute employs a dedicated team of veterinarians that are continually seeking to improve animal welfare and refine animal use. My group consults closely with this team and takes full advantage of the extensive resources provided on their website to ensure we are following current best practices. These resources include comprehensive guidelines and standard operating procedures for most common rodent procedures. We have also consulted the NC3Rs research strategy paper by Prescott MJ, Lidster K (2017).

Project	**138. Factors that regulate normal development in Zebrafish and their dysfunctions in inherited disorders**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
	X	Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overarching goal of this project is to understand how cells in embryos differentiate to form various tissues and organs, and how internal organs such as the heart are positioned in the body. Similar to postcodes which provide information about where mail is delivered by the postal services, gene products have codes that provide information about when and where the products are delivered in cells and tissues, how they are transported, and when they are activated. We are working to understand the logic of these codes, and figuring out what goes	

	wrong in human diseases affecting either the codes themselves, their sorting machinery, and transport processes within cells and tissues. We work on how this affects specific organs including the heart and brain. Our work is relevant to human birth defects. Through this work, we will focus on the role of particular codes in messengers, the proteins that recognise the codes, and the transport machinery that delivers the messengers to the correct location in cells and embryos during tissue and organ development and positioning. This requires study of complex tissue interactions in embryos and larvae. Thus, it is not feasible to recreate this process accurately in cell culture. We use the animal model of human disease, Zebrafish, as the primary system to study these processes through a variety of approaches (genetics, genome engineering, imaging, computational modelling, proteomics, and chemistry). Zebrafish is highly similar to humans, with counterparts for 70% of all genes and 83% of disease-causing genes. Zebrafish develop externally, which makes their eggs amenable to manipulations and we can also observe them by making films. Therefore, this is an ideal non-mammalian vertebrate to study developmental processes.
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 is of significance to understanding the basis of human congenital birth defects.
What species and approximate numbers of animals do you expect to use over what period of time?	The zebrafish, Danio rerio, is the main species that will be used in the majority of experiments. Related Danio species will also be used occasionally. Zebrafish: 14,000 fish over 5 years ; Other Danio species: 1000 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	All procedures used are of “mild” severity. Expected adverse effects: Anaesthetising fish can occasionally produce bleeding from the gills, which is short lived. These fish may recover but they will be observed carefully for

the end?	signs of breathing difficulty or other distress. Fish that struggle to recover in fresh water will be killed by schedule 1. Where the technique also involves brief removal from the water for detailed observation, the fish will be kept damp by being wrapped in a moist sponge having first established adequate anaesthetic depth has been achieved; this is a standard technique and in our experience most unlikely to cause detectable harm. For fin-clipping, an appropriate anaesthetic will be chosen which is suitable for the procedure and in line with best practice. Anaesthesia will be carefully monitored throughout the brief procedure by visual observation. Observation will continue until the fish recover consciousness and are able to swim normally. Adult fish heal very well naturally, and the amputated fin will regenerate within a few weeks to its normal size and shape. Infection following fin biopsy is rare, and has not been reported by experienced workers. Operated fish will be monitored carefully at regular intervals upon return to the aquarium system. If infection occurs, the fish will be killed by a Schedule 1 procedure. If any doubt exists as to the severity of the adverse effects, then the named persons will be contacted and their advice will be followed. In rare cases fish may carry dominant mutations that have deleterious effects on development and survival. Animals displaying abnormalities (such as tumours) will be identified by regular screening and immediately killed by a schedule 1 method. Spontaneously induced deleterious mutations may occasionally arise in inbred fish. These will also be killed by a Schedule 1 method as soon as detected. If newly obtained mutations appear wild-type during embryonic stages, small numbers of animals (10 homozygotes) will be raised to test for adult phenotypes. Such animals will be checked every day for abnormalities initially and after two weeks every workday. If significant abnormalities are found such fish will be killed by decapitation under terminal anaesthesia if this is required for further analysis. The animals will be killed by schedule 1 at the end of the experiments.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project aims to understand the function of RNA elements, binding proteins, and the cytoskeleton in regulation of cell-cell signaling during tissue and organ development and positioning. This requires study of complex tissue interactions in embryos and larvae. Thus, it is not feasible to recreate this process accurately in cell culture. Animals are therefore absolutely necessary for this work. The analysis is focused on zebrafish transgenic lines or mutant lines that we will obtain or generate. The zebrafish embryo has special advantages for imaging studies, genetics, genomics, compound screening and proteomics.Our choice of animal therefore reflects the 3Rs since fish are the simplest vertebrate model system in which these studies can be performed, and our experiments are on embryos that have a lower level of awareness in comparison to adults.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We estimate that we will use 14,000 adult zebrafish throughout the duration of the project, and the majority of these are generally only used for matings. Some fish will be genetically modified to generate transgenic or mutant fish lines. These numbers are determined by stock keeping requirements, and a minimal number is required for each line to ensure maintenance or for generation of the required transgenic line. We are also optimising methods for generating new knock-in and mutant lines. This will also lead to reduced numbers of animals being raised. All unwanted lines and lines not in active use will typically be maintained as frozen sperm samples.
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 the experimental manipulations described in this proposal require easy access to embryos, and fish are a good model for the manipulations we have proposed in comparison to mammalian embryos. Thus, the work helps to address the 3Rs by using alternatives to mammalian models. Experiments will be carried out on embryos immediately after fertilization, and since the animals are under 5 days post fertilization, they do not fall under ASPA protection and are not as aware as adult animals or mammals. All embryos used for experimental purposes will be

	humanely killed at the end of the experiment, and will not be raised beyond 5 days post fertilization. Adult animals that have reached the end of the breeding life (~about two and a half years of age) will be humanely killed by an overdose of anaesthetic.

Project	**139. Farm animal veterinary product 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)	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 licencing of new veterinary or agricultural products for the prevention and treatment of diseases in livestock and the reduction in the prevalence of zoonotic organisms in the human food chain.	
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 benefit of the project is the provision of data to facilitate sound regulatory decisions, e.g. on clinical trial approval or marketing authorisation for new medicines or other substances to improve the health and welfare of farm animals. Another benefit is a reduction in the need for the use of antibiotics in livestock production.	

What species and approximate numbers of animals do you expect to use over what period of time?	This project uses domestic livestock species, which for the purpose of this project are defined as those species generally regarded as farm livestock, i.e. cattle, pigs, sheep, goats, camelids and poultry. Cattle – 3300; Sheep – 725; Pigs – 3000; Poultry – 2550; Goats – 300; Camelids – 200. The use of these animals will be spread over the 5 year life of the 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?	Animals are dosed/treated by the intended/likely route of target animal exposure, and observed regularly to monitor appearance, behaviour and clinical health. Typical investigative procedures are similar to diagnostic procedures that might be used medically to monitor progress of a human patient (e.g. collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function). Most animals are expected to experience no adverse effects, or only mild effects such as slight weight loss. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss or reduced activity. The studies are not expected to be of greater than moderate severity. Experienced observers, with access to veterinary advice and care at all times, monitor clinical signs of all experimental animals at regular intervals in order to quickly identify any animal requiring veterinary treatment. Any animal failing to respond to treatment will be killed humanely to prevent unnecessary suffering. At the end of the studies animals will be humanely euthanased or for animals involved in on-farm studies will be returned to the farm stock.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal (in vitro, in silico) studies do provide valuable preliminary and supporting data to refine and reduce animal studies, however, definitive assessments of systemic exposure, efficacy and safety can at present only be achieved in studies using intact animals, and this remains a mandatory legal requirement; currently, there are no scientifically, ethically or legally acceptable non-animal alternatives available. The EU, FDA, USDA and EPA regulatory authorities require a series of pre-clinical efficacy, safety and residue tests in animals prior to approval of new compounds. These tests are described in regulatory documents. Products that are to be tested will have already undergone evaluation in non-animal alternative tests (replacement) in

	previous in silico and in vitro studies and that data will be taken into consideration before conduct of in vivo studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The careful refinement of experimental models ensures that only the minimal number of animals required to obtain statistically significant and biologically relevant outcomes will be used. Independent advice on the experimental design is provided by trained statisticians in advance of any experimental work being conducted. In addition, proposed experiments are reviewed by an ethical review committee to ensure that the minimal number of animals is used and that the studies and procedures are justified. Where there are not pre-existing specific models the minimum numbers of animals will be used to ensure the statistical significance of the resulting study data as determined by pilot studies. Where minimum numbers of animals are specified by regulatory requirements these will only be exceeded to meet the necessary statistical criteria or where a repeat study may be avoided by the use of larger groups. The species used are common production animal species in the UK and the wider world and therefore findings from this research are highly applicable internationally as well as nationally.
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.	Products are tested in the target species for any treatment product or the possible target of exposure for any agricultural products, the choice of animal is determined by the product use and regulatory requirements. Relevant, reliable and reproducible disease models have been developed and refined to be the least severe necessary for valid results to be obtained. Considerable care and attention has gone into refining the techniques employed to monitor the immune responses during animal studies in order to reduce the degree and duration of any suffering to a minimum. Trained teams of observers monitor animals at regular intervals, accurately evaluating the responses of individual animals and seeking veterinary intervention where necessary. Sequential testing, with review of findings at each stage and modification of subsequent stages as necessary, maximises opportunities for refinement to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals. Where appropriate, positive reinforcement training (treat rewards) is used to encourage co-operation in (and minimise any stress of) handling/procedures. Environmental enrichments appropriate to the species are used within the animal facilities.

Project	**140. Fish Physiology in an Era of Climate Change**	
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)	Climate change represents a significant challenge for fish. The purpose of this application is to study the pressing environmental challenges facing fish today including rising water temperatures, reductions in aquatic oxygen levels, and the presence of man-made pollution in water systems, to better understand the tolerances of fish so that we can implement better and more timely resource management.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	The aim of this work is to benefit fish populations by determining the impact of environmental challenges on physiology. From an ecological perspective, physiological tolerance thresholds are	

benefit from the project)?	likely to determine geographical distribution of fish in oceans, abundance in fresh water lakes and rivers, and influence migratory success. However, the environments of fish habitats are changing rapidly. Given the known impact of climate change on fish populations and their geographical distribution, understanding the single and combined effects of environmental change in a vulnerable group of fish (sharks/elasmobranchs) and using a model system to probe specifics mechanisms (zebrafish), is both pertinent and timely. The **short** term benefit will be realised by challenging the current 'upper limit-based' models of environmental tolerances. Specifically, by determining the incremental change in the environment where 'things get worse' we believe we can provide better thresholds for environmental policy makers whilst gaining insight into the intrinsic tolerance mechanism of the fish cardiovascular system. The zebrafish studies will also allow us to understand how specific changes in a fish's metabolism or heart function (via short term genetic alterations) will allow us to pinpoint cellular proteins and mechanistic pathways that underlie the environmental tolerance of fishes. This will provide the mechanisms for the organismal tolerances that in the **medium** term can used to create more bespoke management models. This will be achieved by sharing our data a conferences and with resource management/stakeholders to help prioritise areas for work. These benefits are conceivable within the 5 year duration of this licence. Collectively, the **longer** term benefit of the knowledge generated from this licence will improve survival and welfare of fish populations both in wild systems and in aquaculture. This could occur via better management strategies including cool water refuges, weir adjustments/ and fish passes which take into account realistic physiological tolerance limitations. If the fish are the prime beneficiaries then the millions of people who depend on them for livelihood and nutrition are secondary beneficiaries.

What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 1000 catsharks and around 1500 zebrafish over the course of the 5 year study.
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?	To understand the environmental tolerance limits of the fish, they will undergo a series of environmental challenge tests. These include non-invasive measuring metabolic rate, swimming biomechanics and looking at how the environment affects behaviours like exploration and decision making. Adverse effects from all of these tests are expected to be minimal. However, in a few cases (<10%) we will examine the effects of the environmental challenges in fish that are instrumented with implanted ECG electrodes or blood pressure cannulas for measuring cardiovascular variables concomitant with environmental tolerances. In this case, the fish may feel discomfort due to instrumentation however, where ever possible such parameters will be measured under anaesthesia. Similarly, fin clipping, and injection of substances like hormones (i.e. adrenaline) that modulate growth or cardiac function may cause short term discomfort. But we expect fish to recover fully. There are 2 environmental challenges that are expected to be stressful over a short time period as the fish temporarily lose equilibrium at warmest temperatures or low oxygen levels. However, we expect all fish to recover quickly from this when returned to normal water conditions. At the end of the protocol, the fish will be humanely killed and their body tissues will be sampled for further analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This work has to be carried out on fish because, as aquatic ectotherms, their vulnerability in relation to climate change differs from other animal models (i.e. terrestrial vertebrates or invertebrates). Additionally, as fish are the prime beneficiaries of the output from this work, understanding tolerance in relation to fish biology is paramount. In all cases the in vivo work covered by this licence will be supported by in vitro, ex vivo and in silico studies of environmental tolerance. Wherever

	possible, these non-invasive studies will be used to assess tolerances if possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental design has been discussed with, and approved by, statistical advisor in order to minimise the number of animals required though good design.
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.	Shark populations are in decline world- wide. Therefore we have chosen to work with the lesser spotted catshark, a coastal, intertidal catshark, to understand how their physiology is impacted by climate change. It was important to choose a coastal species as this is changing at a more rapid rate with respect to temperature, hypoxia and pollution than that of the open ocean environment. We have also chosen zebrafish to deeply probe the mechanisms which confer environmental tolerance in fish due to their fast growth, their ease of care and easy access to genetically altered animals. Fish will be monitored daily for signs of illness and any animal displaying unexpected signs of distress will be returned to their home tank and monitored closely. If a fish experiences persistent discomfort (as assessed by erratic swimming behaviour, failure to right itself in the water column, or excessive ventilation, or poor body condition) then this will be considered the endpoint and the fish will be killed humanely by schedule 1 procedures.

Project	**141. Functional analysis of target genes of neuropsychiatric disorders including Autism and Schizophrenia**	
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 are interested in studying psychiatric disorders, such as Autism Spectrum Disorder (ASD) and Schizophrenia (SZ). These disorders have mutations in target genes which may influence brain development, behaviour, molecular networks, and gene expression. The aim of this project is to use respective mouse models which have targeted gene mutations. These mice display characteristics of human ASD/SZ. We will study the genetics, behaviour, anatomy and molecular biology of the mouse model. All of these aspects will help us to understand the changes to brain structure caused	

	by targeted gene mutations. We will also study each of these aspects at different time stages in mouse development. Overall this will allow us to investigate how these genes regulate behaviour during brain development.
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 are increased science knowledge and potential new insights into neuropsychiatric disorders such as ASD. We are studying genetic variations that cause typical ASD behavioural deficits e.g. social interaction. Developing the mouse model could help to develop future ASD drugs to alleviate symptoms. We hope this will help families and caregivers of those with ASD.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use up to 5000 mice over a period of 5 years. A mouse model is chosen here because changes in genes are similar to those observed in human disorders.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will be bred and maintained at the facility under standard protocols. Mouse pups will be required to generate primary cell cultures. The pups will be rapidly humanely killed so no adverse effects are expected. Mice will undergo behavioural and neurological testing including one or more anxiety/fear-related tests. For example, testing social interaction between animals. Animals may be subject to mild suffering such as stress from the tests and food restriction for motivation in tests. The mice will be weighed daily and returned to an ad lib diet if their pre-restriction body weight reduces by more than 15%. We will monitor for stress-related behaviour e.g. excessive grooming. We will try to reduce stress by performing only one test a day. We will also monitor cumulative stress from tests. Animals will be killed by a humane method and tissues taken for analysis after death.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We do routinely use non-animal alternatives and in fact the majority of our studies do not involve animals. We have performed these Petri dish studies as much as possible and have now reached the limit of what information we can gather before having to progress to more complex scenarios. In

	order to obtain information that is relevant to human health, we need to observe the behaviour/anatomical/genetic changes in an Autism mouse model. This cannot be accurately replicated with cells in Petri dishes and does not yield information completely relevant to human disease. During this project I will undertake regular reviews of the literature to keep informed of alternative approaches that could be used to replace animal use within this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will continue to use cells in Petri dishes to refine hypotheses before taking studies into animal models. This means that we can be as sure as possible that the molecule we are interested in is likely to influence this research. Moreover, for each new experiment, we will undertake pilot studies to carry out statistical power analysis that would be required to achieve statistical significance.
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.	A mouse model is the best model to answer our objectives. Only with this mouse model, we are able to generate changes in genes similar to that seen in human diseases. In relation to human disorders, common ASD and SZ characteristics can be tested through mouse behaviour tests for repetitive behaviour, hypersensitive responses and memory/social impairment. When using this model, where we can accurately investigate gene effects, we will need far fewer studies. Welfare concerns are kept to a minimum by using highly trained staff to conduct all our studies. We also take advice from the local veterinary team and the animal technologists with the facility.

Project	**142. Gene Function in Tumorigenesis**	
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)	Cancer is responsible for more than one in four deaths in the UK and four cancers, lung, bowel, breast and prostate, account for almost half of all cancer deaths in the UK. Colorectal cancer is the second commonest cause of cancer related death in the UK with over 16 000 deaths annually. Cancer incidence is also increasing and highlights the need for improvements in cancer prevention strategies, diagnosis and more effective treatments, particularly in the context of an ageing population. To achieve the required improvements in cancer diagnosis and treatment, a more detailed understanding of the molecular mechanisms that	

	promote cancer formation, its growth and spread. are required. This will lead to the identification of novel biomarkers for diagnosis and diagnostic imaging as well as new drug and treatment strategies.
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)?	Pre-clinical investigation and translation using animal models of human cancers will, in time, facilitate a more personalized approach to cancer treatment where the right treatment is given to the right patient at the right time. This will lead to an improvement in outcomes and quality-of-life for cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used in this project up to 2250 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 animals used in this project may be genetically engineered to develop spontaneous tumours or be experimental tumour models which use implanted human cancer cells or tissues . The development of tumours in these animals is likely to cause some adverse effects of a moderate severity. Tumour growth and development will be closely monitored to ensure that adverse effects are minimised and humane endpoints are observed, as well as the moderate severity limit. All animals will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cancer is a very complex disease involving all spects of human physiology, including different cell and organ systems along with the cardiovasular system, nervous system and immune responses. In order to fully understand the molecular mechanisms that drive this complex disease, it is essential at some point to study gene function and tumorigenesis in the context of the whole living organism. This is equally true for the development of novel therapeutics and treatment approaches.
2. Reduction	We strive to use the minimum number of animals possible in a number of ways. Firstly, extensive in

Explain how you will assure the use of minimum numbers of animals	vitro analysis is performed prior to animal experiments to ensure that appropriate studies are undertaken and the likelihood of success optimised. All new studies use pilot study groups to ascertain any given effects and also to provide data for statistical analysis to determine sample sizes in further studies. Non-invasive imaging will be used wherever possible to reduce the numbers of animals used by enabling longitudinal studies to be performed and therefore reducing the need for large cohort 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.	The range of mouse models available for cancer research and the ethical framework in place makes the mouse one of the most important models for cancer related research. The availability of genetically altered animals means that models have been created and are available where tumours arise spobtaneously in specific organs and therefore closely resemble the human disease. We select the most appropriate cancer models available to achieve our specific objectives. Non-invasive imaging will be used where possible to accurately detect and measure tumour growth. This will facilitate use of more humane endpoints as sensitive detection and measuring of tumour size/growth will inform decision making.

Project	**143. Gene-Drug interactions in melanocyte development and melanoma**	
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)	A striking discovery in the past decade has been that cancers are made up of different cell types, and that some of these cells are more dangerous than others. Our objective is to understand how stem cell populations (the body's master cells that give rise to many cell types and tissues) can contribute to cancer development when they become damaged. We have evidence that one cancer stem cell type is responsible for initiating new tumors in the body, and may contribute to resistance to therapy. Based on our findings, we are designing new therapies to target these cells in cancer. We are	

	specifically interested in the skin cancer, melanoma, and developing new drugs that might be useful in treating melanoma. Melanoma is the most deadly form of skin cancer, and rates are rapidly rising in Scotland. If caught early, melanoma can be cured by surgery. However, once the disease spreads most patients still die of the disease. Our work is based on identifying new drugs to target melanoma, and to understand how the cells within a tumor contribute to disease progression and respond to therapy
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 science is basic science and translational science. We expect to learn about the biology of melanoma and other cancers, and then apply this knowledge to the development of new therapeutics. We work closely with clinical collaborators to test our findings in the clinical context, and some of our work has been part of the basis for a clinical trial.We will share our findings with research and non-scientific community to through published scientific papers and international meetings
What species and approximate numbers of animals do you expect to use over what period of time?	We use zebrafish (Danio rerio), and we plan to use a maximum of 30,500 fish over 5 years. Most of these animals will be generated through breeding protocols and will not experience pain or suffering.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There are two aspects of our work that may cause moderate severity levels. First, we are developing zebrafish that have mutations or will be transplanted with mutated cells, and some of these fish develop cancer. Most of the fish are otherwise healthy, in spite of the tumour growth on the skin. Over the course of our experiments, the zebrafish will be exposed to some procedures that may be harmful, including anaesthesia, cell transplantation, electroporation and irradiation. These procedures are well established in the scientific community, and we will work closely with our zebrafish facility staff and University vets to minimise suffering for the zebrafish. Adverse reactions can include inflammation, bleeding and tissue damage, and we will carefully monitor zebrafish for signs of stress or distress (such as difficulty swimming or

	rapid gill movements). We minimize the development of these adverse effects by training our new staff in all techniques, using state-of-the-art equipment and by performing techniques under anaesthesia when appropriate. Any fish that show adverse reactions that cannot be controlled or reversed will be killed by a humane method to immediately end suffering. Second, we will be treating fish with anti-cancer agents, and the fish may develop adverse effects to the drug. This can include skin irritation, gill and skin inflammation and unexpected off-target effects. We will limit the potential of these effects by using the lowest concentration of drug we can. We determine the drug concentrations by first testing the drugs on human cells in a dish (that are not animals and do not suffer), and then by testing on zebrafish embryos before they are protected as animals (before 5 days of development). This provides a reasonable dose range to test drugs on adult fish that will have maximal target engagement while having minimal off-target effects. Any fish that is no longer able to swim well, or has evidence of suffering, or additional unexpected signs of disease or illness will be killed in a humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals because we are unable to address our questions in any non-animal system and have it directly relevant to human disease. We need to study the function of the genetic mutations and the potential drugs in the context of a living organism. Sometimes, we can address some of our scientific questions using cell cultures, or other systems such as yeast or in vitro, and we have the experimental set up in our lab to do these experiments when we can.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We ensure that the minimum numbers of animals are used by preparing our experiments ahead of time and using proper statistical methods, keeping healthy animals, using the fewest animals needed for reliable results, and when possible using fish in the embryonic stages. This license also includes a new method for generating genetically modified zebrafish that

	will reduce the number of animals needed to maintain the genetic mutation. Finally, we will use cryopreservation (the conservation of sperm and eggs at very low temperature) as a method to reduce the number of living animals needed to keep our genetic lines.
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 zebrafish because they develop cancers that are highly similar to human cancers. Like us, they are vertebrates, and they share many genetic and anatomical features with humans. Zebrafish are not mammals, so they are less similar to us than other animal systems, such as mice. However, we cannot use non-vertebrates (such as worms or flies) because these animals do not develop cancers that have the clinical features of human cancers. We have three dedicated staff looking after the zebrafish to maintain their health. Any fish that is no longer able to swim well, or has evidence of suffering, or additional unexpected signs of disease or illness will be killed in a humane method.

Project	**144. Generation and differentiation of blood and the cardiovascular system**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		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?	Our overall aim is to understand how vertebrate embryos make a blood and cardiovascular system as they develop and in homeostasis, and how the genetic programming is corrupted in disease.	
Why is it important to undertake this work?	Each tissue type is a result of a cellular hierarchy of precursor cells going all the way back to the stem cell. Therefore, to understand the workings of the blood and cardiovascular systems (and to be able to identify where things have gone wrong in disease or malfunction), we need to be able to assign or map regulatory networks to each stage of development of precursor cells in the embryo and to follow their activities as they mature in the adults. A more profound understanding of the mechanisms controlling tissue-specific gene expression will shed crucial light on congenital and developmental human diseases,	

	and it will also enable pluripotent cells to be differentiated in a directed way for potential use in tissue engineering, disease modelling and drug screening in the future. The information gained from this research may have impact on the area of therapeutic approaches to human disease using stem cell replacement technology. One way of directly assessing the impact of disease perturbation on tissue-specific gene networks is to model the disease in animal models. Zebrafish offer many technical advantages for analysis of gene activity while at the same time having the same genes, cell types, tissues, organs and biological circuits as humans, ensuring that the lessons from this model can be directly applied to human health. Moreover, several zebrafish models of disease have been described. We will initially use a zebrafish line, generated in our lab, that faithfully models MonoMAC syndrome, a human disease that progresses from mild haematopoietic defects to Myelodisplastic syndrome and in some cases, to leukaemia. We will explore how these cells escape normal controls over their proliferation and differentiation with a view to providing novel targets for therapy.
What outputs do you think you will see at the end of this project?	We list here the expected outputs from each of our main objectives (Primary purpose: advance science; secondary: disease control): 1. Identifying the extra-cellular signals, their nuclear transcription factor targets and the regulatory relationships between them, will enable a more profound understanding of the mechanisms controlling tissue-specific gene expression. 2. Determination of the cellular hierarchies that lead to the production of blood and cardiovascular cell types will identify the stable intermediate states for the genetic regulatory networks identified in specific objective 1. 3. Identifying adult stem cells and elucidating their programming will enable a better understanding of their normal and defective behaviour and its control. We will directly compare the genetic circuitry of normal HSCs and progenitors with those found in leukaemic models in zebrafish to identify novel targets that could be used to inform patient stratification or to design new therapeutic approaches.
Who or what will benefit from these outputs, and how?	In this project we are investigating the principles of haematopoietic stem cell maintenance, differentiation and survival driven by transcription factors or enhancer

	elements in the genome, in the context of a developing organism. This will be of great interest to other researchers in the fields of haematopoiesis, developmental biology and stem cell biology, but also appeal to researchers with an interest in transcriptional regulation and gene regulatory networks underlying cell fate decisions. Because the interest and applicability of the zebrafish as a disease model has grown massively over the last few years, our research will be of great interest to that community. Loss of function in key haematopoietic regulators in humans leads to haematopoietic disorders that may lead to leukaemia. Geneticists and clinicians with interests in normal haematopoiesis, myelodysplasias and acute myeloid leukaemias will be especially interested in our research as it may have a more immediate impact on the genetic screening routinely used in the clinic.Thus, our project will be of great interest to haematology researchers, geneticists and cancer biologists. These outputs will benefit all of these stakeholders as the research is published in peer-reviewed journals or presented at national and international conferences. The outcomes arising from this investigation will have a direct impact at international level. Our project is fundamental discovery science, but the knowledge generated will be crucial for our understanding of the molecular mechanism underlying haematopoietic disorders in humans and provide insights into onset of disease and the transition from normal haematopoiesis to leukaemia. Ultimately, this will pave the way towards earlier detection of disease, better disease stratification and the development of novel therapies for this disease. The beneficiaries of this research include patients suffering from haematopoietic disorders, cancer patients suffering from MDS or leukaemia and health professionals. The benefit to these stakeholders are expected in the long-term and will likely not be felt immediately after completion of this project.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	The discoveries we make during the course of this project will be presented at national and international conferences and published in peer-reviewed journals as appropriate. This will help to disseminate new knowledge but also to encourage new collaborations that might result in a new body of knowledge that is more than the sum of its parts. Any data that is not used for specific publications will be made publicly available

	(after a suitable period of embargo) in local (REDACTED) or international repositories (PubMed Central, Figshare, etc) to ensure maximum exposure and usage of the data generated.
Explain why you are using these types of animals and your choice of life stages.	Zebrafish are genetically very similar to humans; both vascular and haematopoietic processes are well conserved between zebrafish and human. They are a less sentient species than other models such as rodents, and easier to ascertain the impact of genetic modifications, made easier by the advent of CRISPR/Cas9 genome editing. We are interested in the process of formation of haematopoietic stem cells from endothelial cells, a process that takes place well within the first 5 days post-fertilization. Because zebrafish develop externally, there is no requirement to sacrifice the mother (like in rodent models). We are also interested in how the haematopoietic system reaches homeostasis and how it fails in disease. To address this, we need to analyse the haematopoietic compartment of adult zebrafish following specific genetic perturbations (e.g. model the loss of GATA2 found in MonoMAC syndrome patients).

Typically, what will be done to an animal used in your project?	Typically the animals will undergo fin clipping, either at 3dpf or as adults to select the ones with the relevant genotypes (Protocol 1). In either case this will be done under anaesthesia and analgesia as agreed with the veterinary surgeon and will cause only transient discomfort and no lasting harm. Most adult zebrafish in this protocol will be used to generate animals for Protocols 2 or 3 and embryos by natural mating for analysis up to 5dpf (i.e. before free- feeding). The genes involved in leukemia are normally expressed throughout development but when that expression is perturbed they are likely to cause disease in adults. We wish to determine how cancer- causing genetic mutations impact on normal blood stem cell lineage specification and differentiation in the adult (Protocol 2). In these cases, animals will typically undergo fin clipping under anaesthesia/analgesia once and allowed to grow up to 24 months of age to study disease onset and progression where relevant. As our interest lies in the early biological events of disease progression, animals will usually be killed by Schedule 1 before a major departure from their usual state of health is seen. Post confirmation of death, biopsies may be taken for further analysis. Where a gene of interest is likely to play a crucial role later in development (post free-feeding), juveniles (up to 31dpf) may be immobilized with an anaesthetic (non-recovery) in order to acquire a high resolution image with more specialized microscopes such as for example spinning disc or laser scanning microscopes (Protocol 3). In this protocol animals may undergo anaesthesia/analgesia once (before 5dpf) for genotyping and then undergo terminal anaesthesia for imaging up to 31dpf. In protocol 4, we aim to generate new genetically altered animals as necessary. Typically, embryos be microinjected with a perturbing material (e.g. an antisense morpholino oligonucleotide or CRISPR/Cas9 to reduce the production of a particular regulator). Embryos will then be allowed to develop to adulthood and mated to wildtype to assess transmission of the required genetic modification. Occasionally, they may undergo fin clipping or imaging under anaesthesia/analgesia once to help identify the best F0 founders.

What are the expected impacts and/or adverse effects for the animals during your project?	Typically, animals in this project will be used for breeding and generating embryos for analysis and thus the main source of adverse effects is likely to be from the use of anaesthetic prior to genotyping or from the genotyping itself. Adverse effects from the anaesthetic - Upon recovery from anaesthesia, animals should return to normal swimming and behaviour within 30 minutes. In rare cases (<1%) where animals may show impaired swimming behaviour upon recovery, they will be immediately killed by a Schedule 1 method. In rare cases (<1%), animals may show damage to scales due to fin clipping or infections due to loss of mucous surface from swabbing. These may result in abnormal behaviour or swimming. In these cases, animals will be immediately killed by a Schedule 1 method or in the case of individual fish of particular scientific interest, advice will be sought promptly from the assigned Home Office Inspector. Adverse effects from the genetic modification - Genetically altered animals may display a harmful phenotype due to the genetic modification that may include redness in the skin and oedema. These should not affect their swimming or feeding behaviour but are indicative of an underlying phenotype. In these cases, animals will be immediately killed by a Schedule 1 method or in the case of individual fish of particular scientific interest, advice will be sought promptly from the assigned Home Office Inspector.
What are the expected severities and the proportion of animals in each category (per animal type)?	Most animals will be under a mild severity protocol as they will used essentially for breeding and generating embryos for analysis (~87.5%, or 17,500 animals over 5 years, Protocols 1,3,4). Some specific strains (for example the homozygous gata2a mutants) may be required for analysis of disease progression in adults. Other animals of specific mutant backgrounds mat be required for breeding and generating embryos for analysis where the ocurrence or penetrance of eventual harmful phenotypes are not known. To allow for these cases, we expect that ~12.5%, or 2000 animals over 5 years will be under a moderate severity protocol (Protocol 2).
What will happen to animals at the end of this project?	killed

Why do you need to use animals to achieve the aim of your project?	This project aims to study the formation and differentiation of blood stem cells and their journey between haematopoietic niches which requires a complex organism with an intact whole body system. There is no viable alternative available that faithfully mimics this process in non-animal models and thus we require the use of animals.
Which non- animal alternatives did you consider for use in this project?	We have considered iPS cells and ES cells for this project. However, in order to study tissue differentiation during embryonic development and homeostasis in the adult, it is essential to determine what happens in a developing embryo and how this translates into the adult. iPS or ES cells are not suitable for these type of studies.
Why were they not suitable?	The developmental processes that we are studying are a constantly evolving system that requires complex temporal and spatial regulation to give rise to the blood and cardiovascular systems. Many cues directing these processes are received as cells interact with one another and migrate throughout the animal or particular organ/tissue and change with time and between different tissues. Thus these processes cannot be modelled in cell culture or in isolated tissues. However, as this information accumulates, comparisons can be made with in vitro differentiating systems such as embryonic stem cells or iPS cells to determine the extent to which they will be able to replace animal experiments in the future.
Enter the estimated number of animals of each type used in this project.	zebra-fish: 17500

How have you estimated the numbers of animals you will use?	The animals in this project will be used to generate embryos for analysis before 5dpf, juveniles for imaging before 31dpf and adults for analysis of haematopoietic phenotypes up to 24mpf. For experiments where we analyse embryonic phenotypes (gene expression, fluorescence intensities, etc), the required number of embryos was calculated assuming a small effect size (Cohen's d=0.5) to ensure we can detect small differences in our data. Sample sizes were calculated to provide 80% power where $p<0.05$ (t-test or ANOVA power analysis). For example, in a typical experiment we compare haematopoietic gene expression between wildtype and microinjection of a perturbing material. Here, to detect an effect size of 0.5 at 80% power and $p<0.05$, we determined that the sample size should be 22 embryos for each group being tested (untreated vs treated, total sample size = 44 embryos). On average, we test 10 genes per experiment in a given week= 440 embryos. To ensure statistical robustness in the face of potential losses and variation of rates of development (estimated at 25% of the embryos), we aim to have 600 embryos per experiment. For experiments where we investigate the phenotypes in wildtype versus mutant or transgenic adults (e.g. number or type of haematopoietic cells at a particular stage), we have either estimated a small effect size (Cohen’s d=0.5) or calculated effect sizes based on actual cell counts. Sample sizes were calculated to provide 80% power where $p<0.05$ and the allocation ration between groups was 1:1. Power analysis was perfomed by either unpaired t-tests (for two-group comparisons) or ANOVA power analysis (for multiple group comparisons). Here we also estimate a 25% loss of adult animals due to the onset of potential harmful phenotypes, and this was accounted for in our estimation of the final numbers required. Four tanks (or 30 breeding pairs) per zebrafish strain will allow sufficient animals for breeding and maintenance of specific strains and to generate enough animals for experimental purposes. We have approximately 40 strains and aim to generate another 10-15 during the course of this project and thus estimated a total of 17,500 animals. Power calculations are performed using the EDA tool (NC3Rs website, allows t-test power calculations) or GPower (performs ANOVA power analysis and others not available with the EDA tool).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	To maximise the scientific productivity from single spawnings, experimenters routinely share the embryos produced in protocols 1- 2. We have also performed various power calculations (as explained above) to ensure we produce the correct number of animals for statistical robustness. We will also reduce inter-tank variability by grouping animals of the same genotypes from different tanks where possible. For example, if 20 animals are required from a colony of 60 (split over 4 tanks), we will take 5 animals from each tank to get the required 20 for further analysis.
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 continuously review our usage of the available fish strains and will categorize them into classes depending on their usage i.e high, medium, low and will accordingly reduce the number of fish we keep per line. If a particular strain is no longer required we will either export it for long term storage at the REDACTED or terminate it using a Schedule 1 method. In addition, the REDACTED provides an aquatic management software that allows the tracking of fish usage, including monthly reports and querying numbers of specific strains. We will also perform post-hoc power calculations to ensure the experiments are powered properly and perform pilot studies where appropriate.
Which animal models and methods will you use during this project?	In this project, we will use zebrafish at immature life stages (prior to 5dpf) and more mature stages (from 5dpf onwards). Wherever possible, experiments will be conducted prior to the free-feeding stage and therefore not protected under the Animals (Scientific Procedures) Act 1986. Where zebrafish are required to grow past the free-feeding stage, they will only be used for breeding, imaging or collection of tissues, but only after terminal anaesthesia by a Schedule 1 method. Fish embryos are transparent and easily obtained in large numbers by natural mating and the mother will be able to produce eggs on subsequent occasions. In addition, gene function can be perturbed in a relatively high throughput fashion to expand our knowledge of genetic circuitry at a rate not possible with mammalian models. This includes studies both in embryonic but also adult forms of zebrafish. The use of such animal models from the lower vertebrates is a significantly less severe scenario from the animal's point of view since obtaining embryos does not imply surgical invasion of the mother with subsequent death as in mice. Thus, most of the analysis of gene function and cellular behaviour can be done non- invasively and ex-utero. Having the possibility to grow large

	numbers of embryos mean we can robustly generate enough embryos to grow to adulthood for further analysis of cell numbers, morphology or behaviour. Investigating disease progression in zebrafish adults represents an experimental refinement as they don't possess the same ability to perceive pain than higher vertebrates like mice. In addition, all analyses in adults will be performed in organ biopsies after killing by a Schedule 1 method, thus limiting any pain and distress to a minimum.
Why can't you use animals that are less sentient?	Zebrafish have short enough life cycles to make genetic experiments possible. Thousands of mutant lines are now available enabling the study of many signalling pathways and nuclear transcription regulators. The embryos are also transparent, making cell tracking experiments very feasible. Where tested, most of the genetic circuits are conserved through to humans thus results are translatable. On the other hand, in vitro alternatives, such as embryonic stem cells, are not yet proven to truly mimic normal development or disease progression in a complex multicellular organism. Although less sentient animal are useful to investigate other aspects of the haematopoietic process, they are less complex and evolutionarily more distant from human and thus less appropriate than zebrafish for our project. Zebrafish have successfully been used to model disease progression like the development of solid tumours (e.g. melanomas) and leukaemias and have provided a better understanding of these disease states. While the development of these diseases may cause a degree of suffering and distress, the higher brain structures such as the neo- and mesocortex required to consciously perceive pain are only present in mammals (for review see Rose et al., 2012) and thus zebrafish is most appropriate model to use that is considered less sentient than alternative mammalian models like mouse or rat.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We regularly attend zebrafish-specific conferences where any new developments are relayed to the community. We will keep abreast of new opportunities for refinement and reduction in our experiments by continuously monitoring published research to ensure we always apply the best possible statistical methods, animal husbandry and experimental design. We have also developed a close relationship with the NACWO and animal technicians to ensure that all contribute to implement any new advances in the 3Rs effectively. We are in direct contact with the regional NC3Rs programme manager to ensure we are up to date on the 3Rs and will use the NC3Rs website to stay updated on relevant news and workshops. We will follow the PREPARE guidelines and all publications will be

	in accordance with ARRIVE.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	Wherever possible, we aim to genotype animals by fin clipping before free-feeding; larval stage embryos are not as sentient as adults and their pain perception and avoidance reactions are not fully developed. In that regard,we have implemented an protocol for fin clipping that includes both anesthesia and analgesia to minimize any potential discomfort during and after the procedure. Some genetically modified animals (e.g. homozygous mutants) may have the potential to develop a harmful phenotype as adults. If they show signs of suffering that in any way compromises their wellbeing, they will be immediately killed by a Schedule 1 method. Understanding the nature of these symptoms is part of our research goals, so in cases where the animals are of particular scientific interest, we will isolate the animals and monitor them weekly for a maximum of 2 weeks after the appearance of the known symptoms. If they fail to recover, they will be killed by a Schedule 1 method. For genetically modified strains of scientific interest where animals may develop a harmful phenotype, we will first do a small scale pilot study to ascertain the onset and penetrance of the phenotype. This will determine whether more frequent monitoring (e.g. weekly from 3 months of age) or different experimental endpoints are required to ensure that animals do not suffer pain, distress or lasting harm. As per above, if the animals show any signs of suffering at any time that in any way compromises their wellbeing (for example fish that do not grow, behave, swim and feed normally), they will be immediately killed by a Schedule 1 method.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We will follow the PREPARE guidelines and all publications will be in accordance to the ARRIVE guidelines. We will also keep abreast of the literature to inform our protocols/procedures for genetic alterations, imaging and anesthesia/analgesia and thus ensure that they are performed in the most refined way possible.

Project	**145. Generation and maintenance of transgenic mice**	
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)	There is a lot of information publically available on human and mouse genes and their genomes. This genome information is found in every cell in the body and provides everything that is needed for the body and its organs to run properly. Scientists use this information to try to understand how genes or disease causing mutations work (damaged genes), they work in a lab using cell models to perform their experiments. However they are often left with an incomplete picture of what would actually happen in a complete organism like a human. Mice are very similar to man and allow us to look more closely at these genes in a whole animal, with hope of better understanding the	

	disease and how it could be treated in humans. We now have the technology to very specifically target genes and alter them in the mouse, making very useful mouse models of human disease. This is a very skilled job and other scientists' often lack the skills/expertise to use this technology. This project allows us to make mouse models for other scientists. By having highly trained staff in place, in a core facility dedicated to this technology we can ensure that we don't use more mice than is necessary making new mouse models for scientists.
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)?	We have lots of information from modern genomics on the genes and their mutations (damaged copies of a gene) that have impact on human diseases. By creating animals models for scientists to study we will have a better understanding of human disease and how it could be treated. This project will also allow the technology to be improved so that it becomes more efficient in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We will work by creating new mouse models. Each year we hope to produce 240-300 new mouse models. To do this we will use ~72000 animals each 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?	The techniques used in this project have been designed to minimise suffering and animal numbers. The techniques are all well used and the likelihood of adverse effects is known to be low. Each mouse model produced is monitored throughout its life for signs of ill health. Any concerns are carefully monitored and if they become clinically apparent the animal is killed. Making a new mouse model begins with producing embryos. Embryos are produced by injecting hormones into young females, this causes momentary discomfort. After we have used technology to change these embryos so they will grow into a new mouse model, they are put back into an expectant mother mouse by an operation. The expectant mother thinks she is pregnant as she was mated to a sterile male and has become ready to carry a pregnancy,

	however as the male is sterile no baby mice will be born unless we put embryos inside her. This operation is performed under general anaesthetic and pain relief will be given. Baby mice are born 19days later, when they are old enough a small ear clip is taken in order to check that they are our expected new mouse model. This procedure is only associated with momentary discomfort. The overall severity limit of this project is expected to be moderate, but the majority of will be mild. Mice used in this project will either be transferred to another project so the scientist who asked for them can begin their studies or they are humanely killed. We also encourage scientists to add their mice to the public mouse library (REDACTED) so that they are available to the rest of the scientific community and don't need to be remade elsewhere.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Genes and their disease causing mutations can be studied in the lab using cell systems, however we are often left with an incomplete picture of what would actually happen in a complete organism like a human. Mice are very similar to man and allow us to look more closely at these genes in a whole animal, with hope of better understanding the disease and how it could be treated in humans. The new mouse models made will also allow for future developments in the use of cell lines, made from the tissues supplied, hopefully reducing the need for animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We do our best to improve how we make mouse models so that fewer animals are sacrificed to produce them. We review our data often and check that we are not using more animals than we need and also make sure we do not breed more than is required to secure the new mouse model. This project licence allows us to keep a number of highly trained technicians able to make new mouse models in a lab that specialises in this technology. This reduces the number of

	animals that would otherwise be used. New mouse models made will be frozen so there is no need to continually breed them if they are not needed, this keeps them safe for future scientists that may need them and means that we don't need to remake them.
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 most appropriate animal model for this project because our intended aim is to work out the function of mammalian genes and proteins. Mice are very similar to man and allow us to look more closely at genes, and damaged genes, in a whole animal. We have developed a tool kit that allows us to make new mouse models by changing their genes. This gives us information that will help us better understand disease and how it could be treated in humans. We will minimise the welfare costs to the animals by using the minimum number of animals at all times. We will constantly review the techniques we use and introduce new refinements at the earliest opportunity. [For example when possible we now use non-surgical methods to return embryos to the expectant mother mice. We also no longer need to surgically sterilise male mice instead we use mice that are born genetically sterile.]

Project	**146. Generation of novel antibodies with therapeutic potential**	
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 allow us to develop and evaluate novel antibodies (proteins in the bloodstream that fight infections) for use in the treatment of human disease. There is a pressing clinical need to identify new types of treatment for human disease (for example, the development of novel antibiotics) and engineered antibodies have considerable potential for this.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit	This project will develop engineered antibodies that will be suitable for administration into humans and will not be rejected by the body or cause an adverse reaction. These antibodies	

from the project)?	could be used to treat a wide variety of human diseases such as cancers and autoimmune disorders including multiple sclerosis, inflammatory bowel disease and arthritis.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice to develop the engineered antibodies. This species is the most appropriate as efficient methods exist to allow us to generate mice that carry a genetic alteration (transgenic mice) within the antibody genes. Approximately 4500 mice will 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?	The mice that carry the engineered antibodies are not expected to experience any adverse affects. The immune system is still fully functional in these mice so they will not be compromised in any way and are not expected to be at any increased risk from infections. The greatest risk of an adverse effect will be during the immunisation protocols to validate the genetic model. While most substances that will be used to immunise the mice are expected to only generate a mild response, similar to that found from a standard vaccination, it is sometimes difficult to predict the biological response. Immunisations will be performed as early in the day as possible so that mice can be monitored during the day. Following an immunisation, mice will be closely monitored and any animal that shows a deviation from normal health will be humanely killed. We may use adjuvants such as FCA but when we do this is only by sc route and in small volumes and if adverse effects are more than minor or transient then the animal will be killed. The majority of the mice used (2000 mice) will be killed to obtain pre-implantation embryos for genetic manipulation. The second largest group of mice (1500) represent those that form the breeding colony. Only a sub set of these will be used for immunisations (600, mild). It should be noted that over the duration of the project, the great majority of the mice (95%) will only undergo mild procedures such as breeding, injections, blood sampling from a vein and ear notching. The small minority of mice that have a surgical procedure such as a vasectomy will
	be given post-operative pain relief as standard.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use mice as these have an immune system and antibody structure that is similar to humans making them a good model system for these studies. Unfortunately, there is no cell culture (*in vitro*) system currently available that can be used to generate appropriately modified antibodies with the diversity, specificity and stability properties that using a live animal (*in vivo)* model offers. Antibody production in a live animal is necessary to obtain the most potent and effective antibodies. Currently there is no *in vitro* system that can be used to model the complex nature of the *in vivo* model. The advantages of antibody generation *in vivo* include achieving a better diversity of antibodies and this also allows quality control checkpoints to ensure the selection and enrichment of cells that produce antibodies with therapeutically desirable properties.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The breeding colony of mice will be maintained as a minimum number by good husbandry, effective colony management and cohort assignment for immunisations. Breeding pairs will be routinely separated once enough offspring have been generated to avoid overbreeding or wastage of animals. To minimise animal numbers the choice of the immunisation method and co-administration with substances to stimulate the immune response (adjuvants) will be partly based on previous experience, literature searches and in consultation with experts in the field. Small pilot studies on non-genetically altered mice may be performed to identify the most efficient delivery method before conducting larger experiments. Relevant statistical tools (such as power analysis) will be used to design the studies. We have access to statisticians who we will consult when planning the *in vivo* studies to design experiments that use the minimum number of

	mice compatible with a rigorous statistical analysis. We will use randomisation of mice into each treatment group when testing different immunisation methods and the person doing the data analysis will be unaware of the treatment groups to avoid unconscious biases.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are a small and easily handled species with a highly characterised immune system and well-defined biology. Mice are also short lived, have rapid generation times and are easier to look after than other larger animals. The mice are kept in high quality specific pathogen free facilities with access to food, water and environmental enrichments. I have been successfully using a non-surgical method to transplant embryos into the uterus of females for many years and this represents a considerable refinement as the mice do not have to undergo a surgical procedure. For some parts of our research we also use a genetically modified mouse that carries a mutation and is sterile and we would like to try using this rather than male mice that have been made sterile by a surgical procedure. The speed of antibody response in non-genetically altered mice and the optimal routes for delivery of different antigens are very well characterised. For example, good antibody production is found for soluble proteins by injections beneath the skin or into the space surrounding the internal organs. The route of injections used in this project (eg under the skin or into the bloodstream) are expected to cause no or minimal adverse effects whilst inducing effective antibody responses in most cases. We will minimise harm to the experimental animals and improve their welfare and well being by ensuring acclimatisation before any procedures are performed, accustomise them to being handled, using reduced injection volumes and using a check-list of health observations. Freund’s complete adjuvant (named after Jules T Freund) is a substance that is mixed with the antigen and can stimulate the immune

	response. Freund's complete adjuvant contains inactive mycobacteria and we have found that this gives such an excellent immune response that it only needs to be given once thus reducing the chances of an adverse reaction. Small pilot studies will be conducted to ensure that the methods used provide for the maximum animal welfare in relation to the study objectives. Pilot studies involve using a small number of mice to test the safety and efficacy of a method before using it on a larger number of mice.

Project	**147. Generation of pharmacokinetic parameters**	
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)	The ultimate aim of the project is to bring new medicines to market that improve patient outcomes and quality of life. Many potential new drugs fail to reach market or have to be withdrawn as they have poor properties. These include: ● They are poorly absorbed so it is not possible to give a dose large enough to treat the disease. ● They are metabolically unstable i.e. the body breaks them down too quickly for the drug to have an effect. ● When the body metabolises the medicine, it	

	forms a new compound that is toxic. ● The medicine fails to reach the site of the disease and has no effect. ● The body removes the medicine through urine and faeces so quickly it is unable to have an effect. The objectives of this project are to help our clients identify compounds that have good pharmacokinetic properties i.e. they are well absorbed, remain in the body long enough to have an effect, are not metabolised in anything that is toxic and reach the site of the disease. This will be done as much as possible through non-animal (in vitro) methods. However, all of these pharmacokinetic properties are affected by a complex interplay of organs, enzyme systems and physiological structures that cannot at present be replicated with in vitro systems. Therefore, to enable a potential medicine to progress to clinical trials in humans it will have to be dosed in an animal experiment to allow the measurement of pharmacokinetic properties in a whole body system.
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 benefit humans by helping to accelerate the development of new medicines for unmet medical needs, particularly where work is being undertaken on the behalf of clients that lack the necessary facilities to perform in vivo testing. The information generated will reduce the development time of new therapies and treatments that have the potential to reduce human suffering.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice. Approximately 2000 mice and 1500 rats over a 5-year period. The exact number of animals and the split between rats and mice will be dependent upon external factors such as the number of clients, the success of those clients in designing suitable drugs and the disease being targeted.
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	To reduce animal suffering, protocols have been designed to have the lowest severity limits possible. To improve animal welfare extensive use of environmental enrichment will be supplied and all protocols will have strict limits on the level

happen to the animals at the end?	of discomfort the animals can experience before being humanely killed. All of the animals used on this Project will experience a single injection and have a series of blood samples, taken, typically over a 24-hour period. If tissues are required, then animals will be humanely killed and tissues and/or blood will be taken after death. In some cases animals may be singly housed and/or temporarily placed in mesh bottomed cages, to allow collection of urine and faeces. The duration of single housing will be kept to the minimum duration to achieve the scientific aims. Minor, temporary discomfort is anticipated upon injection/dosing. Adverse effects due to the injection are anticipated to be rare. Any animals exhibiting continued discomfort will be humanely killed. All studies will end with the animals being humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal studies are necessary because the processes involved in determining how well a drug is absorbed, where it goes to, how it is metabolised and how it is removed are difficult to replicate in laboratory tests (in vitro). Although not identical small mammals (e.g. mice and rats), are similar enough to Man to be able to use them as replacements for humans. Species selected for this Project represent the lowest form of animals in which these types of studies can be conducted and still give relevant information . The use of laboratory tests (in vitro) and computer models prior to animal studies can and do minimise the numbers of animals used. These approaches are becoming increasingly powerful tools in the design of new drugs. These approaches can help in understanding a molecule's suitability as a medicine and screen out compounds that do not have the right properties. However, the suitability of a molecule as a medicine depends on many factors, and it is the combination of all these factors that determines if a compound can be used in humans. Animal studies need to be conducted to determine the actual properties of a new molecule in a body
2. Reduction	Most of the animal studies will use 3 animals per route of administration, this is the smallest number

Explain how you will assure the use of minimum numbers of animals	of animals that can be used and still give reliable data. The main route to minimise animal usage is the use of micro-sampling in mice. This allows us to obtain more than one blood sample from a single animal. Therefore, in a typical study we would need 6 animals if using micro-sampling compared to 48 animals in a more traditional study. Multiple tissue samples will be taken from the same animal after it has been humanely killed to minimise animal numbers. Another method to reduce the number of animals used is to dose several different types of compounds in animals early in a project. This can often result in whole groups or types of compounds being eliminated and prevent a much larger number of compounds being dosed to animals later in 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) to the animals.	Only rats and mice are used on this licence. Although using non-mammalian species would be desirable it is not possible as they are too different from humans. Although rats and mice are not identical to humans, they are similar enough that they can be used to predict human results. Species to be used in this project will be determined by the disease being investigated and which are most like man in relation to the disease. In order to improve general animal welfare and the scientific integrity of experiments, where possible the following considerations will be met: ● Animals will be kept in social groups. ● Group sizes will not exceed ASPA recommendations. ● Animals will receive environmental enrichment for example: a selection of nesting material and refuges/hiding places. All animals will be allowed to get used to their surroundings before use.

Project	**148. Genetic and developmental basis of morphological variation in REDACTED fish**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		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 proposed project is to identify genes responsible for differences in skin colour between species of REDACTED fish. Genes are portions of the genome, which is a particle that contains all the information needed to build and maintain an organism. REDACTED – Fish are a very diverse group of 2100 fish species, with very distinct colour patterns. In this project we will identify which genes underlie the colour diversity observed in this group of fish. More specifically,	

	we will study body pigmentation and anal fin markings that are present only in *REDACTED* –fish males. More colour and a higher amount of anal fin markings makes males more attractive to females and increases their chances of reproduction. To identify genes responsible for coloration differences we will test if it is possible to interbreed different species with different colour patterns using *in vitro* fertilisation methods. The genomes of the progeny with mixed colours will be sequenced to identify the genes causing colour differences. Once candidate genes are identified we will use techniques that delete and/or modify genes to study how their function affects pigmentation.
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 uncover genes underlying differences in colouration. This will contribute to knowledge of nature and evolution. Using techniques that delete genes, we will describe how the deletions affect skin colouration. These genes define how colours are produced both in fish and other vertebrates (e.g. birds, reptiles, mammals and humans). Taken together, we will describe the function of genes that are important to REDACTED fish colouration and conclusions arising from these findings can be extrapolated to other vertebrates. Our work will be disseminated through publications in scientific journals that will be available to other researchers and the general public. We are among the first research groups to use these gene deletion techniques in REDACTED fishes, therefore the success of our project will benefit the whole REDACTED research community. We will share our expertise with other laboratories which will then be able to apply the same methodologies.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use several REDACTED species and throughout the duration of this project (five years) we plan to describe the function of 15 genes. For this purpose, we will generate 15 groups of fishes that harbour gene modifications (mutations). Each group is technically defined as a strain. There are more than 15 genes affecting colouration but due to

	time constrains during the next five years we will only be able to tackle the function of this subset. We will use methods that involve in vitro fertilisation (joining eggs and sperm in a tube to obtain a fertilised egg) and injection of REDACTED eggs with particles that are able to modify specific gene portions. To collect eggs and sperm, we need 10 individuals per species and per strain. We plan to collect eggs and sperm from three different species (total of 30 individuals) and from 15 gene deletion strains (150 individuals). To generate and maintain the different gene deletion strains we require 300 individuals (total of 4690 individuals for the 15 gene deletion strains).
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?	What we propose to do to the animals will minimally impact on their health and welfare because we will only modify genes that are involved in different skin colours. Adult fish will be anaesthetised and we will perform a gentle abdominal massage to collect eggs and sperm. After collection, we will perform in vitro fertilisation or store the sperm for future usage. In vitro fertilisation will originate REDACTED embryos that will be maintained in aquaria in our facility. We will inject fertilised eggs with particles that modify specific gene portions. These modifications should only affect the colour of the fish, but might also result in harmful growth malformations (e.g. fish embryos that do not grow, embryos that lack organs, juvenile fish that swim and behave abnormally, etc.). If the latter occurs affected animals will be humanely killed as soon as the malformation is detected. Animals that are not fully recovered at the end of the procedures will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To understand the relationship between mutations and differences in colouration, we modify specific genes to assess their function on the colour of juvenile and adult fish. We therefore cannot avoid using animals for the purpose of this project. Throughout the project, we will implement

	strategies to replace animal use: 1. For each humanely killed fish we will dissect and store all its tissues (e.g. brain, heart and skin). This tissue archive will be made available to other researchers, which will replace the use of live animals in their laboratories. 2. We will develop a sperm freezing protocol, which will allow us to store and revive REDACTED mutant strains, via in vitro fertilisation, replacing the need to keep adult fish with gene deletions in our aquatic facility. 3. We will develop a sperm freezing protocol, which will allow us to store and revive REDACTED mutant strains, via in vitro fertilisation, replacing the need to keep adult fish with gene deletions in our aquatic facility. 4. We will develop a sperm freezing protocol, which will allow us to store and revive REDACTED mutant strains, via *in vitro* fertilisation, replacing the need to keep adult fish with gene deletions in our aquatic facility.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will strive to use the optimum number of animals and throughout the duration of the project will continuously consider if the benefits of the number of animals used and procedures performed outweigh the potential harm. We will plan, conduct and report our experiments according to the PREPARE and ARRIVE guidelines. For the egg and sperm collection, animals used will be kept to a minimum required to perform successful *in vitro* fertilisation and sperm and eggs will be frozen and stored to reduce the need to use more individuals. For the generation and breeding of REDACTED genetically altered strains, only individuals with confirmed gene modifications will be used. Gene modifications are confirmed by collecting tissue biopsies (e.g. by removing a very small piece of fin or collecting

	skin swabs), which are then used for genetic tests. Fish harbouring genetic modifications that are not actively used in experiments will be preserved by sperm freezing which will reduced the number animals used in the facility. If we need to resurrect a line, we will perform *in vitro* fertilisation using previously frozen eggs and sperm.
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 proposed to use are large, robust and thrive in an aquaria environment and will withstand the mild regulated procedures with the minimum of stress. In order to continuously minimize animal suffering we will always use up to date technology and refine the housing environment. *REDACTED* will be housed in aquaria mimicking their natural environment, which is a sandy area rich in vegetation and hiding stones. Sand, artificial plants, plastic tunnels and clay pots will be used to enrich their aquaria environments. Group housing decreases stress and aggression levels in fish. We will maintain adult fish in high-density groups to reduce aggression, thus avoid unnecessary stress or injuries from chasing or fighting. Researchers and animal technicians will pay close attention to the fish in every tank, in order to control stress and aggression levels. We will continuously run trials on optimising feeding regimes and control water quality to ensure optimal husbandry.

Project	**149. Genetic and developmental origins of craniofacial malformations**	
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)	"Craniofacial malformation" describes an abnormality of development of the face or skull. This may have many serious consequences, affecting functions such as vision, breathing, hearing, eating, mental functions and cosmetic appearance. The primary focus of this project is craniosynostosis, the premature fusion of one or more of the cranial sutures, narrow gaps between the bones of the skull that allow normal growth during fetal life and childhood. Fusion of a suture prevents further growth at right angles to the	

	suture line, causing growth distortion. Craniosynostosis may be caused either by alterations in genes (mutations) that interfere with cranial suture function, or by environmental factors such as external pressure on the developing fetal skull. Treatment requires major surgery, involving the removal and repositioning of the skull bones. Advances in knowledge might in the future enable the prevention of craniosynostosis, either through better genetic counselling and predictive testing or through new medical treatments. To understand why craniosynostosis occurs we need to know how the cranial sutures develop and how their continued function (maintenance of suture patency, and ongoing addition of bone at the suture margins) occurs during growth. These questions can only be studied in living tissues, and it is ethically impossible to study these processes in humans. This project has two major objectives, which we will address using particular genetically modified strains of mice that recapitulate the equivalent genetic variants found in humans with craniosynostosis or other craniofacial malformations. First, we will ask whether craniosynostosis or other craniofacial malformations occur in association with a particular genetic modification in the mouse, in a similar fashion to that previously observed in humans. This can provide important additional evidence supporting that the mutation is the likely cause of the clinical condition. Second, we will monitor how the skull develops in embryos and after birth, and study how this relates to changes in the expression of genes, proteins or cellular processes. This helps us to learn both how cranial sutures normally develop, and how gene mutations affect these normal processes to cause disease.
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)?	Based on this work we hope to obtain evidence supporting or refuting the involvement of a specific genetic mutation as a cause of craniosynostosis or other craniofacial malformation in patients. More broadly our work may also provide clues to identify new disease-

	causing genes and improve genetic diagnosis and the counselling provided to families in which these conditions have occurred.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 6550 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?	The genetically modified mice with craniosynostosis that we study are usually able to feed, drink and reproduce normally. They will be bred so that we can examine the development of the skulls of embryos and young mice at different ages. Rarely, overgrowth of incisors occurs; this can be treated by trimming the teeth. Pregnant mice may be killed at defined stages for examination of the embryos and in addition some may administered substances orally or by injection to track individual cells in the offspring. Overall most animals will have no adverse effects (mild experience), but a minority of animals will have a moderate experience related to one or more of the events described above. All mice will be killed once their experimental or reproductive purpose has been completed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The cranial sutures are complex 3-dimensional structures containing multiple cell types. These structures need to stay open for several weeks for bone growth to occur. Currently there is no artificial system able to recapitulate these complex developmental processes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When analysing new mutant strains, if available we will make use of animals available from storage banks. In cases where genetically modified strains of particular interest for scientific study are not already available, we will collaborate with expert teams licensed to generate appropriate genetically altered lines, which could be transferred to this project. Useful strains will be preserved so that they do not need to be made independently by other researchers in the future.

	For experiments in which we wish to compare the effects of a treatment with a control group, we will use 8-10 mice in each group. This is a minimum number that will give an effect that is statistically significant, where difference in effect of the treatment between the two groups is relatively large.
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.	Amongst model organisms (including chicks, amphibians, and fish), only mammals have a pattern of cranial sutures similar to these structures in humans. Amongst mammals, the mouse is the most suitable for intensive study because of its small size, short reproductive cycle, and the availability of well-developed genetic technologies to manipulate the mouse genome, and associated resources of genetic strains. The models chosen are those that recapitulate the phenotype of the equivalent human disorder (for example, craniosynostosis) most reproducibly. Experimental methods chosen are widely used and yield the most precise data. We have fifteen years of experience in the husbandry of mice with disorders of skull development. The health and nutrition of these mice will be monitored regularly with a simple scoring system, with particular attention to the occurrence of incisor overgrowth (see section on adverse effects). A soft diet will be provided. Where it is necessary to give treatments by gavage or injection, the minimum volumes will be used and the route chosen to minimise distress to the animal. These procedures will be undertaken by competent staff using best practice techniques.

Project	**150. Genetic and environmental effects on behaviour in fish**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		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)	Behaviour can be influenced by the genetic make up of an animal and also by the environment. Some behaviour can be passed on to offspring and so is inherited, though we do not know the extent of this for many behaviours. Animals can be influenced by various environmental stimuli throughout their life and we are interested in knowing whether these can affect their behaviour such as how anxious or aggressive they are, their interactions with other individuals and their ability to learn simple tasks. Some of these effects could be long term and changes in behaviour could be passed on to offspring but this needs to be investigated.	

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 proposed work is primarily fundamental research on fish behaviour. We propose to measure various behaviours and determine whether various environmental stimuli can influence behaviour associated with anxiety and if this influences future generations.
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish 17580 Guppies 3200 Nile tilapia 920 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?	This project will involve measuring and assessing the behaviour of three different species of fish. Some experiments will expose zebrafish and guppies to physical (e.g. touch), visual (e.g. model of a predator) or chemical (e.g. predator water) stimuli and assess their behaviour as adults after exposure as well as their offspring. Some behavioural tests will measure aggression using their response to a mirror image as a gauge of this. It is unlikely that the animals will suffer any pain or suffering but if they do, they will be removed from the treatment immediately. The expected level of severity is mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The nature of the work, which is on animal behaviour, necessitates the use of live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before each experiment is conducted, a detailed protocol will be written covering: (i) a statement of the experimental objectives; (ii) a description of the experiment, covering such matters as the experimental treatments, the size of the experiment, and the experimental material; and (iii) an outline of the method of analysis of the results. Factorial designs are preferred, and power analysis is used where appropriate.
3. Refinement Explain the choice of species and why the animal model(s) you will	These fish are easy to keep and produce lots of offspring during reproduction. They have had their genome sequenced and they share many genes with humans. These factors make these

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.	fish good organisms to use when investigating behaviours that are also relevant to human conditions. The behavioural tests, involving single or repeated exposures to the physical, visual or chemical stimuli and measuring aggression are not expected to cause more than mild severity adverse effects.

Project	**151. Genetic determinants of renal cancer progression**	
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)	Renal cell carcinoma is the most common type of kidney cancer affecting 8,000 people and causing 4,000 cancer related deaths each year in the UK. Despite recent advances in our understanding of the underlying molecular biology and the development of novel therapeutic agents it remains an incurable disease once it has spread, i.e. formed metastases, outside the kidney. The vast majority of metastatic cancers are refractory to treatment and are therefore incurable. Thus, there is a pressing clinical need for further research on the molecular basis of renal cancer. The goal of this project is to understand genetic	

	factors that are required for initiation, maintenance and metastasis of renal cancer. Such knowledge is essential for the development of rational new treatments for this disease.
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 benefit from this work is a better understanding of which genes are important for renal cancer growth, and how at the molecular level these genes promote renal cancer. Thus, the potential secondary benefits of this work will go beyond basic cancer research, and might prove valuable to clinicians by possibly leading to the identification of (i) new drug targets for kidney cancer and other cancer types (ii) new biological markers that can be used to predict how renal cancers behave in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	1700 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?	Tumour induction, which mean injection of cancer cells under the skin, into the vein, into the heart or into the kidney, will in most cases have no significant impact on animals' general well-being. Short-term mild discomfort may be possible directly at the injection site but animals are expected to recover quickly. In some cases, such as where surgical procedures are used, the mice will receive pain-relieving drugs in order to reduce the injection-related adverse effects. In very rare occasions tumour induction may result in sudden death without preceding signs. After tumour induction, animals will be observed closely for any evidence of tumour growth by physical examination, inspection of the injection site and whole animal imaging. Discomfort resulting in moderate clinical signs such as hunched posture and inactivity or respiratory distress will result in individual animals being killed and on occasion the termination of the experiment. For other procedures such as drug treatment most animals will show no more than mild clinical signs. In drug experiments control animals will receive vehicle. Some may show moderate signs such as hunched posture, loss of body weight, lack of grooming, which will require termination of the experiments by killing

	the animals. At the end of experiments, all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Understanding the genetic and molecular mechanisms that support the development of cancer requires investigation in model systems that replicate as close as possible the human disease (cell and tissue of origin, surrounding environment). These parameters cannot be replicated in tissue culture systems in the lab. The laboratory mouse represents the best available model system for cancer owing to various factors including its extensive biological similarities to humans, and an entirely sequenced genome. We will therefore utilise animal models in order to validate findings that we initially make using tissue culture experiments in the lab.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will aim to minimise the numbers of animals used by: (1) Using tissue culture experiments to identify genes that are likely to be important for cancer growth before staring animal experiments. (2) Careful planning and statistical analysis will help us use the smallest number of animals that will ensure robust results. (3) Once experimental end points are reached, cancer tissue from affected animals will be harvested to facilitate continuing, complementary work in the laboratory. (4) Pilot studies on small groups of animals will reduce the number of animals used. (5) Imaging techniques such as bioluminescence, i.e. detection of light produced by a reporter gene that is expressed by the cancer cells, will allow further reduction in animal usage. (6) Whenever possible, the experiments will be randomised and the the investigator will be blinded to the experimental 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.	Mouse models have been chosen as they represent the least sentient species able to generate meaningful data, i.e. that is likely to be directly applicable to the human disease. Animal suffering is minimised by the use of appropriate pain-relieving drugs. Where clinical signs are seen animals will be killed as soon as possible and before they are likely to develop signs of pain or distress that would exceed a moderate severity limit.

Project	**152. Genetic determinants of response to cancer therapies**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We work primarily on lung cancers with some additional investigation of drug-resistant subtypes of other cancers (eg. pancreatic cancer and colon cancer) Our work using genetically altered (GA) mice aims to achieve 2 broad aims: 1) a better understanding of how cancer develops and changes from less to more aggressive disease over time; 2) to identify and validate new treatment strategies that, if proven to be effective in mice, may then be tested in human patients with the same types of cancer. Under Aim 1, we want to investigate how	

	specific genes promote cancer spreading to other organs and how cancers hide from the immune system during their development. In the specific instance of Mesothelioma, we want to investigate how chronic inflammation gives rise to eventual disease. Under Aim2, using our GA mice, we previously identified a combination of existing drugs for treatment of a subgroup of very aggressive Lung cancers. Our future work aims to determine just how effective this combination is, how it works, if it can be applied to other cancers and to identify additional therapy options In general, we will use specific genetic tools to control the start cancer development in adult GA mice. This ability to control the start of the process means that we can investigate how far cancers have developed at any given time after starting cancer development. We will follow how cancers grow using non-invasive imaging techniques, such as ultrasound, fluorescent, or micro-PET/CT/MRI imaging that is similar to techniques used in humans. Mice with cancer will be treated with therapeutic (or candidate therapeutic) drugs, given orally, in food/water, or injected. For longer term treatments (typically 1-3 months) drugs that are well tolerated may be administered using surgically implanted mini-pumps (less than 5% of mice). Less than 5% of mice will be treated with micro-irradiation that will be limited to the part of the body with cancer. Monitoring the response to drugs typically involves the same techniques as above, sometimes at increased frequency.
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)?	We are focused primarily on cancers that are difficult to treat and have very low survival rates in humans. These include: Lung Cancer: 5 year survival < 10%; Pancreatic Cancer: 5 year survival < 5%; Mesothelioma: 5 year survival <2%. Our work will identify new treatment strategies that can be quickly adapted for use in human patients. Indeed, we have already identified an unexpected drug combination and are in the process of negotiating a Phase 1 clinical trial, in collaboration with partners in the pharmaceutical industry and local doctors. Our work will additionally tell us more about how

	these aggressive cancers develop over time – we expect that this will identify new indications of early cancers that could lead to the development of clinical tests to detect cancer before it becomes very aggressive.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 15,000 mice over a 5-year period. Almost all of these mice will be genetically altered (GA) and bred on-site. Less than 1% of GA mice will be obtained from licensed suppliers. Less than 5% of mice will be non-GA. Our experiments typically involve multiple altered genes that are transmitted from parent to offspring. Approximately 50% of bred mice will not have a scientifically useful combination of GA genes (genotype) and will be humanely killed without further procedures upon completion of genotyping.
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 small percentage (<5%) of mice will develop up to 3 moderate or 1 substantial symptom (eg. weight loss up to 20%) while on protocol. All such mice will be humanely killed upon reaching such defined end-points. The vast majority of mice will experience sub-threshold to moderate levels of discomfort (for most procedures, no more than that of an injection). Approximately 50-75% of our mice are investigated before they show any outward symptoms of disease. Those that are allowed to develop more advanced disease typically show the following mild/moderate symptoms for each of the listed cancer types: Lung Cancer & Mesothelioma: Irregular breathing; Subdued behaviour; Moderate weight loss; Loss of appetite. Pancreatic and Colorectal Cancer: Subdued behaviour; Loss of appetite; Weight loss; Pale feet; Hunching. Mice that begin to show these symptoms are subject to increased visual monitoring. We use Body Conditioning Scoring combined with regular weighing to assess if mice are healthy enough to continue on the study, or if they require humane killing by an approved method. All mice will be humanely killed upon conclusion of study. Specific procedures are included for certain cancer types: GA mice modelling mesothelioma may be administered asbestos or other fibres (approximately 75% of such mice), via injection

	into the chest or abdominal cavity; GA mice modelling colorectal cancer may be administered substances that cause bowel inflammation (less than 20% of such mice) and the same model may be monitored by colonoscopy (less than 10% of such mice).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use power calculations to ensure that enough animals are used to allow us to reach sound conclusions and terminate investigations as soon as these numbers are reached. In consultation with local statisticians, cohort sizes will be determined according to the equation N = $(1.96+0.84)^2$ x $sigma^2$ divided by $delta^2$, allowing for a <5% chance of a Type I error at 80% power, where sigma is the combined standard deviation and delta is the expected difference. In many instances, sigma and delta will be unknown a priori, requiring pilot studies on small cohorts (typically 5 mice per cohort) to establish standard deviations and approximate differences. Once these variables are established, the power calculation will be performed to determine if cohort sizes are adequate or need to be increased accordingly. To minimise the generation of mice with unwanted genetic alterations, we will use selective breeding schemes that enrich for useful GA combinations.
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.	GA mice have the advantage of closely resembling human subjects at the level of gene and protein activity; cell, tissue and organ function; and respond similarly to treatment with anti-cancer drugs as do humans.GA mice that allow us to study many of the genes and proteins that are changed or lost in human cancers are already available, enabling us to investigate specific GA combinations that differ from one cancer to another.

	We continuously monitor our protocols and procedures to identify possible areas for refinement, including discontinuation of any procedure deemed to cause undue stress; to be redundant; or for which simpler or less intrusive techniques emerge.We keep up to date with current practice, informed by animal welfare websites (eg. NC3Rs), relevant publications, as well as our local NCTO and NVS.

Project	**153. Genetics and treatment of acute lymphoblastic leukaemia**	
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)	My laboratory works on a blood cancer called acute lymphoblastic leukaemia (ALL). This is the commonest cancer in children with children cured after prolonged intensive chemotherapy. The disease uncommon in adults, but the incidence rise with age. Adults have a much worse outcome than children. Our overall goal is to find out why childhood ALL differs from adult ALL. Our work to address this uses specimens we have obtained, with appropriate consent, from patients participating in clinical trials. If we are fortunate, we sometimes can preserve patient leukaemia cells in the freezer. They are a	

	very precious resource and do not grow well outside the body. So, our first aim (1) is to expand patient ALL cells by injecting them into mice intravenously or into the mouse bone marrow whereupon they will seed and expand, allowing us to use and share them after collection them from the mice. Our second aim(2) builds on our observations that ALL and its therapies impact the so-called "bone marrow microenvironment" namely the non-cancerous cells that surround and support the growth of the abnormal cells. We have shown that some elements of the surrounding tissues behave in such as manner as to donate mitochondria - which are the energy-generating, power houses of a cell – to the cancer cells. The mitochondria help the cancer cells to survive and evade our usual treatments. We require a more complex model system to get the clearest answer of how this might impact a human with ALL and how we can modify that. Our main observations are how these treatments and the different subtypes of the tumor impacts how the 'microenvironment' supports the ALL cells and most of our work is on cells and tissues from the mice. For the third and final aim of our proposal, (3) we propose to develop a safe, vaccine strain of virus to treat ALL. This virus has already been tested in humans in other cancers. We are hoping to design clinical trials specifically for patients with ALL. We will get the best results for our patients if we know how to combine the virus with various existing treatments, understand how the virus can spread throughout non-tumor tissues and how the body's immune cells interact with the virus to fight the cancer. We propose mouse models to study this so we can observe how many cell types work in coordination. We know we must sometimes demonstrate how things work in mice for regulatory authorities to allow human testing. During these experiments, we can make observations of tumor growth and progression directly on the mice, but we also use the cells and tissue that we collect at the end of experiment to gain a more detailed picture of what has happened.

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)?	We will make best use of the precious resource of human ALL cells by propagating them in mice. We will develop a potential new treatment for ALL. We will learn more about why existing treatments do and don't work but studying their effect not just on cancer cells but on surrounding tissues. Our data and resource will be shared with a large group of scientist and clinicians that we already work closely with. Our data may be used for regulatory authorities to give us permission to do human studies.
What species and approximate numbers of animals do you expect to use over what period of time?	We have requested the possibility to use up to 5000 mice (including genetically-altered 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?	When we breed the animals for our work, they do not suffer, subthreshold or at a maximum so-called 'mild'. When we inject the mice with tumor cells and administer drugs and antibodies to them, they feel transient discomfort. Sometimes we have to anaesthetise them in order to use a technique to 'see' the tumors through light given out by specially modified tumor cells which can then be detected with a very sensitive camera. The mice are put to sleep briefly and recover quickly. Sometimes we take a sample of blood or bone marrow - again the mice feel transient discomfort. Ultimately, mice who have been given cancer will become unwell. They are monitored very closely for their normal behaviours of eating, drinking, grooming and moving around and are weighed a minimum of once per week and more if needed. If they reach a pre-specified level of ill health – termed the humane endpoint, they are euthanised using an appropriately kind method before they suffer. All of this work is classified as having a 'moderate' impact on the mice.
Application of the 3Rs	
1. Replacement	We always seek alternatives to mouse work - in addition to the moral and ethical concerns, taking

State why you need to use animals and why you cannot use non-animal alternatives	care of mice is hard work and very expensive compared to other types of experiments, so we have a very practical incentive not to use them. However, sometimes we have to use murine studies as proof of concept for the field to accept our findings, regulatory agencies often require murine studies in support of a proposal or where murine studies are currently only scientifically accepted way of evaluation for example, seeking tumor propogating cells or gaining the maximal benefit from patient-derived material by generating so-called patient-derived xenografts, generally known as PDXs. One specific alterative that we are considering for some of our studies is tumor spheroids. We have actively sought a collaboration with the team of a colleague who co-invented 'tumouroids', 3D in vitro cultures of cancer which mimic the composition and architecture of solid tumours. Tumouroids are being developed as platforms for therapeutics and for stratified medicine and we seeking funding to determine we can develop models of ALL in tumoroids, by comparison with murine models, in collaboration with our colleague.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction We are very careful to use the smallest number of mice give a valid result. Myself and my group all are trained in statistics and in addition we consult an experienced statistican to ensure that we have the ‘power’ to detect the effect size we anticipate seeing. Experimental rigor will be ensured by using randomisation – namely randomly assigning mice to groups, blinding namely the day to day workers who look for the endpoints not being aware which groups the mice have been assigned to and other means to reduce bias and minimise variation to ensure we have repeatable and robust data.
3. Refinement Explain the choice of species and why the animal model(s) you will	Mice are the lowest group in which pre-clinical models for oncolytic (namely virus which kills cancer) therapies in cancer have been developed or in which we can observed the

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.	relationship between the tumor and the microenvironment. In particular, we need to use mice with deficient immune systems to allow human tumor engraftment. We use the least invasive method of tumor seeding possible in the least immunodeficient strain to allow the most efficient engraftment of the tumors. We minimse the number of procedures and use the smallest volumes possible when injections are given. CD46 transgenic mice This immunocompetent mouse strain which expresses the human measles virus receptor CD46 is required for any testing of immune response or toxicity of oncolytic measles virus since mice are not permissive for measles virus infections. This model is the lowest vertebrate species possible to model this situation. Other models that are traditionally used to model MV pathology are primates which are the only animals other than humans that are readily infectable by MV. By using transgenic mice, we can avoid being asked to do this work in primates

Project	**154. Genomics of communication and social life in a songbird model**	
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)	Like humans, songbirds communicate through vocal signals which affect how they develop and interact socially. This research aims to understand the biological mechanisms: what biological processes are engaged in the brain, when songbirds experience salient vocal and social signals?	
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 research may reveal new principles of brain function, which could be useful in therapies for communication disorders and in evaluating animal welfare.	

What species and approximate numbers of animals do you expect to use over what period of time?	Zebra finches: 2226 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?	Animals may experience mild discomfort from blood collection, altered nest temperature, and/or from short periods of social isolation (mild severity, up to 48 hours). In one experiment, animals may experience prolonged social isolation up to 30d (moderate severity). Animals that are not euthanized for analysis of brain tissues and extracts will be returned to the breeding aviary.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	
2. Reduction Explain how you will assure the use of minimum numbers of animals	We evaluate the statistical power of each experiment when it is designed, and use the minimum number of animals needed to draw meaningful concluions from the results.
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.	Communication through learned vocal signals only occurs in humans, songbirds and a few other animal groups (which are more difficult to study, e.g., dolphins). As a hardy, domesticated species, the zebra finch has emerged as the dominant songbird in laboratory research. Most of our work involves simple breeding and observation in naturalistic conditions; when regulated procedures are required, the animals are closely monitored for their welfare. Our objectives include the development of refined research procedures to avoid or mitigate effects of social isolation.

Project	**155. Germline genetic inheritance and reproductive 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 overarching goal of our research programme is to improve reproductive health and prevent transmission of disease in humans through the application of basic science and translational research. The proposed research consists of two areas of investigation: 1. **What are the causes of the decline in fertility during female ageing?** A high proportion of eggs ovulated by older women contain either too many or too few chromosomes (thin strands of DNA), resulting in a high incidence of infertility, miscarriage and birth defects. This is due to errors occurring in the equal chromosome segregation required	

	during the first division of the egg. Our previous work in mouse eggs have shown this to be due to a reduction in proteins essential for holding the chromosomes together, until the exact point at which they are required to divide. We want to investigate further how these proteins are lost, so we can then look as to whether it is the same in human eggs. 2. **Development and optimisation of IVF-based techniques to reduce the risk of disease in children of women who carry mitochondrial DNA mutations.** Mitochondria are tiny organelles which produce most of the energy in our cells. Mitochondria contain their own DNA which is passed from mother to child. Mutations in mitochondrial DNA can cause life threatening diseases, the severity of which depends on the levels of mutated to normal mitochondria, which can vary widely in an affected woman's eggs. We aim to address this problem by developing technologies to prevent the transmission of mitochondria from mother to child. We have pioneered the development of pronuclear transfer (the transfer of the genetic information from an egg with high levels of mutated mitochondria, to a healthy egg from which its own genetic material has been removed) to prevent transmission of mtDNA disease. We have been granted an HFEA licence to offer the technique in clinical treatment. A major goal of the next phase of the research is to further improve the efficiency of the procedures in establishing pregnancies and to increase efficacy in preventing transmission of mitochondrial DNA disease. For this we will need a better understanding of mitochondrial biology in eggs and early embryos. We plan to use mouse eggs and embryos to develop and optimise these techniques.
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 programme of work outlined here will add to our knowledge of reproductive biology in mammals and has the potential to lead to ground-breaking treatments to reduce reproductive risk for older women and to improvement of treatments for women affected by mitochondrial DNA mutations.

What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse reproductive cells and tissues to understand basic mechanisms and to develop new techniques. We estimate that we will require 5000 females and 500 males during the 5 year project. A number of experiments involve the use of genetically altered mice, which we will breed. However, only those with the correct genotype will be suitable for use in 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?	We will breed and maintain genetically altered mice. The genetic modifications we propose to study are generally confined to the reproductive cells. Apart from infertility, it is highly unlikely that there will be an adverse effect on the general health of animals. New strains will be monitored for any such effects. For investigations into ageing females may be aged up to 18 months. Aged mice will have extra health checks to ensure their well being. Genetically altered and wild type females may undergo drug induced production of multiple follicles by hormone injection in order to produce more oocytes or embryos. Mice may experience some discomfort for only a small period of time. Some genetically altered strains will also require injection mediated gene disruption. In general, our experiments involve minimal interventions, which involve minimal suffering. However, on some occasions we may also use animals as embryo recipients (with vasectomized males), or for ovary transfer for ageing studies. These procedures will involve surgery. Animals will be killed by a humane method at the designated establishment, and tissue taken for study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The production of reproductive cells (eggs and embryos) in the lab is a mayor challenge. The possibility of generating eggs from embryonic stem cells is an area of intensive research to which we will continue to pay close attention. Although promising, current techniques still involve the use of animals for some steps in the procedure (Hayashi, et al., 2017) In addition, the

	stages of eggs development particularly interesting for our studies have not yet fully been replicated in the lab (Nagamatsu, et al., 2019 and Shimamoto, et al., 2019).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our core approach is based on live cell imaging of individual eggs and early embryos, which have been manipulated to either overexpress, or silence expression of relevant proteins. This approach has the advantage of yielding data from individual eggs/embryos. Where knockout (genetically modified strain in which a gene is inactivated) mice are used, we will minimise the number of mice required to maintain colonies by creating banks of frozen embryos. The estimated number of mice used is based on our current experience of designing these type of studies, such as knowing how many cells are needed for an experiment and knowing the average number we can get from one mouse. Our experimental design involves obtaining data from a minimum of three animals per experiment with data from ~30 individual oocytes per experimental group. However much of our work involves development of new approaches and techniques and the number of mice required in the development phase can be difficult to predict. As a rough estimate, we would use 10-20 mice for the development of each new experimental procedure. Using drug induced production of multiple follicles for oocyte (especially in ageing studies) and embryo harvest increases the number harvest from each mouse, and therefore reduces the number of females required. Where at all possible, we co-ordinate experiments such that tissue from one mouse can be used for more than one of the objectives
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	Mouse is the model of choice because it is by far the best characterised model of the formation of eggs and sperm, and early embryo development in mammals. The processes by which eggs and embryos develop are similar to

general measures you will take to minimise welfare costs (harms) to the animals.	those observed in humans. They generally produce large numbers of eggs and most of our work involves generating data from individual eggs and embryos. This allows us to minimise the number of mice used in our studies. A major advantage of the mouse model is that, as in humans, egg quality declines with advancing female age. Some of our work therefore involves ageing females up to 18 months. During this time, mice are subject to extra monitoring to ensure that they are in good health. Overall, our experiments involve minimal interventions, which involve minimal suffering. The majority of our work does not require any interventions up to the time when the mouse is humanely killed. Some experiments involve injection of hormones to enhance embryo production to increase the number of eggs available from aged females. Following this animals will be humanely killed before taking eggs and embryos. In the case of genetically modified mice, we sometimes inject a drug that enables us to silence the gene of interest at a specific stage of development. In addition, we may need to perform surgery for embryo transfer or ovarian transplantation. Pain relief and post-operative care will be given for experiments involving surgery. Recent refinements to our procedures arose from the finding that the number of eggs obtained after giving hormone injection to young female mice was the same as those who had not been injected. Therefore, we have mostly abandoned this procedure, except in the case of experiments involving aged mice.

Project	**156. Glial roles in brain plasticity**	
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 project will investigate the role of a cell type called astrocytes in controlling how nerve cells in the brain communicate with each other. Sensory information (touch, pain vision) is transmitted to the brain and deciphered by nerve cells which signal to each other by connections called synapses. This happens in the outer part of the brain called the cortex. The connections change depending on the sensory information. This ability to change is called “plasticity”. For instance, a large part of the cortex of rodents is devoted to information transmitted from their whiskers, and cutting whiskers causes changes in the connections between nerve cells. This as an example of “plasticity”. Scientists believe that these changes are similar to those that underlie	

	learning and memory and those that happen due to stroke or limb loss in people. It is also known that in Alzheimer's Disease there is a problem with the way that these plastic changes happen. We aim to study how astrocytes are involved in cortical plasticity, which happen between nerve cells, and also what sort of changes happen to astrocytes.
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)?	Understanding the mechanisms of plasticity is essential as there is hope that being able to manipulate plasticity when there is damage would help us to treat conditions that affect the brain such as epilepsy, blindness, deafness, phantom limb pain and memory deficits in diseases such as Alzheimer's.
What species and approximate numbers of animals do you expect to use over what period of time?	Around 2500 mice and 500 rats will 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?	The project will involve some cranial surgery under deep anaesthesia, the placement of recording electrodes in the mouse brain, and the expression of light activated proteins. Following surgery, the animal will be given analgesia and following a period when the proteins express in the brain, the animal will be placed under terminal anaesthesia and after death slices will be taken from, or recordings will be taken while the animal is alive. No adverse effects of pain and suffering are expected since procedures are conducted under general anaesthesia. Recording from already implanted electrodes will not cause pain and suffering. Moderate severity level is expected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	These studies are aimed at understanding the mechanisms underlying plasticity, particularly in the cortex, which is the part of the brain important for consciousness. Because it is complex structures like the cortex that undergo plasticity in the brain, it is not possible at present to reconstruct the connections and complex architecture of the

	brain in culture. Also, the aim of the study is find out how plasticity changes when input from the outside world changes. These changes must take place in a living animal before the cells can be investigated. It is therefore necessary to use mice and rats.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have discussed with statisticians and performed calculations to determine the minimum number of experiments required for our experiments. In addition, many brain slices will be taken from the same animal and different methods combined in the same experiments to increase the quantity and validity of the data. The use of viral expression of proteins greatly reduces the number of animals used compared to generating different transgenic mouse colonies expressing different proteins. In vivo recordings of the same brain region in the same animal over time reduces the number of animals used and provides quality data. Repeated recordings will not cause distress to the animals since in the case of head restrained recordings the animals will be able to “move” since the supporting sphere or roller will enable the animal to behave as if it was moving across a surface. In the case of freely moving imaging and recording, animals will have wires attached to already implanted probes but will be able to move freely around their cage environment and have free access to water and food.
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.	Because the studies are aimed at finding out about cortical plasticity they must be conducted in mammals since animals such as invertebrates do not have such brain structures. The most appropriate species are the mouse and rat because a large amount is known already about plasticity in these animals, and there are long established methods for generating plasticity which we will also use. Mice will be used because this is the main model where transgenic variants are available, that is animals which have been engineered to not have a particular gene or have had a human gene inserted – enabling us to more accurately study human disease.

	Rats will be specifically used for absence epilepsy studies because the rat strain GAERS is the most studied absence model since it expresses most human symptoms. In other studies we will also use rats, partly because the rat brain is larger and it is sometimes more efficient to record from specific areas using certain methods, but also because it is important to verify our findings in different species, if findings happen in more than one species there is a better chance that they may also happen in humans. Our results will therefore build upon and advance existing knowledge. All surgical techniques will be conducted with general and local anaesthesia, and following this animals will be monitored for distress, though in our experience we have never observed distress following procedures.

Project	**157. Haematological development and functional characterisation in tumour models**	
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 investigate how parts of a cell's genetic instructions (found on DNA) can influence the development and function of the cells that circulate in the blood such as immune cells, red blood cells and platelets. The cells are very important for normal health and in fighting infections and it is now becoming evident that they can be used to target cancer. When their development or function goes wrong this can result in an inability of the body to generate certain cells and defects in fighting infections (immunodeficiency) or a lack of control which results in the body attacking itself (autoimmune	

	conditions). Through large human sequencing studies, such as 100,000 genomes run with the NHS, we are beginning to identify more parts of DNA that could be responsible for these conditions and be important for development and function of these cells. However, even if we identify these regions from human studies we need to be able to confirm that these are the cause and to then understand how they regulate these processes. In this work we plan to help identify these parts of DNA and gain insight into how they can regulate the development and function of blood cells. One particular area of interest we have is how these cells function towards cancer cells and if we can manipulate this to develop new therapies. We will investigate this using a model of primary tumour growth and also a model of when tumours spread to other places around the body, so called secondary tumours.
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)?	One benefit will be to provide evidence to confirm or eliminate particular parts of DNA as being causal for human diseases such as immunodeficiency or autoimmune. This will help in designing screening tools to diagnose these conditions in the clinic and by understanding how a particular alteration can cause a disease could eventually aid in the development of new therapies. For other scientists these studies will provide information regarding the function of some of these regions within blood cells which will allow for additional follow up studies. We will also generate large datasets and mouse lines that other scientists can use rather than generate their own. Investigating the interaction between blood cells and cancer might lead to the identification of new ways to help a patient's own cells fight off cancer cells which could be developed into new cancer therapies. This could be in making ways to make a patient's cells survive and function better within a tumour, identifying ways to boost their function towards killing cancer cells or finding new ways in preventing them from being converted into cells that dampen the activity of other blood cells.
What species and approximate numbers of animals do you	We plan to use mice in this study and up to 72,000 over 5 years.

expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	As we will be generating genetically altered mice (where part of the DNA has been affected) approximately one third of the mice we will use is in breeding to be able to perform our experiments. These mice will have small pieces of tissue taken to allow us identify them and determine if they carry the mutation we desire. On occasion we may take small blood samples from them (typically the tail) and these are classified as mild severity. In another one third we will analyse the mice that we have generated and we shall take blood samples, and after humane killing, examine tissue samples. Some of these mice will have the function of their immune system checked, this will be achieved by injecting the mice with substances such as vaccines or cells, prior to analysis of their blood and tissues after humane killing. As these blood cells move around the body we may alter this either by injection or placing substances in the food or drinking water. For some of these mice we may collect larger blood samples when the mice are deeply anaesthetised prior to increasing the anaesthesia administered and humanely kill and/or flush their body with saline to remove all the circulating blood from tissues to enable us to study the cells within tissues as a method of humane killing. All these procedures are done under deep anaesthesia and are classified as mild severity. After collection of blood samples from the tail on rare occasions mice may develop an infection or bad scarring, in this case they will be humanely killed. The final third of mice will undergo procedures that are considered to be of moderate severity. Some of the mice will have their blood cells replaced by performing a bone marrow transplantation, here a host mouse is exposed to a source of radioactivity which kills all their blood cells, which we then replace with cells harvested from another mouse. These mice are briefly susceptible to infection while their blood cells recover and so we give them and enhanced diet to assist recovery and ensure they are healthy prior to this procedure. We will collect blood samples from these mice to monitor the recovery of the blood cells and analyse tissues after they are humanely killed. On very rare occasions they can develop sickness related to the procedure and

	will be humanely killed if their condition does not improve. We know some of the factors that can give rise to this sickness and mice destined for this procedure are subjected to extra health checks. The remaining mice will be used in our cancer studies and will be injected with cancer cells to their side to generate a small lump or into their tail vein to give rise to small masses in the lungs and/or liver of the mice. Some of these mice will be briefly anaesthetised so that we can look at the cancer cells via imaging methods and we can track how they develop. The small lumps on the side of the mice are measured at least every other day to track their size and mice are humanely killed when they reach 1.2cm2. A subset of these mice will be used in our therapy studies and we will investigate ways to target cancer cells. This could be achieved via administration of purified mouse or human immune cells (which we may manipulate in the laboratory) or substances to boost the function of immune cells for example an anti-cancer vaccine or treatments that are currently used to treat patients. When the mice reach the endpoint of the study, defined by a humane endpoint such as tumour size or timepoint, they will be humanely killed prior to tissue collection. Some mice could have small blood samples collected while they are alive so that we can track the effect of a treatment or larger volumes collected after they are humanely killed. Mice typically do not show any altered behaviour when administered cancer cells, however on some occasions they may not have control of the cancer cell growth and while we can easily monitor those where the tumours are on the side of the mouse for the lungs this would be evidenced by rapid breathing and the mice humanely killed. We select the cancer cells to be administered to be the most suitable for our experiment which give rise to masses that are well tolerated. Very rarely the mice may scratch at their lumps causing the skin to be broken and if this is observed the mice are humanely killed. Some of the immune system treatments, as they are designed to provoke the immune system, can give rise to symptoms such as increased temperature and diarrhoea. This will be closely monitored and when it exceeds certain thresholds the affected mice will be humanely killed. The models and experimental protocols that we are using are well established and designed to
	cause the least pain, suffering and distress.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The cells of the blood system and their interaction with tumours is very complex and requires the interaction with other cell types for which it is not possible to use a non-animal alternative. We will use existing data sources rather than duplicating where these exist and also harvest additional tissues for use in alternative lab-based experiments where possible. Should new lab-based or computer based models exist which generate comparable data these will be adopted.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When a suitable mouse line exists we will import the line rather than generate a new line. All lines that we generate in this study will be archived in a suitable international repository to be used by other interested researchers. The gene expression data that forms part of the identification of parts of DNA that we are interested in will be shared in open access repositories to enable other researchers to use our data rather than generate additional data sets. We will use various statistical approaches to determine the minimum number of animals to use in an experiment and where possible combine experiments. We will also harvest additional tissues from mice at the end of experiments to use in laboratory-based assays to reduce numbers needed. Proper design of the experiments and controlling for sources of variation such as the age and sex of the animals will also help increase the robustness of the experiment and result in an overall reduction in animals needed. When publishing our data we will follow the ARRIVE guidelines to ensure comprehensive reporting and will release all data including where we do not find any alterations as this can be as informative to prevent assays being repeated in other laboratories.
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	Mice represent the best choice of animal for these studies given the similarity between the human and murine immune system. Furthermore, they are very amenable to genetic manipulation allowing us to perform the studies we plan. The use of mice in cancer studies is well established

the general measures you will take to minimise welfare costs (harms) to the animals.	and the models we plan to use are considered 'gold standard' and are used in the development of therapies that are now used in the clinic and resulting in good responses in some patients. In all our experimental work we will minimise the number and severity of procedures applied to the mice, this could be via the selection of the substances that we administer causing the least number of side effects, administering substances together if possible, or switching to oral administration in diet/drinking water to minimise injections. We will use cancer cells that are fully characterised and will investigate alternative methods to monitor the growth of the cancer cells in the mice to minimise stress from handling. When we are performing experiments requiring mice to be anaesthetised for a period of time we will use agents that allow for a rapid recovery and that have minimal build up. Mice will also be kept warm while asleep and if they are asleep for a while administered fluids to help speed up recovery and reduce dehydration. When we perform bone marrow transplantation mice are thoroughly health checked to eliminate those who may not tolerate the process as well. They are also provided with wet mash to aid recovery and antibiotics to reduce the chance of infection. We will use published data to define the best agents to use in our studies, picking those that are the most clinically relevant where they exist. The use of a sophisticated mouse tracking system allows accurate tracking of all health concerns associated with the mice to be used in this study and to enable rapid investigation where they occur at a higher than expected incidence. All people who work with the mice in this study are thoroughly trained and continuously assessed for their ability to perform these procedures, with procedures refined following advice from the vet/NACWO or other international guidance.

Project	**158. Hormonal regulation of hippocampal synaptic function in health and CNS 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 key objectives are: 1). To examine how communication between brain cells is regulated by the hormone leptin and how this alters during development and ageing. 2). To examine whether leptin and related molecules might be therapeutically active in preventing molecular and behavioural changes in animals that model human Alzheimer's disease.	

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 increase our understanding of how brain cells communicate with one another, and how this process is regulated by hormones like leptin in the healthy brain This study will also examine the therapeutic potential of using leptin and smaller leptin-like molecules in models of Alzheimer's disease; a devastating and incurable brain disease. This study could ultimately lead to the identification of novel agents to treat Alzheimer's disease.
What species and approximate numbers of animals do you expect to use over what period of time?	This study will use rats and mice. We expect to use 1550 mice and 750 rats over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We study the electrical activity of brain slices or cell cultures harvested after mice or rats have been killed humanely. Altered leptin signalling in rodents can be associated with obesity and excessive water intake and consequent urination. Changes in the opposite direction can lead to weight loss. We shall observe closely for signs these events and animals will be killed humanely before they can cause significant welfare harms. At the end of experiments all animals will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of mice and rats is appropriate as the cellular events underlying learning and memory are well characterised in these animals. There are currently no non-animal alternatives or models systems that mirror these cellular processes and/or complexity of the brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We can prepare up to 12 brain slices or cell cultures from one animal. For most experiments, we will need samples from 5-6 animals but, by coordinating the work carefully, we can run several experiments simultaneously, thus reducing the overall number of animals required.

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 rats and mice are the most appropriate species as the functioning and complexity of the brain are not only well characterised but also very similar to the human brain. To ensure there is minimal welfare cost to animals, the health of all animals will be monitored routinely to ensure no significant deviation from normal health. All animals will be killed in a humane manner in order to obtain brain tissue. The available rodent models from Alzheimer's disease do not appear to involve any significant overall welfare issues.

Project	**159. Host, pathogens and microbiota interactions in mice.**	
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)	Diarrheal disease is the second leading cause of death in children under five years of age. Multiple microbes may be responsible for these infections, but the majority of diarrheal cases are associated with bacteria called Escherichia coli and Salmonella. These bacteria can be found in contaminated water and food. After they have been eaten, they colonise the gut, cause inflammation and stop food absorption leading to malnutrition, which can be fatal, especially in children in the poorest countries. Today, mechanisms used by the bacteria to	

	infect the host are not well characterized because most of the findings using in-vitro cell culture assays do not correlate with what is happening in the host. The main aim of the project is to gain a better understanding of the infection strategies used by these microorganisms so that effective treatments and prevention strategies could be developed and tested. The project is divided into three interrelated research streams: • Investigation to define virulence factors implicated in disease • Evaluation of the efficacy of new vaccines • Determination of the health benefits of the microbiota.
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)?	Infectious diseases are major causes of morbidity and mortality worldwide, especially in developing countries. This study will contribute to the improvement of health on several levels. First, we will determine the mechanisms of mucosal bacterial infections. Those mechanisms involve complex interactions between the host and the pathogen as well as a complex immune response mounted by the host to combat the pathogen. By using an in vivo system, we benefit from monitoring the immune response throughout the whole infection cycle and are not restricted to a short-term infection of a few hours as limited by an in vitro model. This is a prerequisite for the development of effective and rational control measures and for the identification of novel anti-bacterial drugs. Second, we will develop, and test vaccines based on key pathogenic proteins secreted by bacteria and novel bacterial vaccine delivery systems. This vaccine can prevent the disease in children but also reduce the colonisation of animal minimising the risk of infection from contaminated water and food. Third, we will determine the health benefits of probiotics during infection. Consumption of probiotic products by adult humans is increasing, although the health benefits are less defined. We will assess the effect of probiotic treatment, either before or during challenge with pathogenic bacteria, on the wellbeing of mice, using the same criteria as those used for evaluation of vaccines.

What species and approximate numbers of animals do you expect to use over what period of time?	We will used around 7000 female 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?	In the context of the enteric infection, the most severe adverse effect for the animal is diarrhoea, dehydration and loss of weight. In the context of the acute or chronic inflammation induce by DSS, the most severe adverse effect for the animal is blood in the stool, dehydration and loss of weight. The mouse models have been well characterised and clinical signs of infection are readily recognisable as will have moderate severity limit. Care will be taken to minimise suffering and the human endpoints described will be strictly adhered to. Possible, rare, adverse effects include damage to the trachea, oesophagus, or airways during oral gavage, there is a risk of weight loss due to the infection. In addition, repetitive anaesthesia can lead to stress and non-recovery. We monitor the animals closely and mitigate the adverse effect as much as possible. Animals may be killed by exsanguinations under non-recovery anaesthesia, followed by cervical dislocation (schedule 1) or by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of animals is an unavoidable consequence of studying the complex physiological processes involved in models of human infection. We study the role of proteins released by gut bacterial pathogens in infection; this involves generation of alterations (mutations) in the gene of interest. While it is essential to obtain full characterization using cultured cells in the laboratory (*in vitro*), the true role of a protein in infection can only be revealed using relevant animal models (*in vivo*). Generally, in host pathogen interaction scientists find that in vitro infection data do not reflect the in vivo reality of the infection. Even though in vitro models are primed tools to study cell biology, it is not a true model of host pathogen interaction and this is where the differences are. Indeed, it has been

	shown that when models of infection strategies developed solely *in vitro* can lead to misleading and unrealistic conclusions. We use a defined set of criteria to assess the role of bacterial protein in infection. When possible and appropriate, we use bioluminescent bacteria that allow us to study colonization dynamics and tissue distribution while reducing significantly the number of animals. Better understanding of how virulence factors function *in vivo* provides a rational for development of specific treatments and vaccine. As far as vaccines are concerned there are no alternative but to test them in animal models as, by definition, their efficacy can only be assessed in the context of a complex immune response found only in animals and testing the effect of probiotic bacteria can only be done using animal models; there are no other alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use a bioluminescent imaging technique to reduce the number of animals used for testing and adhere closely to the 3Rs principles. Application of this technique allows us to pre-screen the kinetics the colonisation and the localisation of the bacteria over time and decrease the number of animals used from 20 to 5. Pilot experiments will routinely involve 5 animals per group and per time point. The aim of the pilot experiment is to test if we can observe a phenotype and will serve as a proof of concept. We will only be carried out them when a minimum of two bacterial strains can be tested in parallel with positive and negative control groups. The pilot experiment will be exploited to the best by collecting data for a maximum of scientific parameters. Several mutated bacterial strains are routinely tested in the same experiment so that positive control mice and negative control mice (uninfected) can be shared, thereby reducing the number of animals. All experiments are repeated two to three times (including pilot experiment) to achieve scientific validation and to ensure reproducibility.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse models described have been well characterised and clinical signs of infection are readily recognisable. One of the pathogens we are using, is a mouse specific pathogen which can only infect mice but has the same virulence factor than the close related human pathogen. It is one of the best enteric models as it recreates the same infection process than in infants. The other alternative to study the human enteric pathogens is to treat the mice with antibiotics to disrupt the gut flora of the animal increasing the suffering and stress of the animal. Every injection will use the most refined route of administration known in the literature. After infection, mice are monitored daily for visible signs of illness (cachexia, ruffled fur etc) and weight loss. Care will be taken to minimise suffering and the human endpoints described will be strictly adhered to. The main bacterial infection model we use is self-limiting and the severity is moderate.

Project	**160. How does innate immune activation cause neurological damage?**	
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 immune system fights against a constant barrage of viruses and bacteria to keep us healthy. In some people, the immune system goes wrong and starts to fight against itself – this is called autoimmune disease. Systemic Lupus Erythematous (SLE, or lupus) is an autoimmune disease. Most patients with lupus make too much interferon-alpha (IFN-α), which is has extremely potent antiviral effects. However, when too much IFN-α is being made when there is no viral infection, then it causes severe damage to many human organs, including the brain. Brain disease in lupus is an	

	understudied area and how it develops is not well understood. Previous research has shown that IFN-α can damage the tiny blood vessels that run deep inside the brain. These 'microvessels' are essential for bringing nutrients and oxygen into the brain and for removing waste products. Our research focuses on how IFN-α leads to blood vessel damage, with the aim of being able to prevent it in patients with lupus. Whilst lupus is a rare disease, insights from this research will also have implications for brain disease that occurs because of blood vessel damage in the ageing brain, e.g. vascular dementia. Current mouse models of lupus are complex and development of brain abnormalities in these mice is not well understood, therefore in the course of this work we will develop more defined models to help our understanding of the disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By understanding how IFN-α affects the brain, we may be able to identify medicines to treat diseases which involve the production of too much IFN-α. This project also aims to increase out understand in an area that is currently not particularly well understood. Mouse models developed in this project will be of wider interest to researchers studying autoimmune disease. Outputs from this project will be published in order to widely disseminate any findings, and will be shared with researchers and clinicians at conferences.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, approximately 3200 mice for use in breeding, maintenance and experiments 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?	We will be making and using mice that have been engineered to make too much IFN-α only when we 'switch the gene on'. These mice will be maintained as far as possible with the gene switched off. When we activate the gene for experimental purposes we expect to see some adverse effects due to the detrimental effect of IFN-α, which will be moderate in severity. These

	will increase with age and mice will be closely monitored throughout the experimental period. At the end of the experiments, mice will be culled by a humane method and tissues will be taken for analysis after death. A small proportion (less than 10%) of mice will undergo further experimental procedures in order to understand more about the effect of too much IFN-α. Drugs/compounds administered to interfere with IFN-α signalling or to allow us to see biological processes (e.g. blood flow) are not expected to cause any adverse effects and the injection route is not expected to cause any more than temporary discomfort. Blood samples may be taken from mice in order to look for changes in blood cell types over time. Blood sample volumes and the blood sampling procedure will be done to ensure minimal risk of adverse effects. Pressure is applied to the injection site to stop bleeding
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives have been used in pilot studies to investigate this problem. We can culture blood vessel cells, brain cells and immune cells but the interaction between these in a 'real world' environment is missing. Since lupus is a complex disease, the next step in understanding the disease is to develop an animal model which will complement previous and ongoing work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimenter and animal house staff will be blinded to genetic status by using a coded system for cage labelling. Blinding will avoid confounding and bias that would require a higher sample number
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.	This model is an advance on what is currently available to model lupus, it is a simpler system so the results will be less variable and more reproducible. Mice will generally be maintained with the gene switched off which will reduce any adverse effects to mice outside of specific experiments. Within experiments, mice will be closely

	monitored for signs of distress and if this occurs they will be culled.

Project	**161. How does sinus node disease maintain atrial fibrillation? A study of electrical and structural changes 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)	Atrial fibrillation is the commonest sustained heart rhythm problem and leads to reduced quality of life and risk of stroke. It is harder to treat when the normal pacemaker of the heart (the sinus node) is also diseased. This project aims to investigate why sinus node disease makes atrial fibrillation harder to treat and to take steps towards developing personalised treatments for these patients.	

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)?	Improved understanding of the interaction between sinus node disease and atrial fibrillation will allow us to be able to design different treatment techniques to improve the outcomes in these patients. It will also provide additional knowledge that will allow us to take steps towards testing gene therapy for the treatment of these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to 20 goats over 4 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 of the animals will have a neurostimulators or pacemaker device implanted under general anaesthetic. During recovery from this operation they will suffer moderate levels of pain and discomfort which will be controlled using painkillers. The device will be used to cause atrial fibrillation in 8 of the animals and, depending how they respond some of these animals may feel out of breath. If this happens they will receive treatment from a Vet. After 12 weeks, the animals will have a general anaesthetic and the electrics of the heart will be measured in detail. While still asleep they will be humanely killed and the heart will be removed for further microscopic analysis. During the course of the study, if any of the animals are seen to be in pain, or distress that can not be treated they will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Computer models of atrial fibrillation are under development, but the heart rhythm disorder is not completely understood and one of the aims of this project is to advance this knowledge. We already work with a computer modelling group to develop computer models of heart rhythm disorders and the data from this research will be shared with the computer modelling group to help further advance these models. Some research will be done on humans during surgery or cardiac procedures, but to understand the causes of the electrical

	abnormalities tissue samples need to be analysed for scarring or disarray and it is not possible to take these samples from humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental design has been discussed with a statistician to ensure that the minimum number of animals will be used while ensuring sufficient statistical power for meaningful results.
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 of complex heart rhythms like atrial fibrillation require study of a heart that is of a similar size to humans so results are translatable. This is because the nature of heart rhythm patterns are affected by the size of the heart, so results from small animals are not as relevant. Prior studies of atrial fibrillation in goats and sheep have detected electrical signals that are very similar to those seen in humans and have advanced the understanding of human atrial fibrillation. The number of procedures performed on each animal is being kept to an absolute minimum and any painful procedures performed during this study will be performed under anaesthetic and with rigorous pain relief as advised by a veterinary surgeon. The goats will be kept in an environment where they will be able to express their normal behaviour so they will have the facility to climb and to live in groups. The animals may occasionally need to be on their own for short periods, but this will be kept to an absolute minimum. They will be frequently monitored for signs of distress or illness and will be treated promptly should these be observed.

Project	**162. Identification and validation of novel therapeutic targets in human 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 main purpose of this project is to assess the effect of chemical inhibitors, either administered alone or in combination with radiotherapy and chemotherapy, that interfere with cellular processes critical for the growth of rare cancers affecting young people: neuroblastoma and adenoid cystic carcinoma. A further aim is to understand the pathogenic role of a specific form of DNA mutation in a rare form of infant leukaemia**.**	
What are the potential benefits likely to derive from this project	Neuroblastoma. This is a childhood cancer affecting very young children and infants in	

(how science could be advanced or humans or animals could benefit from the project)?	which about half of the patients die. Current treatment approaches cause distressing side effects in patients of a young age. We are therefore focusing our attention on assessing the efficacy of less toxic drugs previously untested in neuroblastoma. Adenoid cystic carcinoma is a head and neck cancer with a horrible prognosis that responds poorly to all common chemotherapeutic drugs. We have recently identified chemicals used in clinical trials for other tumour types that inhibit adenoid cystic carcinoma cells in non-animal experiments. We would like to establish an animal model of this cancer and assess whether the newly identified drugs cause tumour regression, offering new hope to cancer patients. Leukaemias come in different forms and the subtype we are studying occurs in children and has a terrible outcome. A major problem is the lack of patient samples since the tumour is extremely rare. Furthermore, we have no means of testing new therapies since we lack the scientific knowledge of the molecular targets in specific subtypes of leukaemais. Studying the DNA alterations found in this cancer will be beneficial because we and others could use this knowledge to test therapeutic approaches.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use about 600 mice in 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 mice will undergo a number of different procedures during the various experiments none of which should cause more than moderate discomfort. We will inject tumour cells under the skin of mice to induce the growth of small tumours, after which the animals will be dosed with drugs that have been previously used in rodents, or are already in clinical use, thus minimising the risk of unexpected side effects. Treatments with the potentially toxic chemotherapeutic drug cisplatin will be conducted after trials on small groups of animals, which will only undergo transient and moderate discomfort. There is some unavoidable discomfort for the animals when they will be injected with tumour cells and substances, but this will be controlled by use of

	small needles. The mice will be humanely killed when the identified endpoints, chosen to minimise the suffering of the animals, are reached. Therefore, the expected level of severity is moderate or mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cancer development is regulated by intrinsic and extrinsic factors. Single features of cancer cells such as their ability to proliferate, invade and respond to drugs can be studied using methods alternative to animals. We have used and will continue to use these in vitro analyses as proof of principle studies before starting an animal experimentation. However, the full complexity of cancer growth can only be replicated using an animal model, since contact with the tumour microenvironment and the host immune system is often critical for tumour growth. The host-tumour interaction, which is a fundamental aspect of cancer growth, cannot be adequately investigated in vitro. The focus of our research is translational and it is essential to demonstrate that the potential therapeutic substances previously validated in vitro are effective in the context of a living organism. This step is required before any drug is considered for clinical trials in human, making the use of animals a necessity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise animal wastage, prevent the unnecessary use of animals showing adverse effect and to ensure numbers are inextricably linked to research requirement, the project licence holder will: ● Ensure high standards of animal care, welfare. ● Ensure that Personal Licensees working on this project are appropriately trained and suitably competent to enable a high success rate to be achieved and thus minimise the number of animals used. ● Ensure that statistical methods will be implemented to calculate numbers required to obtain significant results while

	at the same time minimise the number of animals.
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 suitable species because there are already many mouse models of human cancer in existence, because the immune system is highly analogous to the human system, there is high degree of structural homology between human genes and their mouse counterparts and because extent and effects of tumour growth is readily measurable. The mouse model of human cancer that will be used in this PPL are widely used by the scientific community and considered a good approximation to human cancer. Animal suffering is minimised by limiting upper tumour size to 12mm maximal diameter in subcutaneous tumours. Furthermore, no animal will be allowed to develop overt leukaemia

Project	**163. Identifying new treatment strategies for lymphoma**	
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 overall aim of this project is to identify and evaluate new treatment strategies for lymphoma.	
Why is it important to undertake this work?	Lymphoma is the fifth most commonly diagnosed cancer in the U.K. While many lymphoma patients are cured with chemotherapy (anti-cancer) drugs, they can experience significant long-term effects from the treatment which include reduced fertility, heart failure, increased risk of infection and of getting another type of cancer. We are trying to identify new treatments to attack lymphoma cells and the non-cancerous cells that support them. By targeting specific genes or proteins important in lymphoma growth, we hope to find new treatments that work without the toxic side-effects.	
What outputs do you think	Lymphoma patients are currently being treated with anti-	

you will see at the end of this project?	cancer drugs that can leave patients with long-term side effects. We hope to provide lymphoma patients with smarter anti-cancer drugs that reduce their risk of these side-effects. We will share our findings at national and international conferences, through collaborations with other researchers, and by publishing in peer-reviewed journals.
Who or what will benefit from these outputs, and how?	The short-term impact of our outputs is to add to the knowledge of the alternative ways lymphoma can be treated. In particular, our studies in REDACTED (could identify Establishment) protein, in which there is currently little known about its mechanism and its role in diseases such as lymphoma. In the longer term, we hope to provide these alternative treatments to lymphoma patients who don't respond to current treatments and to provide less toxic treatments for those who do currently respond to the cytotoxic chemotherapies being used.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	To maximise the outputs, we are collaborating with different groups internal and external to our organisation that will lend their expertise and knowledge to our work. We will publish our work in peer-reviewed journals and present at conferences to disseminate new knowledge including reporting of unsuccessful approaches. We will also share best practice of new techniques and protocols we have developed and validated to collaborators.
Explain why you are using these types of animals and your choice of life stages.	Mice used are aged 4-8 weeks of age at the start of the experiment which may continue for up to 12 weeks. At 4-8 weeks the mouse immune system has not fully developed so that they will not readily reject the implanted tumour cells.
Typically, what will be done to an animal used in your project?	Animals are injected with tumour cells just under the skin on the back of the mouse or through an injection in the tail vein, or by injecting cells in the abdominal area. The tumours can take between two weeks up to 12 weeks to form. The tumour growth is monitored by several methods:1) measurement of a visible tumour with calipers (a sliding ruler that "pinches" the tumour and provides precise measurements in millimetres); 2)measuring the amount of human cells from a small

	drop of blood taken from a superficial vein; 3)feeling the abdominal area for the presence of hard lumps. With some cells, we can monitor the tumour growth using a machine that will take live images of tumour cells that are tagged with fluorescent colour. Animals may be given anti-cancer drugs or placebo ("fake drug") by injection or by giving it orally through a long thin tube. Treatments will not exceed 6 weeks. Mice will be humanely killed at the end of the experiment. We will analyse their tumours and organs and blood.
What are the expected impacts and/or adverse effects for the animals during your project?	Mice will experience tumour growth which will not cause any pain but may cause animals to lose weight, have a less plump appearance, may sit in a hunched position, or may not act as lively as normal. These symptoms usually appear at the later stages of the tumour development and may last up to a week before animals are humanely killed.
What are the expected severities and the proportion of animals in each category (per animal type)?	About 90% of animals are expected to get lymphoma and may also receive anti-cancer treatments to see if the lymphoma tumours stop growing or shrink. In the other 10% of animals, we will find out if mice tolerate the doses of anti-cancer treatments we want to test in experiments using mice with lymphoma.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Patient samples used for experiments in the lab provide a snapshot usually at later stages of tumour development. We use lymphoma mouse models to study lymphoma development and to evaluate the effectiveness of targeting a specific molecular target with drugs. We will use non-animal methods to validate our treatment approach but these conditions are artificial. They do not give an accurate picture of the effects that targeted treatment will have on the growth of lymphoma especially at the doses you could give to patients. Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use. We will periodically survey current literature to identify
	new methods for replacement of animal experiments.

Which non-animal alternatives did you consider for use in this project?	Non-animal approaches we are not using – 3-D tissue culture of tumour cells, bioprinting, organ / human/ mouse-on-a-chip systems .
Why were they not suitable?	We have not been able to develop 3-D tissue culture of tumour cells as many of the tumour and non-tumour microenvironment cells do not survive. Bioprinting and organ/human/mouse-on-a-chip are technologies that we have explored but these require significant investment in time and money to validate as they are not yet accepted as replacements for animal experiments.
Enter the estimated number of animals of each type used in this project.	mice: 2700
How have you estimated the numbers of animals you will use?	We aim to characterise a maximum of 8 patient samples and 5 lymphoma cell lines each year which will require a maximum of 100 mice per year (total 500 mice). We aim to do about 10 treatment experiments per year and from power calculations at 80% power, we will require 400 animals per year (total 2000 mice). Estimating that we will test around 10 anti-cancer drugs each year, we will require 40 mice per year (total of 200 mice) for testing the safety and amount of drug we need to give mice to get the right level that will effect the tumour.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	We have used the NC3Rs' Experimental Design Assistant to design experiments and taken advice from the mathematician / bioninfomatics post doctoral researcher in our lab.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	We will use pilot studies to estimate variability and perform power calculations to calculate sample sizes. We share tissue and use tissue from previous animal experiments to answer questions we had not thought yet to asked. Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment. The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.

Which animal models and methods will you use during this project?	We will use mouse models to engraft human or mouse lymphoma cells. Mice with internal tumours have been refined through pilot studies where we are confident of window of treatment and experimental time point where most mice do not show any adverse effects. We use immune compromised mice in order to achieve engraftment and have found that we can humanise these models or inject human immune cells with tumour cells in order to study interactions with immune cells. These models are characterised in pilot studies to identify any unexpected adverse effects. In addition the pilot studies will help us to develop score sheets and robust humane endpoints so that harms can be contained within our need for a certain level of harm to answer the scientific questions.
Why can't you use animals that are less sentient?	Mice are the least sentient species that will allow us to achieve our objectives. The overall structure of the immune system in mice and humans are similar and mice will get lymphoma and allows us to test if a molecular target can be therapeutically targeted by treating through routes that are comparable to route of administration for humans.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Literature searches, attendance at vendor's information sessions, seminars and conferences to find out about new technology and new approaches that we could implement. We will periodically survey current literature to identify new methods for refinement of experiments.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	Through our experience and previous work, we have refined treatment doses and route of administration of four of our most commonly used anti-cancer drugs for lymphoma so that we know the maximum tolerated dose, the minimum effective dose, and sub-optimal doses. This minimises the possibility of adverse effects from chemotherapy. We have refined an immune competent model of lymphoma which has been well characterised and

	reliably engrafts with low variability. We will periodically survey current literature in order to keep up to date with any improvements in protocols and techniques which may reduce, replace or refine experiments using animals. We will publish all in vivo data in open access journals and in accordance with the ARRIVE guidelines for reporting. Animal welfare is a key consideration in all of our protocols and we will be guided by our NACWO and NVS in always ensuring that we are using best practice and the most refined techniques. All staff involved in animal experiments will review the literature on animal welfare provided by the local AWERB. Following every experiment and regularly during group meetings we will review our procedures from a welfare standpoint to identify any potential for refinement.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We will follow the Workman et al, 2010 for experimental design, best practice, and humane endpoints for cancer research in animals, and we will publish in journals that adhere to the ARRIVE guidelines published by the NC3R's, we consult Simon Bate's book, The design and statistical analysis of animal experiments, for experimental design, statistical analysis, and sample size calculations. Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment. The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.

Project	**164. Identifying the molecular and cellular mechanisms involved in tissue regeneration, stem cell activation and differentiation**	
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)	The purpose of this project is to understand the molecular mechanisms by which adult tissues maintain and repair themselves, from how cells become activated to how these differentiate and reinstall the original state. During disease, these mechanisms become deregulated. Therefore, understanding the underlying mechanisms of maintenance	

	and repair is crucial to improve our knowledge on the basics of disease, either tissue degeneration (e.g. in fibrosis), or cancer initiation and progression.
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 is expected to provide novel information about the properties on how cells sense tissue damage and respond to it by proliferating and differentiating and which of these mechanisms are deregulated in disease. Cell proliferation is essential for maintaining the homeostasis of tissues and for tissue repair. The balance between proliferating to repair a damaged tissue and finalizing that proliferation once the tissue is completely repaired needs to be tightly controlled to prevent the formation of tumours. This project has the potential to increase our knowledge on liver scarring and fibrosis as well as on tumour initiation and progression. Consequently, this project could lead to the discovery of new targets and therapeutic strategies for treating these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 4508 (2 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 expect 50% of the animals to experience mild discomfort. This will be related to intraperitoneal injections of inducing agents (e.g. the drug tamoxifen, used to induce deletion of specific genes) and will last less than 1 day. From the studies of tissue regeneration, we expect the adverse effects to be moderate. Mainly ~50% of the animals will be experiencing moderate discomfort due to the induction of a repair response in the liver. As we always aim at the tissue to repair, this will last no longer than 48h. To model human liver regeneration upon tissue resection following a liver cancer treatment, some animals (~10%) will undergo surgical

	procedures; e.g. removal of part of the tissue. These animals will experience a moderate discomfort that will not last more than 5 days, like a person would experience after surgery. Animals will be provided with pain relief to minimize discomfort, monitored daily and humanely killed if unexpected signs of discomfort arise. To induce tumour formation in the mice, we will be using systems that induce the tumour in the adult. This might result in moderate discomfort to the mice. Animals will be monitored daily and assessed for tumour development; e.g. gain or loss of body weight, sign of ill health (hunched posture, piloerection, etc.), jaundice (yellowing of skin). When tumour develops, the animals will be humanely killed before it can spread to other tissues. In all cases, animals will be humanely killed after the experiments, either after induction of a tissue repair response or after generation of tumours. We will analyse the tissue for the presence of particular genetic makeups by using molecular, histological and tissue culture techniques (e.g. appearance of tumours, or tissue repair after damage induction).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The necessary animal studies in this Project will exclusively involve mice. To date, there are no alternative methods to fully understand tissue regeneration, fibrosis and tumour initiation in the context of the whole organism and the study of these processes in human context is impossible due to ethical reasons. Nevertheless, this Program of work will make extensive use of the organoid culture technology (a technology in which cells are plated in a 3 dimentional matrix to mimic the tissue of origin) I have developed over the past 10 years, to address as many fundamental

	questions and screen as many compounds as possible without inducing any harm to animals. The use of this physiologically relevant culture systems allows this project to comply with the 3Rs (replacement(of animals), reduction(of numbers used) and refinement(of techniques to minimise harms)) by keeping the mice numbers to be used to minimum.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As mentioned, we will make extensive use of 3D cultures in a dish for screenings to identify potential candidate factors prior to testing these in animals. Also, all procedures planned are very well established in my lab and don't require additional trial or 'pilot' experiments prior to full experimental studies. Again, the use of mice in this context enables reducing the number of animals that would be required than if we were otherwise setting up protocols in other species, as greater numbers of animals would be needed to first establish the test model before further experiments could be completed.
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 mentioned, all studies in this Research Program will involve exclusively inbred strains of mice from different genetic backgrounds. Liver regeneration has been studied extensively using mouse model, thus we will also use mice, as they are the best model available. The experiments will involve creating and analyzing genetically altered mice and performing liver regeneration studies following injury to the liver. Chemical injury to the liver can result in discomfort to the animals that will last less than 48h. We will use the minimal doses that give the effect we need for the experiment. Where possible, the drugs will be given orally, either supplemented on the diet or drinking water, to prevent any stress to the

	mice. However when this is not possible, drugs will be given by other routes including injection. Surgical removal of liver tissue under anaesthesia will result in discomfort post operatively but this will last no more than 5 days and will be minimised by the use of pain killers. We will always remove the minimal amount of tissue that can provide meaningful results. Any animal in distress that cannot be promptly alleviated will be immediately humanely killed.

Project	**165. Imaging development and disease in mice and zebrafish**	
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 study how multicellular intricate organisms develop from single cells. Our overall goal is to identify genes involved in early embryo development and determine what their role is.	
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 reason of this work is to provide basic understanding of fundamental mechanisms of embryonic development. With this knowledge we can potentially help refine medical practice to improve in vitro fertilization (IVF) success rates. Understanding how cells develop normally in the embryo may also help us understand the development of cancer cells.	

What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years we will use about 7000 mice and 7800 zebrafish.
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?	All our regulated procedures involve breeding, maintenance and generation of genetically altered animals in order to study embryo development and disease progression and therapy. In some of our regulated procedures animals will be surgically manipulated. All the surgeries will be carried out following common veterinarian practice; in the unlikely event of post-surgery complications, they will be addressed according to NVS opinion or the animals will be humanely killed. In our experiments we will use top-notch microscopy techniques that are non-invasive and do not cause adverse effects to the animals imaged. In all cases, animals will be humanely killed if there are signs of pain, distress or suffering above agreed limits. At the end of procedures, the majority of animals used in this licence will be humanely killed. In a small number of cases, animals that have suffered no more than mild severity as result of procedures during one study detailed in this licence and which are not suffering or likely to suffer, may be re-used on a limited number of occasions in the same mild severity protocol under this licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We work with mice and zebrafish. Each species has distinct advantages with respect to the techniques and knowledge available for genetic, embryological and cell biological analyses. The complexity of embryonic development, which arises from multiple interactions between different cells, involving short- and long-range signalling molecules, and complex cell migration events over time, requires in vivo analyses. Currently there are no alternatives to the use of animals for the study of early embryo development. However, in our experiments, complementary to the use of animals, we will use in vitro models and assays based on isolated embryonic stem cells and commonly used cell lines to understand the effect of the

	introduced genetic alterations. Our cancer detection and therapy probes will be first tested in cell culture systems to assess their therapeutic potential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	With the use of appropriate statistics we carefully design experiments to make sure that we use the minimum number of animals required to give clear scientific answers. We also make extensive use of *in vitro* assays, in particular cell culture. This greatly helps experimental design and also reduces the use of animals.
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 selected for our studies as a model species for several reasons. Firstly, there is more information available about this species than any other in genetics, molecular biology and reproduction. Secondly, the short reproduction interval allows studies to be completed more quickly than in any other mammal. Finally, more consistent observations can be expected as inbred strains are maintained in a closely controlled environment. In addition, zebrafish is selected as a model species for our studies for several reasons. Firstly, zebrafish are vertebrates and therefore share a high degree of sequence and functional homology with mammals, including humans. Secondly, zebrafish embryos and larvae are completely transparent meaning that it is possible to follow the impact of manipulations or pharmacological treatment using non-invasive imaging techniques. Finally, this species produces larger number of offsprings in each generation than rodents and genetic changes can be easily introduced. Moreover, all of our animals are housed under pathogen free, environmentally controlled conditions and are routinely monitored for the presence of pathogens that could potentially lead to infections. In addition, all the animals will be closely monitored during and after experiments. Procedures liable to cause distress or pain will be performed under anaesthesia as appropriate and animals experiencing traumata or adverse effects will be humanely killed.

Project	**166. Immune interactions in infection, inflammation & cancer of the serous cavities**	
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)	In this project we want to understand how the mammalian immune system is able to recognise that it has been infected or that a cell has become cancerous. Specifically we want to understand how this happens locally within the space around the lungs (where cancers often grow and a parasitic worm can live) and whether this is controlled by the interactions of immune cell clusters that are within specialised fat tissues within the chest and abdomen.	
What are the potential benefits likely to derive from this project	Most of the work we do is fundamental research designed to reveal how the cells of the immune	

(how science could be advanced or humans or animals could benefit from the project)?	system interact with the rest of the body. By defining the mechanisms underlying infection and cancer of the body cavities this work will enhance our knowledge of immune responses and open new roads for the development of therapeutic tools in the treatment of human disease. The findings will be disseminated to the wider research community at both the local (internal meetings within my research group and collaborators), national (at meetings such as the annual congress of the British Society for Immunology) and international level via presentation at scientific conferences and by publication in world leading scientific journals.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6,000 mice will be used over the 5 years of the project. The numbers of animals used will be reviewed periodically throughout the duration of the license.
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 animals will experience only mild adverse effects. Parasite infections and products mimicking parasite infection are generally well tolerated. In order to investigate the interactions of tumour cells, fungal allergens and inflammatory agents (including fibres like asbestos) with the pleural cavity, animals will experience moderate pathological symptoms, in the form of inflammation in the lung & body cavities. Such inflammation does not cause the animals great amounts of distress, and any which appear generally ill (and reach a score of 5 on our scoring guidelines), or have difficulty breathing will be immediately sacrificed and all animals will be killed before they exceed moderate severity limits. At the end of the experiment, all animals will be humanely culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All aims of this project rely on the highly complex interaction of cells with the local environment at the site that an infectious, inflammatory or cancerous response is occurring, the cross-talk between immune & supporting cells and their environment cannot be adequately modelled in a culture dish.

	Although lower organisms (Drosophila/Zebrafish) do have primitive cellular systems, these do not have the complexity of mammalian systems. Mice have comparably complex immune cell populations to those of the human within the body locations we are interested in better understanding. Colonies of mice exist that possess defined changes within their DNA resulting in specific modification of immune cell function and a wealth of reagents are available for the analysis of the mouse immune system, as such, mice provide an unrivalled system to answer the questions posed in this project. Furthermore, no alternatives exist for modelling parasite migration through the body, as such we cannot replicate these studies without using a mammalian model. Whenever possible we will use cell culture systems to address defined questions. Concurrent to the use of mouse models, we will analyse the immune cells in pleural fluid from a group of patients who have cancer of the body cavity linings (mesothelioma) enabling a direct comparison of our model and that of clinical specimens
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use inbred mice (inbred mice are as closely related to one another as is possible) which reduces inter-animal variability and thus overall numbers required; if the animals are as alike as possible the responses we measure are expected to be less variable. We have carefully calculated the minimum number of mice required for the experiments described in this project to ensure that the findings generated via experimentation are not likely to occur randomly or by chance, but are instead likely to be attributable to a specific cause. We consult statisticians whenever necessary to ensure this is the case. The group and collaborators will plan experiments carefully, ensuring that as many questions can be answered using the tissues and cells isolated from each animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	In the proposed project, we will utilise different families of mice which are unable to make specific factors involved in the function of immune cell subsets. Mice are the best

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	characterised experimental animals with a wide array of tools available for their study. Throughout, we will ensure that the least invasive methods of dosing and sampling are applied, including the use of anaesthesia for humane restraint when appropriate. Pain relief will be used in all situations that warrant such, for example before collecting blood from the tail. All mice will be housed in individually ventilated cages to reduce risk of infections by opportunistic pathogens. Animals under procedure will be closely observed, clinical signs (including hunching, grimace, normal movement, body temperature) will be monitored using a scoring system and used to determine when the humane endpoint has been reached and will be adhered to at all times to minimise harm. We will use a model of pleural cancer in which cancer cells are directly injected between the ribs into the space around the lungs, rather than being seeded from a solid tumour growing somewhere else in the body. This refinement will reduce the severity of phenotypes experienced by the animals.

Project	**167. Immune priming and the tissue microenvironment**	
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)	Currently a subset of patients can get dramatic results from therapies which target the immune response to fight cancer but a majority cannot. We have shown that even before immune cells reach the tumour they have been activated inefficiently but we don't know why this is nor do we know how to improve this. This project investigates how immune responses against tumours are generated and seeks to understand why they are often ineffective. To do this we have 3 main objectives. Firstly we will determine which cells play important roles in initiating immune responses against	

	tumours. We will compare this to those involved during responses to flu to see whether these are the same or if there are differences which may be functionally important. This will allow us to focus on relevant cells to try and improve the immune response. Secondly by comparing immune responses against tumours to robust responses against viruses and situations in which the immune system is dampened down we will seek to find signals which block the full potential of the anti-tumour immune response. We will then manipulate the tumour to alter these signals and to see how these improve the initiation and perpetuation of anti-tumour immunity. Finally we will seek to understand whether the immune suppression we see in the tumour influences tumour spread. We will then manipulate the tumour as previously mentioned and see how this influences the site of tumour spread and the initiation of immune responses at those sites.
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 aims to address a few questions which will have distinct potential benefits. Firstly we aim to determine why the immune response to tumours and metastases (when a tumour spreads) is not optimal. By looking in the tumour, mainly skin or lung cancer, we will determine factors which tumour use to protect themselves from the immune system directly. This knowledge will be shared through publication and through talks to ensure that we and others can use this knowledge to look for treatments which would improve outcomes in patients with lung or skin cancer. Secondly we will investigate how the tumour or metastasis influences the lymph node and so blunts the immune response even more dramatically. This will improve our knowledge of how immune responses are generated and will help generate other potential targets for improving immune responses to cancer. Finally we will test if improving the generation or function of the immune response to cancer will improve responses to existing therapies. This will help to determine whether this is a potential way to extend the benefits of immunotherapy to more patients.
What species and approximate	This project license requires the use of mice. In

numbers of animals do you expect to use over what period of time?	addition to non-genetically modified animals we will also use genetically modified mice to generate mice with human like disease and to generate immune deficits so as to understand the immune system better. We expect to use 19,000 mice over 5 years, the majority of which, approx. 90%, will have some form of genetic modification.
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 mice are likely to experience moderate severity adverse effects. Mice with tumours may show some signs of ill health being less mobile, showing some changes in posture and being less social but if any animal experiences more than moderate severity they will be humanely killed. Mice with vaccines or flu may show a brief period where they are less social and are a bit less mobile but this will only last a couple of days before they return to normal behaviour. All mice will be humanely killed at the end of these protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying how immune responses are generated in complex organs. As such we cannot use purely in vitro systems as they don't replicate the complexity found in an organ and we cannot use non-protected animals as we need a vertebrate model (and really a mammal) so that they have an immune system similar to the human one.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise numbers in a few key ways: 1. Ensuring we use the fewest number of animals to show a significant response 2. Getting as much information from every experiment by taking multiple tissues 3. Making sure we don't breed more mice than we need 4. Developing ways of answering questions in vitro where possible
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Mice are the best model for studying the immune system as many strains have been developed which have modifications to their immune system. Furthermore mice have similar immune systems to

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	humans. We will constantly look for refinement opportunities to improve our techniques to reduce suffering. We will also seek alternative techniques which would provide similar information where possible. We also are using well established techniques which have been refined over time. There is a lot of experience at the institution of all the tumour models proposed and so staff are well trained to recognise symptoms and adverse effects to ensure undue suffering isn't caused. Flu will be given at doses which only cause transient illness and mice will return to normal behaviour soon.

Project	**168. Immunisation of Rodents**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	To provide a commercial service of rodent immunisation for the purpose of production of novel monoclonal antibodies for commercial for-profit clients and academic not-for profit organisations. The antibodies will be used in the development of therapeutics that will be used to combat disease as well as for use in basic and applied research.	
Why is it important to undertake this work?	To produce novel antigen specific monoclonal antibodies at the behest of our clients that otherwise would not be available. The chances of a project being successful and producing useful antibodies increase from the technical expertise provided by the PPL holder. The antibodies will be developed by the clients into novel therapeutic molecules and biochemical reagents to assist their development from lab to clinic and so improving human health.	

What outputs do you think you will see at the end of this project?	The outputs of this project are • the delivery of novel antibodies tailored to a client's specific needs. • the antibody secreting hybridoma cell lines that will be provided to the client whose establishment will allow for the permanent sources of antibody production. • the knowledge and technical innovations gained during the course of a project.
Who or what will benefit from these outputs, and how?	• The client will receive the antigen specific monoclonal antibodies at the end of the project. • The client will receive the antibody secreting hybridoma cell lines at the end of the project. These will represent an unlimited source of monoclonal antibody for the client who will use them in developing novel therapeutic molecules and tools to assist their development from lab to clinic. • Patients with diseases will benefit from novel therapeutic medicines developed from the antibodies that may also be used to detect disease states and monitor medical treatments.
Will this work be offered as a service to others?	Yes
How will you look to maximise the outputs of this work?	I will disseminate any improvements made in hybridoma technology by publishing or meeting and collaborating with other antibody service providers. Negative results, i.e. failure of an antigen to provoke an immune response or produce specific antibody secreting hybridoma cells will be highlighted in publications, talks, posters and discussions with future clients. Clients will be sent proforma forms asking them if they can inform the PPL holder of any literature, talks or posters that mention research that utilise the antibodies. The antibodies may be further developed by the client for the identification of new therapeutic targets or into medicines for combating human diseases.
Explain why you are using these types of animals and your choice of life stages.	Mice and rats have been used in immunology for over 100 years and their immune systems are now understood down to the cellular and DNA level. Mice are the preferred animal for generating monoclonal antibodies due to the availability of proven immunisation

	strategies that do not cause adverse effects, ease of breeding, handling and housing and relatively low amounts of antigen are required. Crucially the mouse cancer cell lines exist that allow the formation of antibody secreting hybridoma cell lines. The complex mouse immunoglobulin genes that encode the antibody proteins have been sequenced so the unique variable immunoglobulin regions that determine the specificity of the antibody can be rapidly isolated and cloned. Juvenile mice from 6 - 8 weeks old will be used as their immune systems are sufficiently developed to mount an immune response but immature enough not to have been challenged with many or any other antigens that may reduce immunisation. Rats are used when antibodies against mouse antigens are required and have the same advantages as described for mouse. Mouse antigens are unlikely to provoke an immune response in mouse as immune systems do not recognise "self" antigens. The rat cancer cells exist that are required for the formation of rat hybridomas. Juvenile rats from 6 - 8 weeks old will be used for the reasons as listed above.
Typically, what will be done to an animal used in your project?	At any stage of the project work below and as required, general or local and/or analgesic agents will be administered. In protocol 1, a pre-immune blood sample will be taken and the animal will be injected with antigen solution subcutaneously (SC). Every other week for up to 8 weeks (i.e. up to 4 times), antigen boosters will be administered by intraperitoneal (IP) injection and a post-immune blood sample will be taken after the second booster to allow evaluation of the immune response. The animals will be left to rest for a period of between 1 to 10.5 months following which it will receive a final antigen boost and 3 days' later, it will be sacrificed and the lymphoid tissues harvested. The time period between the first immunisation and the tissue harvesting will be no more than 1 year. In protocol 2, an animal is immunised up to 12 times SC with antigen and this will be done up to 4 more times over the next 7 - 11 days. On days 10 - 14, it will be sacrificed and the lymphoid tissues harvested.
What are the expected impacts and/or adverse effects for the animals during your project?	For the vast majority of the animals, no significant medium to long-term adverse effects will be observed and the severity category will be Mild. Animals will be placed on heat mats after injection for an hour to alleviate the short term effects of antigen administration such as

	hypothermia. Animals will be closely monitored for the first hour post injection and then hourly throughout that and the following day. In a small proportion of cases, the immune response results in anaphylactic shock due the antigen/antibody complex triggering either histamine or Platelet Activating Factor (PAF) release resulting in low blood pressure and hypothermia. Any animals showing clinical signs greater than Mild considered untreatable for any reason by animal welfare staff will be killed immediately by a Schedule 1 method.
What are the expected severities and the proportion of animals in each category (per animal type)?	Protocol 1: for mouse and rat: Mild (100%) Protocol 2: for mouse and rat: Mild (100%)
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Only an animal with a functioning immune system can produce an immune response required to generate the diversity of high affinity IgG class antibodies with the desired characteristics. The success of immunisation is down to the large library of antibodies that are generated by the interactions of different cell types and complex biological processes. These processes only occur in the germinal centres of spleen and lymph nodes where antigen activated B-lymphocytes migrate in order to interact with activated T-lymphocytes. With current technology, it is not possible to recreate these process outside of a living animal, i.e. in a test tube.
Which non-animal alternatives did you consider for use in this project?	Laboratory methods such as phage and ribosome display can be used to identify antibody-like molecules from artificial libraries. In phage display, libraries of random antibody fragments are produced on the surface of phages, viruses that infect the bacterium *E. coli,* which can be isolated and their structure determined. In ribosome display, DNA encoding for the antigen variable regions is isolated by capturing the antigen/ribosome-mRNA complexes.
Why were they not	*In vitro* methods such as phage and ribosome display do not produce whole molecule immunoglobulin antibodies

suitable?	with the desired high affinity. As the molecular libraries used in the screens are not specific to the antigen (i.e. they are completely random), significantly higher numbers of molecules need to be screened for binding: i.e. 10^{12} to 10^{15} members contrasted with 10^{3} for B lymphocytes isolated from one immunised animal. Molecular libraries are not freely available, vary widely in terms of quality and are expensive to buy. The low affinity and non-immunoglobulin nature of the primary screen positives will be unsuitable for most purposes and so affinity maturation to strengthen binding and cloning into a suitable antibody gene must take place and this process can take up to 2 years as compared to 4 - 6 months for traditional monoclonal antibody discovery. The high throughput screening equipment and scientific expertise required to execute i*n vitro* antibody generation projects is not usually found in a traditional monoclonal antibody discovery laboratory. Antibodies that are produced by *in vitro* methods have not undergone i*n vivo* biological quality control processes and are more likely to contain undesirable characteristics such as protein precipitation and unwanted amino acids that compromise stability and binding stability. Finally, certain popular targets such as integral membrane proteins and antibacterial agents are not compatible with these *In vitro* technologies.
Enter the estimated number of animals of each type used in this project.	mice: 380 rats: 40
How have you estimated the numbers of animals you will use?	Animal numbers are based on experience from studies carried out under previous Project Licences and also consulting with other experts in the field. Up to 12 projects a year over 5 years mainly with mice are envisaged. As this is a qualitative project that does not have control groups, no power calculations or statistical analysis were attempted. An antibody discovery screen requires positive B lymphocytes from 2 animals with strong immune responses against the antigen. While a strong antigen will produce sufficient responses in all animals within a group, weaker antigens can produce responses only in a proportion of the animals. So to ensure a minimum of 2 sufficiently immunised animals at the end of the project, 4 animals will be used per project. In projects where 2 formats of antigen are available, 4 mice per format will be immunised in parallel.
What steps did you take	On advice from experts in the field, published literature,

during the experimental design phase to reduce the number of animals being used in this project?	study of similar PPLs' NTS on the Home Office website and from previous experience, the number of animals per antigen immunisation is set at 4. The methods and technologies for isolating and screening antibodies were improved making more efficient use of animals and allowing a reduction of 5 animals on older licences to the current 4 per project. Finally, immunisation for the purpose of raising monoclonal antibodies is a qualitative experiment so no control animals are required, thus keeping animal numbers to a minimum.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	Animals will be purchased from commercial suppliers as required thus removing the need for a breeding colony. To maximise success and outputs, immunisation strategies will be designed to maximise the number of antibodies discovered per animal. Other experts in the field will also be consulted about animal numbers. To provide enough material for a successful antibody screen, 4 animals are required. The reason is that tissue from 2 animals is the minimum requirement for an antibody screen. However, as weakly antigenic substances may not provoke an immune responses in every animal within a group, 4 immunised animals will on average yield 2 animals with sufficient immunopositive B lymphocytes for an antibody screen. Reducing the animal group number below 4 could result in insufficient numbers of immunised animals and cause the project to fail in its objective.
Which animal models and methods will you use during this project?	Mice and rats will be immunised with antigen in order to produce reactive B-lymphocytes. These cells will be extracted for the purpose of making antibody secreting monoclonal hybridomas. These animals are ideal as they are easy to maintain, have short life spans, are easy to handle and cage, thus reducing stress. They have a long history of use in immunology and so the strategies and routes of administration of antigens have been designed to produce a strong immune response and the high probability of isolating monoclonal antibodies without causing long lasting adverse effects. Two kinds of genetically altered animals may be used for generation of monoclonal antibodies; animals containing humanised antibody genes and animals containing knockout gene alleles that delete a specific protein. The humanised antibody animals yield fully human antibodies that can take advantage of all benefits of modern mouse monoclonal technology. The knockout animals are used when the antigen is already present within the animal that interferes with the anti-antigen immune response,

	i.e. mouse protein antigen or a human protein antigen that is very similar to the mouse protein. The knockout deletion of the gene in the germ line DNA encoding the animal's protein results in an immune system that has not been in contact with the antigen producing a stronger immune response. Both kinds of genetically altered animal can be used as sources for the discovery of human antibodies for therapeutic use. Rats will be used when the antigen has been purified from mouse or that the client requires the monoclonal antibodies to be rat in origin. Protocol 1 will be used to primarily target the spleen tissue to ensure it contains B lymphocytes secreting anti-antigen antibodies. The relatively long time period of the protocol ensures that high quality (lacking liabilities such as propensity to precipitate from solution or unwanted amino acids), high affinity IgG class antibodies are produced. A further benefit is that the immune response can be monitored by the presence of antibodies in the serum. Weak antigens do not produce an immune response in every animal so these non-responders can be removed early in the protocol. Protocol 2 is a shortened time period immunisation that primarily targets the lymph nodes. High quality IgG class antibodies are produced but may not be as high affinity as those from Protocol 1 due to the reduced time for affinity maturation. Typically Protocol 1 will be the preferred option to generate monoclonal antibodies but Protocol 2 is the preferred option for mouse antigens or where a human protein is identical to the mouse version and may not provoke a sufficiently strong immune response in Protocol 1.
Why can’t you use animals that are less sentient?	The production of monoclonal antibodies requires a functioning immune system and rodents such as mice and rats are short lived, have rapid generation times and are the smallest, easiest to handle and cage mammals that are available from commercial suppliers. Juvenile animals of 6 - 8 weeks old are the optimum age as the immune system has matured to be fully functional but as the animals are housed under relatively clean, pathogen-free conditions, it is unlikely their immune systems will have been challenged. It takes weeks for the immune response to mature so terminal anaesthesia would not be suitable.
How will you stay informed about advances in the	Attending lectures organised by AWERB. Consulting with other groups and scientists involved in

3Rs, and implement these advances effectively, during the project?	this type of work. Consulting with the Home Office inspector. Consult the NC3Rs website.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	Prior to initiating a project, all potential antigens will be assessed during initial discussions and the ethical review to identify molecules that could cause harm. Antigens with known anaphylactic potential such as foreign immunoglobulins will be avoided. Antigens will be sourced from reputable commercial suppliers that will be certified free of microorganisms and contaminating substances. Antigen solutions for administration will be prepared using aseptic technique with sterile physiologically compatible buffers such as saline. After immunisation, animals will be placed on a heat mat to alleviate any possible hypothermia and monitored closely for an hour, which usually is the duration of any mild adverse effects and then checked regularly throughout the next 2 days. Animals will be monitored on a daily basis on non-procedure days. Non-severe and recoverable antigen induced anaphylaxis can be treated by administration of anti-histamines such as Piriton. Animals that do not develop an immune response after the second boost on Protocol 1 will be removed from the project and culled by a Schedule 1 method. Antigens producing intermediate strength responses will be boosted an extra time to allow the immune response to further mature. The administration routes and strategies have been shown to cause no adverse effects while still producing very strong immune responses that are critical for producing sufficient numbers of independent antibodies of the right class with high affinities and diversity. Animals on Protocol 2 will be immunised exclusively through the subcutaneous route multiple times over a relatively short period of time. To minimise any pain from multiple pin pricks, brief anaesthesia will be administered during the injections together with a dose of an analgesic (buprenorphine) for the recovery period. A preference for non-ulcerogenic adjuvants will be made. If Freunds Complete Adjuvant is used for weak antigens, it will only be used once, only via the subcutaneous route and the maximum administered volume per animal will

	be 0.1 ml for Protocol 1 and 0.05 ml for Protocol 2. The intravenous route will be reserved exclusively for genetic (DNA) immunisations and no adjuvants will be used. Volumes of antigen preparation for administration will not exceed 0.2 ml and adhere to published guidelines. The maximum allowable blood sample to be withdrawn will be 10% of the total blood volume complying with published guidelines. The PPL holder will continue to refine procedures throughout the lifetime of the licence by staying abreast of published literature and latest developments, online resources belonging to organisations such as NC3Rs, Institute of Animal Technology (IAT) and The Laboratory Animal Science Association (LASA), consulting with other experts and attending relevant scientific meetings such as those organised by the AWERB.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	Blood volume limits: www.nc3rs.org.uk/blood-sampling-general-principles Volumes of administration: Turner, P. V., Brabb, T., Pekow, C., & Vasbinder, M. A. (2011). Administration of substances to laboratory animals: routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science 50, 600–613 Veterinary drug doses: British Small Animal Veterinary Association (BSAVA) Small Animal Formulary.

Project	**169. Immunity and immunomodulation in helminth infection**	
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)		
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 aims to isolate and test molecules which have potential to treat diseases such as arthritis, colitis and asthma, characterised by excessive infiltration of activated white blood cells into joints, the lungs and the gut, as well as autoimmune diseases such as multiple sclerosis, type 1 diabetes and lupus. In addition, development of vaccines against worm infection would represent a major advance in controlling a class of pathogens which have a huge impact	

	on global health, greater than HIV or tuberculosis when measured by disability-adjusted life years (DALYs), a time-based measure that combines years of life lost due to premature mortality and years of life lost due to time lived in a state of poor health.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (2,400) over 5 years. Rats (400) over 5 years. This is to a) provide parasite material and b) to test how parasites and their constituent molecules suppress the immune response, and the ability of these molecules to alleviate inflammatory disorders.
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 procedures we propose do not result in undue discomfort. Animals will be infected with parasites which, at the doses used, do not have harmful effects, and they will be humanely killed before parasites or cells are retrieved and analysed. The model for Multiple Sclerosis results in progressive paralysis of the limbs and possible ulceration at injection sites, but experiments will be terminated before animals reach this point. In this procedure, mice may have minor surgery to implant a device for slow release of a drug to treat the disease. Animals are expected to recover quickly, and will be given painkillers and post-operative care until fully recovered. For imaging experiments, mice will have their hair removed by shaving or creams and will be anaesthetised. When mice are irradiated, they will be kept in sterile conditions to prevent infection with pathogens, will get dissolved food and easier access to food and drink and will be monitored every 2 hours the first day and then twice a day until they recover fully. Animals will be closely monitored and procedures will be stopped if animals reach well-defined endpoints. All animals will be humanely killed after experiments or should unexpected and harmful side effects arise.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The parasites we are studying can only survive in animals, and we therefore need to use animals to provide parasites for experimental work and to study the process of infection. Some aspects of immunity can be analysed in vitro, and we will do this during the project. However the immune response to parasite infection is very complex, as is the process of inflammation that accompanies allergies and autoimmune diseases, leading to tissue damage, and these can only be assayed in an in vivo (animal) setting. Re-creation or modelling of these diseases in vitro is not possible. The

	mechanisms of action of potential drug treatments will be studied wherever possible in vitro, but their ability to treat the diseases requires testing in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will seek to perform as many experiments in the project as possible without use of animals using cell lines, in vitro assays and in silico approaches. Where animal use is necessary, we have calculated the number needed to reach convincing conclusions for each type of experiment, and optimised the timing of sample collection in order to reduce the number of animals used. These numbers will not be exceeded, but experiments will be repeated at least once in order to be sure that the results are reproducible. Where appropriate, imaging techniques will be applied. These allow for analysis of multiple time points in the same animal, thus reducing the number of animals needed to collect the same amount of data.
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 are the natural hosts for the parasites we propose to study, and they cannot survive in other species. The immune system of mice and rats is extremely well characterised, and closely resembles the human immune system for the diseases we study. Skilled researchers will undertake all techniques, and sympathetic animal handling, injection and blood sampling techniques will be used throughout in order to minimise discomfort. Minor surgery and potentially stressful techniques will be performed under anaesthesia, with painkillers administered until full recovery. Animals will be housed in excellent conditions with appropriate bedding and nesting material, and will be monitored and cared for on a daily basis by professionally trained staff. If animals develop unexpected symptoms they will be humanely killed.

Project	**170. Immunity, Infection and Disorders of the CNS**	
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)	Complement comprises a network of proteins in plasma and on cells that collaborate to defend against infection. Complement dysfunction can contribute to many diseases, particularly diseases of the nervous system such as multiple sclerosis and dementia. The primary objective of the project is to develop a comprehensive understanding of the role of complement and related immune molecules in a group of neurological diseases linked by the presence of inflammation. A secondary objective is to inform the development of better diagnosis and treatments for these diseases, an enormous unmet need.	

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 include better tests for disease classification and staging, better understanding of the pathology of neurological disease and the role played by the immune system within that. The potential to test new therapeutic agents for treating inflammatory aspects of these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (approximately 2800) and rats (approximately 300) over the five years of the Programme.
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 each of the disease models proposed in the project, animals are expected to develop a neurological disease; in the case of protocols 1 and 2 the anticipated disease is a relatively acute onset of paralysis akin to human multiple sclerosis or myasthenia while disease in protocol 3 is a slowly developing neurodegeneration resembling Alzheimer's disease in man. Animals will be carefully monitored and when the disease progresses to a point where it has a significant impact on the animal's behaviour and/or well being as assessed clinically and by measuring weight loss, the animals will be humanely killed.
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 the use of animal models in order to unravel mechanisms by which the immune system contributes to the modelled human diseases and to explore which drugs might slow or reverse disease. Animal experiments are supported by a large body of test-tube and cell culture experiments that answer some of the questions and help in the design of animal experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary non-animal experiments can help in the choice of agent, dose of agent and duration of treatment in the animal experiment, reducing the need for initial dose-finding or effectiveness animal tests. Statistical help in study design ensures that enough but no more animals are used to arrive at an answer to the question set.

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.	Tests on the immune system to be of relevance to man require a species that has an immune system similar to man. Rodents are ideally suited in that they have comparable innate immune systems and there are many reagents available to help us measure changes in their immune systems with disease. Harm will be minimised by excellent experimental technique throughout, close monitoring of animals throughout, prompt termination when set severity thresholds are reached, excellent husbandry to ensure easy access to food, water and other environmental supports, and always an attitude that harm must be kept to the minimum necessary for the experiment.

Project	**171. Immunopathology and Immunotherapy of Hepatitis B Virus Infection**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our research is focussed on understanding why immune responses fail during chronic Hepatitis B virus (HBV) infection. HBV is a viral infection of the livers in humans and 350 million people worldwide are estimated to be currently infected, despite the existence of a preventative vaccine. In some cases, HBV infection can persist and establish a chronic infection (Chronic hepatitis B, CHB) that is associated with severe disease complications such as liver cirrhosis and liver cancer, leading to over 700,000 deaths annually (as reported by the World Health	

	Organisation). So far, treatments can control viral spread but only rarely achieve complete cure of infection. There is also a risk of the virus becoming resistant to treatment and toxicity during prolonged treatment. Furthermore, antiviral treatment is expensive, limiting its availability in less developed countries, which have the highest rates of infection. An ideal alternative would be a therapeutic approach that can induce immune responses because the adult immune system has the capacity to control HBV infection. Our aim is therefore to activate the immune system to achieve complete elimination of virus. Through our very strong previous research knowledge and achievements in HBV immunology we are in a unique position to dissect the anti-viral immune response and explore new therapeutic strategies. We will now complement our ongoing work using patient samples with in vivo murine studies. Research on HBV has always been difficult, due to the specificity of the virus for human liver cells (hepatocytes). Although some mouse models have been developed to constantly produce HBV in their hepatocytes, these are not ideal, since HBV is not seen by the animal's immune system as foreign and, therefore, do not elicit an immune response. Therefore, we will make use of a recently described strategy allowing infection of mice with HBV by using the coat of an unrelated virus to deliver HBV into mouse hepatocytes. The induction of cronic HBV infection of these mice is accompanied by an ineffective immune response, similar to the one seen in patients. This model will make it possible to address our 3 aims: 1. To investigate how the liver environment can inhibit anti-viral immune responses. 2. To understand the function of immune system cells, in particular natural killer (NK) cells, that have been shown to play both beneficial and detrimental roles in chronic HBV. 3. To test different therapeutic strategies to induce T cell anti-viral immunity, with the potential to be later tested in clinical trials.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work proposed will enhance our understanding of the immune response to CHB and is an essential step towards the development of targeted immunotherapy of this infection and related conditions. It will also provide new insights into the influence of the liver environment on immune responses in general. The proposed work will further be of relevance to other diseases characterised by chronic inflammation and progressive fibrosis, in particular a major cause of morbidity and mortality in human immunodeficiency virus (HIV) patients co-infected with HBV and Hepatitis C (HCV) and in patients with alcoholic and non-alcoholic liver disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, approximately 800 animals over the period of the 5-year grant, reflecting the work of 2 researchers.
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?	Any animal close to exceeding moderate severity will be culled humanely immediately, although this is not expected. Clinical signs are expected to generally be mild to moderate and signs of toxicity, pain or distress will be monitored using the mouse grimace scale. All animals utilised for our research will be culled humanely at the end of the experiments, to study specific cells and tissue.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Access to relevant human tissue, such as the HBV-infected liver is limited. Whenever possible, we will use an in vitro infection model using human liver-derived cells that are permissive to HBV virus entry and was recently established in our laboratory. However, we are not able to investigate the human immune response in vivo and the use of human cells in culture limits our understanding of the interplay of the components of the immune system. We will use the proposed animal model to investigate the relationship of the different immune components in vivo by studying the liver, spleen

	and other tissues directly. The mouse model will also allow extensive functional analysis of specialised liver-derived antigen presenting cells. We will assess the dominant in vivo effect of NK cells (for which we have identified several potential roles from our human studies in culture). Importantly, the animal model will allow us to test and refine therapeutic interventions. In particular, we will test the efficacy of different molecular modifications of engineered T cells in vivo as a prelude to future clinical translation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The bulk of this study will be conducted using human samples, making up about 60% of our work and only about 40% will utilised the animal model. Whenever possible in vitro studies using human or mouse tissue will be used. New therapeutic strategies will be tested in animals only if we have an indication of their usefulness from our human work. The number of animals planned reflects the work of at least 2 researchers. Since the projects of the two postdocs are linked we will utilise the same animals whenever possible, e.g. to study two different immune cells purified from the same mouse. Control animals might also serve to control for two different experimental questions and the postdocs will work together closely. We will keep up to date with current developments in our field in order to refine techniques, reduce animal usage and avoid unnecessary duplication of published experiments. The study has been optimised to maximise the information gained from a minimum of samples and from different time points from the same animal. Careful consideration will be given to the number of animals needed to give meaningful results to optimise experiments throughout the life of the 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	Ducks and Woodchucks can be infected with a hepatitis virus similar to human HBV, however, their use is limited due to their outbred nature and their immune markers being ill-defined. Mice are well studied and their immune components well defined, but they are not natural

measures you will take to minimise welfare costs (harms) to the animals.	hosts for HBV. A novel route of packaging HBV into the coating of an unrelated virus, which targets the mouse liver, now allows us to chronically infect mice by injecting a well-tolerated volume of packaged HBV virus. This model can be adjusted to either achieve an acute or chronic infection, allowing us to dissect the mechanisms leading to the two different outcomes. Neither acute nor chronic HBV infection have been reported to lead to any severe suffering of their mouse host. Animals will be kept in well-maintained individual ventilated cages with plenty of bedding and nesting material with good quality food and water, which will also reduce experimental variability caused by environmental stresses. Animals will be monitored for weight loss and signs of toxicity, pain or distress following the mouse grimace scale. In the event of unexpected adverse effects, we will use humane endpoints.

Project	**172. Immunoregulation during parasitic helminth infection**	
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)	Infections by large parasitic worms, particularly those that live in the intestine are extremely common, but often under appreciated. Approaching two billion people are currently infected with at least one type of parasitic worm and these infections have been classified as Great Neglected Tropical Diseases by the World Health Organisation. The most heavily infected individuals that suffer most disease are young children, often from the poorest communities. Farm and domestic animals are also naturally and extensively infected with worm parasites and without repeated treatment with deworming drugs suffer ill health and poor growth. In animals, drug
	resistance is high so that in some parts of the world no effective treatments remain and indeed, drug resistance is now developing in humans. No vaccines are available against these infections in humans and few for animals of veterinary importance. New therapies and approaches are urgently required to help control these infections. In order to develop these, Aim One of the project is to generate a much greater understanding of how worm parasites interact with man and animals, especially how they can survive attack by our body's defence system, the immune system. This will enable us to identify key aspects of the immune response that may be defective and those that need to be enhanced to help clear the parasites from the body. Aim Two of the project aims to identify particular ways that we can enhance our immune response to worm parasites and what molecules of the parasite we must target to achieve this. Our work has already identified some of the most important components of the interactions between parasites and the immune system and shown that they operate in a similar way in animal models as in human infections.

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)?	We will generate an in depth academic knowledge for the biomedical and veterinary communities of how different worm parasites survive or are cleared by the body's immune system. This will open up avenues for development of new targets for anti-worm drugs and vaccines by research scientists and pharmaceutical companies. Moreover, there is clear evidence that in areas of the world where parasitic worms infect people extensively, diseases such as allergies (such as asthma) and autoimmune diseases (such as diabetes) are relatively rare, and this is believed to be as a result of being infected by parasites. The information we obtain from Aim One will help us identify parasite molecules involved and mechanisms that may be responsible for this. In Aim Two we will target these molecules and mechanisms to help develop new approaches and treatments that not only have anti-parasitic effects but also beneficial effects for people with allergic and autoimmune disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats. We will use up to 20000 mice and 250 rats over a five-year period.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of animals will undergo infection or treatments associated with infection. Some will be used for studies testing parasite molecules as potential vaccines. For example, mice may be infected with one or more type of parasitic worm, as experienced in the wild and their immune responses to these parasites investigated by taking samples from them at different time points following infection. In some studies, mice will be vaccinated with molecules taken from parasitic worms and then infected with the parasite to see if this will protect them. Some mice and some rats will be infected with parasitic worms to provide parasites for use in our experiments. The majority of infections and treatments lead to mild symptoms that are often transient. The parasites we use are those that naturally infect rodents in the wild and are thus well adapted to laboratory animals, which minimises excessive adverse effects. Animals are monitored closely and after use are humanely killed with the tissues taken for further laboratory research. Any that show adverse effects during the experiment will be humanely killed and again tissue used for further laboratory research.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The parasites that we use cannot grow, develop or reproduce outside of their mammalian host. They cannot be frozen and thawed to use in the laboratory. The control of parasite infection, treatment and vaccination requires an intact animal as the response involves many different cell types in the immune system, at many different body sites and occurs over a period of time. This is a very complex response and cannot be accurately analysed by computers or in cell culture in an incubator. People infected with worm parasites are mostly present in parts of the world with very limited access to very limited resources for research and it

	is very hard to assess their infection histories. Moreover, only samples such as blood can be taken for analysis, which does not accurately measure changes that go on in the intestine. Also, people are often infected with other infections that make it difficult to determine cause and effect from samples taken from people.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In consultation with experienced statisticians we design our studies to utilise the fewest number of animals to ensure trustworthy results. All tissues possible are taken from the animals and used in multiple projects to reduce the numbers used. Where possible we will use cell culture and computer based analysis to help our study design prior to using animals that will also reduce numbers used.
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 natural mouse or rat worm parasites that have the same life cycle and infection sites as human and large animal counterparts. As much as possible, infections, treatments and vaccinations are given via the same route as they would be for humans or large animals. Extensive use is made of information from previous animal experiments to identify the most informative times to treat animals and take tissues from. Where possible, information taken from the limited studies carried out in humans and large animals are used in experimental design to work out the most important questions to ask. Treatments are as minimally invasive as possible. Animals are usually housed in social groups in a cage with nesting and tubes/shelters to enrich their environment.

Project	**173. Impacts of renewable energy systems on farmed finfish**	
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
	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)	There is a recognised need to increase aquaculture production to meet food security demands; this coincides with the increasing demand to expand renewable energy systems. Co-location of fish-farms with wind-farms and/or wave energy converters at sea could address both requirements, limit the impacted areas of the marine environment, and optimise the use of space, installations and associated infrastructures. Nevertheless, there is a risk that the wind/wave energy systems may impact on the fish being farmed alongside. The cultured stocks will be exposed to potential stressors associated with energy production such as human disturbance, (moving) shade, noise, vibration and	

	electro-magnetic fields. The objective of this laboratory-based research is to evaluate whether these stressors could affect the productivity and welfare of farmed fish. The research ultimately aims to evaluate whether fish-farm and wind/wave energy-farm co-location is viable.
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 help to understand the potential impacts of stressors associated with wind/wave energy production on finfish being farmed alongside. It is well known that stressors affect fish physiology, behaviour and welfare but, because co-location is a novel approach, information on responses to the specific stressors and their combination is lacking. Our research will build upon the existing information by providing new focussed data on stress indicators, such as stress hormones, growth, food conversion efficiency and behaviour (e.g. feeding, shoaling, distribution, activity). Ultimately, the research will clarify the magnitude of any impacts on fish productivity and welfare, and aid decision-making in relation to co-location and multi-use platforms.
What species and approximate numbers of animals do you expect to use over what period of time?	Finfish species of importance to European marine aquaculture and widely used in applied laboratory experiments, e.g. European sea-bass (Dicentrarchus labrax), Atlantic salmon (Salmo salar), gilthead seabream (Sparus aurata). The approximate number of fish used will be 5000 over a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The main focus of the project is to expose fish within a controlled laboratory environment to standardised stressors that simulate those associated with wind/wave energy farms such as noise, vibration and electro-magnetic fields. The treatment stressors will be applied over a maximum period of 8 weeks, at a periodicity representative of co-location. The anticipated adverse effects are restricted to minor disturbance of physiology and behaviour which, given the study duration, the fish may habituate to. However,

	because there is potential for cumulative effects, the severity has been categorised as moderate, which will be adhered to through monitoring and intervention if necessary. Laboratory control fish may be used, which will be held under standard husbandry conditions, not exposed to stress and would therefore be classed as sub-threshold. All fish will be euthanised humanely at the end of the experiment
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The primary aim is to assess the impacts of offshore wind farm/wave energy converter stressors on fish productivity and welfare and as such non-animal alternatives are not relevant. However, non-protected aquatic invertebrates may be used in prior experiments to assess and enhance the experimental set-up and parameters.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will depend upon the number of replicate tanks and the number of individual fish per tank. To produce relevant data, we will need to use fish densities representative of commercial aquaculture practices. Numbers can be reduced by use of smaller tanks or by reducing water volume, so animal husbandry experts will advise on fish social and spatial needs. Statisticians will advise on the number of replicates required to achieve meaningful results (e.g. by power analysis), based upon measurements to be taken of individual fish and the whole tank population. Marking will be considered as a mean to track the performance of individual fish, and thereby maintain data quality while reducing 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	Our establishment has significant expertise in working with fish. The species proposed for use represent common European net-pen (cage) farmed fish; farmed stocks will be sourced and acclimated to our laboratory environment.

the animals.	A literature review is being written to define the relevant stressors and the means of applying them. The individual procedures, which the fish will be exposed to, are classed as mild but the overall severity is categorised as moderate due to the potential for a cumulative effect. The exposure period will be limited to a maximum of 8 weeks, and fish will be regularly monitored throughout - for both welfare and for data collection. Quantification of behaviour is non-invasive and can be used to assess welfare in real-time. In the unlikely event that observed effects become more adverse than anticipated (and indicative that co-location is not viable), prompt action will be taken (for individuals or tank groups as appropriate). We believe we have a strong institutional culture of care and have review processes to identify where continual improvements in care can be made. Finally, as mentioned above, a prior study using non-vertebrate animals may be undertaken to refine the experimental set-up and ensure logistics/equipment are fit for purpose prior to using fish.

Project	**174. Improvement of light-activated therapies**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	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 focuses on understanding and optimising the treatment of cancer using drugs that can be activated by light. The light is typically provided using lasers at red wavelengths. Current drugs have several disadvantages such as poor absorption of red light and high uptake in the skin which makes the skin sensitive to sunlight. The use of new drugs and different drug doses and treatment times will be tested in order to improve the efficacy of treatment and limit current adverse side-effects such as skin damage from direct exposure to sunlight. In the longer term, we aim to use the new knowledge to develop a more effective clinical 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 success of this project would enable us to optimise the clinical use of current drugs for cancer treatment, in particular reducing either the amount of drug administered or reducing the laser light exposure time. The treatment will have to undergo a trial in patients prior to full approval and this process will take up to five years following completion of the laboratory studies. In the long-term, improved treatment based on these findings will prolong patient survival and lead to higher rates of cure as well as offering a reduction in treatment times and fewer complications which will be beneficial to the patient's quality of life. In the medium to short-term, the laboratory research will benefit from an improved knowledge of the treatment mechanism, which should reduce animal usage, and publication of the results in peer-reviewed journals and presentations and talks at leading medical conferences.
What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that an average of 6500 rodents could be used over 5 years in this project, estimated at 3900 mice, 2600 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?	Procedures will involve systemic administration of a photosensitiser which will make animal tissue sensitive to light, and/or low doses of a chemotherapeutic agent, which should not be overtly toxic. The use of tumours grown in the breast, oesophagus, pancreas, colon and subcutaneously in the flank for this project are intended to be the smallest tumour size necessary to reach a scientific endpoint, in terms of the extent of tumour damage that can be reliably measured. Significant tumour burden and risk of metastases are not expected to occur. However, animals may experience pain and suffer from weight loss and skin lesions. All surgery will be carried out under anaesthesia, which may cause discomfort, but the number of times an animal undergoes anaesthesia will be kept to a minimum. Targeted sensitisers are to be used during light delivery therefore preventing damage to normal tissue. All surgical procedures carry some risk of excess bleeding and infection. All treatments and procedures will
	be mild

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Much preliminary light-activated therapy using novel drugs will generally be tested *in vitro* for efficacy prior to *in vivo* studies and will continue to be carried out alongside animal studies (cell culture) to test the effectiveness and localisation of different sensitisers, chemotherapeutics and other chemical agents. However, key aims of this programme are to use optical pharmacokinetics to follow the distribution of photosensitising drugs around the body and to study how light-treated tissues heal. This can only be done in live animals. In addition, a significant component of PDT treatment-induced tumour damage is thought to be caused through vascular damage to blood vessels effects. The consequence is that the treatment is dependent to a large degree on effects in vivo. In silico modelling will also be undertaken to understand how the *in vivo* efficacy depends on the treatment doses which if successful could replace further *in vivo* experimentation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be kept to a minimum by observing *in vitro* affects and/or studying drug uptake *in vivo* prior to any treatment *in vivo*. The inclusion of in vivo imaging of the drugs will improve the understanding of the rate of drug accumulation and clearance from the body will result in a reduction in the number of subsequent experiments and thereby reduce animal usage. Where applicable data obtained on a set of animals without drug administration can be re-used for other studies, and reduce animal usage. As few animals as possible will be used to achieve statistically rigorous results. In addition we will ensure that experiments are well designed so as to reduced the likelihood of repetition, and are carried out effectively and that colleagues collaborate wherever possible.
3. Refinement Explain the choice of species and why the animal model(s) you will	Rats and mice are suitable for the proposed tumour model experiments, which are minimally invasive using treatment parameters optimised based on prior imaging and/or pharmacokinetic

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.	data. Using imaging we can refine the models used in order to reduce animal usage and use tumours that are as small as required for therapeutic studie. Animals will be closely monitored throughout treatment and recovery. We will monitor body weight, food and fluid uptake and other signs of distress so as to ensure minimal harm to the animals.

Project	**175. Improving outcomes following nerve injury/repair**	
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)	300,000 peripheral nerve injuries arise every year in Europe, predominantly through work, domestic and traffic accidents, and as a consequence of surgical procedures. Nerve repair can be achieved by stitching the injured nerve ends together, nerve grafting (using a graft taken from another nerve) or using a biocompatible nerve guidance conduit, however outcomes of these approaches vary and recovery is very rarely complete, and about 50% of patients have persistent pain for which there is no reliable treatment. Of patients with injuries in the forearm, less than two-thirds return to employment. Where there is a loss of nerve structure greater than 1cm nerve grafting	

	remains the gold standard. However, it has many drawbacks, such as the need for second surgical site, and loss of sensory or motor supply to the area from where the nerve is harvested. For this reason biocompatible nerve guidance conduits are being developed, whilst there have been advances in this field more research needs to be done before these conduits can replace nerve grafting as the best treatment for repairing gaps in injured nerves. The objectives of this project are to develop methods to enhance nerve regeneration through the development of bioactive nerve guides and the application of nerve regeneration enhancing therapeutic agents; and to further our understanding of the causes of pain following nerve injury. This work will facilitate improved treatment for patients with nerve injury and nerve injury-induced pain.
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 i) identify methods to reduce scarring and inflammation at sites of nerve injury – that are known to limit functional recovery, ii) assist in the development of biocompatible nerve guides that are able to help injured nerves regenerate, and iii) enable us to further understand why some patients develop pain following nerve injury. It could lead to new clinical treatments for the repair of nerve injuries, improving the quality of life of patients with severe motor/sensory defects or chronic nerve injury-induced pain. The outcomes from this project are of considerable clinical relevance in relation to our clinical work REDACTED there is a critical translational route REDACTED. REDACTED. Following nerve injury, a proportion of patients are left with complete numbness or altered sensation of the affected region; this may be accompanied by severe nerve injury-induced pain, which is often described by patients as stabbing or burning in nature. Regions most commonly affected are the lips and tongue, causing significant difficulty with a range of functions including speech, taste and chewing. In addition, many patients develop chronic pain, which is extremely difficult to treat. Our previous work has led to improved treatments for these patients, and we anticipate that the proposed research will enable us to
	improve treatment further.

What species and approximate numbers of animals do you expect to use over what period of time?	This project will be carried in rodents and we estimate that it will use 650 mice and 160 rats 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?	Adverse effects (for both mice and rats) include: sensory and/or motor deficiency, local discomfort, local infection at the nerve injury/repair site. All animals will experience sensory or motor deficits following nerve injury. These differ depending on the site of the nerve injury. Injuries to the sciatic and its branches effect the foot ant and leg, injuries to the trigeminal nerve will effect the oro-facial region. In our experience over many years, there is very little indication that these injuries markedly affect the animals' general behaviour (eg their mobility and ability to feed), their weight gain or their overall condition. The expected level of severity is moderate or below. Animals will be culled using a Schedule 1 method or via perfusing the vascular system with histological fixatives via a major vessel or cardiac puncture, under deep anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The physiological response following a peripheral nerve injury involves a complex series of events, with many interactions that are as yet not fully characterised. As our goal is to improve the overall outcome of peripheral nerve injuries - through the application of therapeutic agents or conduit guidance - we require a live animal model to assess the full range of effects and efficacy within the nervous system, which cannot be predicted using isolated tissues in vitro. REDACTED This tissue is used in our neuropathic pain research, and gives us considerable insight into neuropathic pain mechanisms in man.
2. Reduction Explain how you will assure the use	Biocompatibility of all materials and conduit designs used in nerve conduit construction will be assessed in vitro using cultured neurones

of minimum numbers of animals	and glial cells prior to any use in vivo. This will enable us to reduce the number of in-vivo experiments, as we will not test materials in vivo that are either not biocompatible or do not support nerve growth in vitro. Power calculations will be carried out prior to any individual studies in order to determine the minimum number of animals required to detect a clinically relevant difference.
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 no reason to expect any significant species differences in nerve regeneration between rodents and man, but there are differences in regenerative abilities in lower order species such as reptiles. We therefore believe that rodents are the appropriate choice for these studies, which is consistent with the majority of previous investigations. The majority of the work intended to be performed under this licence can be achieved using mice; however, due to size constraints mice are unsuitable for studies investigating the more clinically relevant trigeminal nerve branches or extended nerve defect lengths. Therefore, we believe that in experiments investigating the trigeminal nerve branches, or peripheral nerve regeneration over relatively long distances, the choice of the rat is justified. The nerve injury models intended to be used are well established, reproducible, and their associated adverse effects are well known. Anaesthetics will be administered during any surgical work and analgesics will be administered subsequently if the animal is deemed to be in discomfort during the recovery period. If unexpected effects become apparent, veterinary advice will be sought and followed immediately, with animals culled if deemed necessary. Preliminary work assessing the biocompatibility nerve conduits has been conducted in vitro and further in vitro assessment may be carried out in the event of changes to either the composition of conduit materials or the conduit manufacturing process. In vitro assessment may also be carried out to help determine potential toxicity and appropriate dosage of

	therapeutic agents that are not well established within the present literature. We believe that these precautions will reduce the potential for unexpected adverse effects to occur as a result of conduit implantation and/or therapeutic treatment.

Project	**176. Improving vaccine immunogenicity**	
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)	White blood cells called T-cells are crucial for generating an immune response to fight infection. In the case of certain infections such as influenza virus, these T cells often fail to offer complete protection against serious illness due to the ability of the virus to change key characteristics which enable it to hide from the immune response. We hypothesise that by making T cell responses stronger, we will endow them with the power to withstand these changes and offer protection against multiple strains of influenza. The World Health Organization (WHO) estimates that flu is responsible for the deaths of between 250,000 and 500,000 people around the world every year. Indeed, influenza epidemics, in the UK alone, currently result in hospitalisation of thousands of people annually resulting in death in approximately	

	600 individuals thus better vaccines are urgently required (http://vk.ovg.ox.ac.uk/vk/influenza-flu). We are also constantly at risk of the emergence of radically altered influenza viruses (pandemic strains) with the potential to claim tens of thousands of lives if effective vaccines are not put in place. In this study, we will test the hypothesis that increasing the strength of influenza-specific T cell responses improves immunity to different influenza viruses. Results of this study may improve the ability of vaccines to generate immune responses against seasonal and pandemic flu.
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)?	Findings of this project will directly inform the design of better vaccines against influenza virus. The new knowledge gained from the study will also be applicable to the design of vaccines against other infectious agents and cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We will study how effective our new vaccines are in mice. The advantages of using mice are: 1) their immune system resembles that of us humans, 2) we have many tools to study different aspects of the immune system in mice that we cannot apply to other systems and 3) flu vaccination has been studied in mice for a long time and results have been translated to improve human health successfully. We will use approximately 4000 genetically modified mice. Approximately 3200 of these will be vaccinated and around half of these will also be challenged with influenza virus. A maximum of 100 chicken eggs will be used to grow the influenza virus required for the study.
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?	Genetically modified mice will be bred for this study. A minority of the animals will be immunodeficient and may, in principle be more infection-prone. However, as a result of good housing conditions, we do expect that mice bred for this study will be healthy and will not suffer from spontaneous infections or inflammatory diseases. Most mice will be vaccinated with the experimental vaccines, designed in the course of the study. These vaccines are modified versions of previous vaccines, differing mainly in their ability to induce immune responses to influenza virus. These responses are not expected to cause harm to the animal. In the case where mice are infected with

	influenza virus, it is likely that some of these, in particular unvaccinated mice, will develop typical symptoms of viral infection. These include listlessness, loss of appetite and weight loss. Animals will be monitored daily during this period and provided with alternative dietary products. In the unexpected event that recovery is unlikely, animals will be humanely killed. As well as mice, chicken eggs will be used to propagate the influenza viruses required for the study. Each egg will be regularly assessed during the procedure in the rare event that damage is caused to the embryo.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The animal work in this project will follow extensive studies on human cells outside the body. We will investigate all aspects of generating more sticky proteins and produce vaccines before starting animal work. However, vaccines can only be tested in an intact immune system as it requires signals from different immune players. Furthermore, flu infection cannot be mimicked outside the body.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are able to minimise animal numbers to obtain meaningful results by drawing on experience and include statistics into designing experiments. We have set a minimum threshold for accepting differences between groups tested.
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 studied flu for many years and have a fully optimised and safe system in place that resembles human infection closely. We have many tools available in mice that enable us to study the immune system in detail which are not available in other models. Furthermore, we are using the latest technologies in order to maximise information we can obtain from a single mouse.

Project	**177. In vivo assessment of novel injectable hydrogel biomaterials for spinal disc repair**	
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)	Lower back pain is the main cause of disability worldwide and affects many people. Lower back disease causes soreness and stiffness in the lower back and there is no specific cure, with many people suffering from lifelong discomfort and loss of function. There are a number of causes of lower back pain but one of the commonest is damage to the intertervertebral discs of the spine. These discs act as shock absorbers between the bones of the spine. When the discs become damaged they lose their shape and shrink, which leads to pain and	

	discomfort in the back. At the moment, treatment of lower back pain relies primarily on physiotherapy and pain killers and there is an unmet clinical need for a direct treatment of disc damage. One strategy for treating disc damage is to inject the shrunken discs with a substance to increase the size of the disc and restore its function. However, currently, products that are suitable for disc enlargement are very thick and need to be injected into the disc through large needles, which themselves cause further damage. The aim of project is to use new, safe, biocompatible substances that can be injected through very small needles, to treat naturally occuring disc disease in sheep. The substances have been developed in our laboratory and shown to be safe for use with cells. We now need to show that these substances can be used to treat disc damage in an animal before clinical trials in humans can be performed. The aim of this project is to investigate 2 such newly developed artificially created substances ('biomaterials') which can be used to increase the size of a damaged intervertebral disc and potentially restore its function. In this way, we are hoping to develop a new therapy for disc degeneration and thus lower back pain.
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)?	Lower back pain is a very common cause of pain and loss of movement. One of the most important causes of lower back pain is intervertebral disc degeneration. If successful this project will demonstrate that up to 2 novel biomaterials can be used to restore the function of damaged discs. In the short term this work will benefit scientists who work in biomaterial research and in the longer term will be of benefit to the doctors who treat people with disc disease and human patients with disc disease.
What species and approximate numbers of animals do you expect to use over what period of time?	36 sheep over 5 years
In the context of what you propose to do to the animals,	The sheep exhibits naturally occurring disc degeneration and, by using these naturally

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	affected animals, we avoid the need for painful interventions to induce disc damage. We will select sheep for this study by taking a radiograph of the spine of sheep older than 4 years (more likely to have disc degeneration). When the sheep have been identified they will be kept as one group and have their activity monitored for 4 weeks using a Fitbit activity tracker attached to their collar. After 4 weeks, sheep will have a general anaesthetic so that a high resolution Image (MRI or CT - the best method to be determined during the project) of their discs can be acquired. This image will accurately identify the abnormalities in the discs and how much damage they have. This will be the damage we are aiming to treat. They will then have a maximum of 3 damaged discs injected with biomaterial or a control substance. We will inject up to 3 discs to make the beneficial effects of the treatment significant to the animal and so that we can monitor changes in behaviour (measured by Fitbit) associated with the treatment. The expected adverse effects of the study are transient soreness associated with these injections and all animals will receive drugs to prevent pain after surgery. After an initial week immediately after surgery during which time the sheep are kept in small groups of 3-6, all sheep will be kept in a grass field for 12 weeks. At the end of the study the animals will be killed humanely so that we are able to collect information from the tissues for the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Developing a successful treatment for a disease or disorder takes many years. The biomaterials that will be used in this study have been tested thoroughly in the laboratory for tissue compatibility before their progression to use in animals in this project and many possible biomaterials that are not suitable for this purpose have been abandoned at the laboratory stage. We now need to use animals is the final part of this study so that we can show that the biomaterials that have progressed into animal work are effective ie can restore disc size and shape and reduce pain. This final part of the work cannot be completed in anything other than an animal as the effect of the

	biomaterial needs to be evaluated in a full biological system in which pain can be monitored. If successful, the biomaterials can then be used in humans subject to appropriate regulatory approval by relevant bodies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will always use the least number of animals necessary to achieve the aims of the project whilst achieving statistically meaningful results. For this study we will use 6 animals per group, a number worked out from similar studies that have already been published. In our research we randomise our experiments – for example we randomise which animals get which treatment and in what order they undergo surgery. In some instances we ask researchers we work with outside our laboratory to randomly allocate animals to experimental groups. We believe that these methods contribute to the robustness of our data interpretation and such robustness reduces overall animal numbers used on studies by ensuring that the results are valid and not biased (and do not need repeating by other groups). In addition we use control animals to ensure that the response that we are measuring is real, providing further evidence that an observed response is genuine and ensuring that experiments do not have to be repeated, using more animals.
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 sheep as our model for a number of reasons. The sheep exhibits naturally occurring disc degeneration similar to that seen in humans and, by using these naturally affected animals, we avoid the need for painful interventions to induce disc damage for study purposes] The sheep has an intervertebral disc of similar structure and anatomy to the human meaning that the results in sheep will be readily comparable or 'translatable' to the human situation. The sheep also has a sufficiently large intervertebral disc to allow injection of the biomaterials through a small needle of similar size to that which would be used in human patients. Therefore, if successful, the new materials could be used in human patients without further experiments being undertaken subject to relevant approvals. The only other animal that could be used in a similar way is the goat, however we have expertise in the anaesthesia and handling of

	sheep as well as having developed our pain monitoring methods on sheep and so we feel that sheep will be provide us with more information than goats would. We will minimise animal suffering by ensuring that animals receive anti-inflammatory medication, similar to ibuprofen, during and after spinal injections and the spinal injections themsleves are given under anaesthesia.All of our animals are kept together as a flock after the first week after surgery. In the first week after surgery they are kept in small groups of 3-6 animals. After this first week, they have unrestricted access to grass fields – they are kept as naturally as possible. We monitor our animals movement continuously through the experiment using a 'Fitbit tracker', attached to a collar worn around the animals neck and any animal that is showing abnormal movement or behaviour that could be indicative of reduced activity/pain can be quickly identified and any necessary treatment promptly given.

Project	**178. In vivo imaging of wild-type/immunocompromised/transgenic 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
		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)	Imaging of disease processes in humans, either to detect disease within the body, predict or measure the effectiveness of a specific therapy, is one of the pillars of the new "personalised" approach to medicine and therapy. The advancement of this field requires the development of novel tracer molecules which can be injected into patients, whereupon they find their way to the disease site and their presence can be detected by scanners. This can then tell us about the chemistry of biological processes within the diseased tissue. Each disease process needs a new tracer, which is developed through an interdisciplinary process involving chemists (to	

	design and make them), biologists (to discover the biological target), medics (to set the clinical challenge and identify the needs of patients) and physicists (to optimise the imaging equipment and image reconstruction). The new tracers have to be evaluated in normal/healthy animals before they can be further tested in animal disease models and then finally be used for the first time in humans.
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)?	Imaging is intrinsically a technique designed to extract biological information from animal studies with relatively low animal numbers. Thus, imaging offers better information quality, information yield per animal, and the possibility to detect unexpected and unsought observations, all of which are highly advantageous in most if not all biomedical and clinical fields. The expected immediate benefit of research under this licence is the ability to make an informed decision whether to test the new contrast agent in humans, or to test further in an animal model of disease under another licence, or to abandon the agent, or return to the laboratory for further modification. Better contrast agents and chemistry will improve the quality of imaging. Making the radiochemistry of labelling tracers simpler and more robust, will eventually lead to wider availability to more patients. Whether directly by the development of new imaging technologies, or indirectly by use of imaging as a tool in basic biomedical research, better quality, availability and applications of imaging technologies will lead to better clinical decision making. Consequently this would lead to better quality of life for patients, reduced drug development costs, and reduced costs for health services. Once diseases have been identified in a patient, imaging also has the potential to non-invasively evaluate therapeutic efficacy, providing rapid feedback on therapeutic or interventional effectiveness. The beneficiaries will be patients, health services and pharmaceutical companies.

What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use at most, 3000 mice, 4000 rats and 100 rabbits within the 5 year period of the 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?	Under Protocol 1 (containing a series of procedures), an animal will generally undergo intravenous injection of a contrast agent which would allow internal parts of the animal to 'light up' when imaged. This injection and the imaging session would be done while the animal is under general anaesthesia to minimise its discomfort and to prevent movement during the imaging session. No adverse events are expected to be caused by the imaging methods used. Any adverse events expected are usually related to induction and maintenance of anaesthesia (animals may die from respiratory depression <1 -2 % and/or hypothermia). However, we endeavour to keep the animals warm and monitor their respiration rate during their anaesthesia/imaging sessions. Where possible and if required, other procedures may be conducted whilst the animal is under anaesthesia to minimise any pain and distress (e.g. blood sampling). During the majority of these types of experiments, animals may only experience the transient discomfort of anaesthesia induction but all other procedures such as administration of contrast agents, imaging, blood sampling and culling, would occur whilst the animal is under anaesthesia and therefore would not experience any pain. Less frequently, withholding of food (but not water) prior to an imaging session may be implemented to mimic imaging conditions in the clinic. However, withholding of food coupled with imaging would not be repeated unless the animal had fully recovered (eating and drinking normally) over the following 28h period. In some experiments animals may be given a 'modifying' agent which may be a substance to block the targeting ability of the novel contrast agent under investigation to demonstrate that the targeting mode is via a specific mechanism. Such agents may be given to the animal in food or drinking water prior to the imaging experiment or injected via other routes such as

	sub-cutaneous, intra-peritoneal, intra-muscular, intranasal, intra-tracheal or via oral gavage. These administration methods may be performed either whilst the animal is under anaesthesia or awake but these would lead to no more that transient discomfort. These modifying agents themselves are not expected to cause any harm to the animals as these agents are generally well understood compounds with regards to dosage, mode and duration of action. Less frequently, when testing novel contrast agents which have been radiolabelled (made radioactive) to allow us to track this compound within the mouse body, we place an individual animal into a single cage with a grid floor. The reason for this is to separate the faeces and urine excreted from the animal. Mice will eat their own faeces, thereby confusing the data by eating radioactive faeces containing the radioactive compound or metabolites. As the excrement is radioactive, we are able to remove this from the cage (from below the grid floor) and account for the radioactivity which is no longer inside the animal. To minimise the discomfort to the mice, we place environment enrichment within the cage (such as cardboard rolls), to give them areas of rest and reprieve. This type of experiment would be conducted for no more than 3 consecutive days per week. Isolating social animals such as mice may cause them stress, but where possible, the mice will be returned to their cage mates in their usual cages after the experiment. Efforts are constantly being made to optimise anaesthesia, administration of substances and avoid unexpected adverse effects &/or deaths. Therefore the worse case scenario in terms of animal experience within this licence would be due to repeated anaesthesia/imaging sessions and transient discomfort from an administration methods performed whilst the animal is awake. We anticipate that most animals under this licence will experience only the distress of being put under anaesthesia, as all other subsequent procedures, including culling, would be carried out under anaesthesia where possible. All animals will be humanely killed at the end of the experiments and tissues harvested for further study.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals have to be used because 1. Agencies which govern testing of novel drugs/probes/methods in man, require animal data which provide information and guidance to help design human clinical trials. 2. To validate mode of action, experiments are required that cannot be conducted in humans for ethical and scientific reasons. 3. Bio-distribution in whole organisms (i.e. tracking the injected agents route/ accumulation and excretion through the body), with intact biological barriers and excretion mechanisms, is key to clinical use. Non-animal alternatives cannot replace the complexities of the interactions of these probes in whole body systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary *in vitro* studies (i.e. performed on cells or tissues grown or maintained outside the body) will eliminate unsuitable candidates which will not progress to *in vivo* (animal) studies, thus reducing the numbers of animals. The use of imaging to determine bio-distribution of novel contrast agents within the animal rather than conventional killing at sequential time points, dissections and organ analysis is a major contributor to reduction of animal numbers. Imaging allows repeated observations/measurements over a period of time (longitudinal study) on the same animal, with humane killing only at the last time-point. Thus, if a longitudinal study involves six time-points, the numbers of animals are reduced to one sixth by use of repeated imaging. Since each animal serves as its own control to compare different time-points, the data obtained are statistically more robust (reduction), requiring fewer animals. Moreover, distribution of contrast agent within organs, not just between organs, is obtained, and unexpected uptakes that may not be detected by conventional methods can be found by whole body scanning. All these attributes of imaging contribute to a

	greatly improved benefit:cost ratio (benefit = data quality and quantity, cost = animal numbers, procedures and their severity). Additionally, we use 'pilot studies' which are small experimental groups of animals which help us to decide quickly how best to design a statistically and scientifically valid experiment. Thereby helping develop better larger study design and reduce possible suffering.
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: Mice and rats are the species of lowest neurophysiological sensitivity that provide the necessary size compatible with the scale of resolution or movement associated with the imaging techniques being studied. Resolution of the whole body imaging techniques is of the order of 0.5 - 1 mm. Distribution within smaller animals will be beyond these limits. Less frequently, experiments will be conducted in normal healthy rabbits prior to further work in appropriate disease models tested under other licences (e.g. Imaging Cardiovascular disease). For example, rabbit is the only available clinically relevant model of atherosclerosis and controlled plaque rupture leading to thrombosis, which mimics the human condition. Currently, this clinical event cannot be reproduced in rodents. Thus rabbits are imaged under this particular licence to provide non-diseased control information. This data is valuable in helping us interpret data derived from the diseased animal model. Generally, inhalation anaesthesia will be used to minimise transient pain and distress and where possible, used for blood sampling, contrast injection, weighing and combined with imaging techniques where it is mainly used for restraint. In addition, there would be full and complete recovery between periods of anaesthesia and/or food withdrawal; rehydrating of animals during long imaging sessions; monitoring of respiration and/or cardiac function and maintaining body temperature during imaging. These steps will all be conducive to maintaining the animal's wellbeing.

Project	**179. In vivo testing of novel targeted treatments in neoplasias with a defective DNA damage response**	
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)	Current therapies for cancer rely on DNA damaging regimens, such as chemotherapy. Tumours with a defective DNA damage response tend to respond poorly to such treatment. This project aims to understand how an aberrant DNA damage response alters cellular behaviour and leads to resistance to chemotherapy. By understanding this process we hope to develop novel therapeutic approaches to overcome this.	
What are the potential benefits likely	Improved understanding of how the loss of a	

to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	DNA damage response contributes to resistance to standard chemotherapies that primarily kill rapidly dividing tumour cells by damaging their genomey. Identification of novel therapeutic approaches to eradicate resistant tumours. In the longer term, the potential of this work to contribute to improving survival times for cancer patients whose tumours have a defective DNA damage response and show a poor response to current therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	Primarily we intend to use immunodeficient mice. However, for some dose-finding experiments we will use non-GA mice and to complement some of the human studies we may use GA mice from which we have generated lymphomas. We will use no more than 3700 mice over the five year duration of this licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will develop tumours as a result of having tumour cells injected into them, either intrafemorally, intravenously or subcutaneously, in the rear flank. Preliminary experiments will be performed using a few mice to determine the optimal route of injection and assess tumour growth kinetics. Tumour development may be monitor by bleeding which will cause stress to the animals from handling and transient discomfort as a result of being bled. With the development of tumours, mice may display lethargy, enlarged spleen, diarrhoea. Weight loss, hunched back and starry coat. Animals will be humanely killed should these happen. We will perform in vitro testing of reagents to determine anti-tumour efficacy, only taking the most promising reagents forwards to testing in animals. We will use the minimal number of animals to provide statistically robust data as determined by power calculations. Wherever possible we will use clinically relevant reagents that have been tested both in mice and man. We will use the most refined methods for delivering these drugs based on published literature. Dosing and treatment regimens will be base, wherever possible on published data and will be initially assessed on a small cohort of mice to ensure

	tolerability. As a consequence of treatment, mice will be stressed from handling and feel transitory discomfort from these procedures. Mice are expected to feel some short-term side-effects including lethargy, anaemia, bruising, loss of appetite, diarrhoea or reduced peer interaction. They may display a hunched back, starry coat and loss of response to their environment. Mice will be monitored daily and humanely killed if they do not show signs of improvement over 48-hours
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Primary tumour cells, such as multiple, myeloma, chronic leukaemia and metastatic prostate cancer, are very difficult to grow in tissue culture. These cells require key factors from their environment to grow and survive. At present, we have not identified the factors that permit long-term culture of these tumour cells. Whilst we will be performing in vitro experiments to rule out drugs that do now have the desired outcome, in terms of killing tumour cells, ultimately pre-clinical assessment of these reagents requires testing in animals. We have undertaken similar studies assessing a drug called a PARP inhibitor in animals which led to a clinical trial to treat people with chronic leukaemia. Animal work will only be undertaken once we have evidence from cell culture systems that they display the desired anti-cancer effect. We routinely review the literature in order to identify novel methods that could be used to replace any aspects of animal work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By designing experiments correctly and taking statistical advice, we will use the minimum numbers of animals required to carry out the work and obtain a statistically significant result. We will also maximise the results that we can obtain from each animal by keeping surplus tissue. Results will be reported according to NC3R's

	'Animal Research: Reporting *In Vivo* Expts.' In order to inform others and, hopefully, avoid duplication of animal work.
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 immunodeficient mice, as we are doing currently. These system models allow for the propagation of tumour cells including leukaemias and lymphomas. So far they have provided a wealth of information regarding tumour growth and in the assessment of novel anti-cancer therapies. Wherever possible we will refine our methods for tumour detection to reduce any adverse suffering of animals, referring to the grimace scales. Where novel reagents are being used, we will initially perform dose-finding and pilot studies to identify optimal doses and any potential adverse effects of the drugs before commencing with studies using larger cohorts of animals. Dosing will be performed base on published data and scientific expertise. We will use the most refined route of administration.

Project	**180. Individual variation in synaptic neurobiology underlying incentive motivation and addiction-like 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
	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)	Using a well-documented animal model of variation in motivated behaviour, we will investigate neuropharmacological methods to: 1) Decrease the ability of Pavlovian cues to excite desire to pursue reward in certain individuals. 2) Decrease the development of addiction-like behaviour in susceptible individuals.	
What are the potential benefits	Studying the neurobiology of motivation and	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	attention in rats may help us to understand disorders such as addiction people. The proposed research stems from our finding that certain rats that are highly-attracted to reward-related stimuli may also display addiction-like behaviours. The brain's dopamine system also functions differently in these animals, and this may result from differential expression of specific dopamine-related proteins. Genetic differences in these proteins have been proposed to relate not only to addiction in people, but also ADHD and autism. Our goal is to investigate how variation in dopamine-related neurobiology in animals has downstream biopsychological consequences that impact addiction-like behaviour. Accordingly, we aim to develop strategies for decreasing potential development of addiction in susceptible individuals. Psychiatrists and clinical psychologists treating patients for mental health disorders will also benefit from our results. For example, patients with substance use disorder may be attracted to environmental cues that are predictive of a drug, and these cues may, therefore, promote drug-seeking. Our findings may suggest that it is possible to reduce the motivational power of these cues, either through cognitive-behavioural or pharmaceutical approaches. Beyond these initial goals, the research has relevance to increasing our understanding of the neurobiology of the brain's dopamine system. Thus, individuals who study a variety of disorders will benefit from the results. Following publication, results will be shared in an online data depository. We will encourage secondary analysis of the data.
What species and approximate numbers of animals do you expect to use over what period of time?	~1344 rats, 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 described below, a significant portion of rats will experience only behavioural testing and will likely not encounter forms of distress. It is possible rats may be fed a specific amount of food at a certain time of day; this will help to motivate animals to pursue a food reward during behavioural tests. The weight of rats will be carefully monitored for abnormal gains or losses.

	1) ~240 rats will only undergo behavioural testing; they will perform tasks in chambers and consume food rewards. We don't anticipate adverse effects of these studies, as they involve the performance and observation of natural behaviours. Therefore, this procedure is considered mild in severity. At the end of this experiment, rats will be humanely killed under anaesthesia. Their brains will be removed, and the tissues will be processed to investigate individual variability in neuronal structure and biochemistry. 2) ~384 rats will undergo behavioural testing and will also receive systemic injections of a drug. This procedure is considered moderate in severity. These injections will be carefully administered. While we do not anticipate any adverse effects of these injections, the health and behaviour of rats will be carefully monitored and documented. At the end of this experiment, rats will be humanely killed under anaesthesia. Their brains will be removed, and the tissues will be processed to investigate individual variability in neuronal structure and biochemistry. 3) ~720 rats will undergo behavioural testing, but will also receive surgeries that enable the injection of pharmaceutical compounds directly into certain brain regions. This procedure is considered moderate in severity. Surgeries will be performed in anaesthetised rats using standard aseptic techniques, with the use of pain-relief medications. Rats will be carefully monitored for signs of distress multiple times per day for at least 1 week following surgery. After recovery from surgery, rats will receive injections of drugs into specific brain regions, with the goal of reducing motivated and addiction-like behaviours. These injections will be carefully delivered over minutes in unrestrained rats, thereby minimising any forms of distress. While we do not anticipate any adverse effects of these injections, the health and behaviour of rats will be carefully monitored and documented. At the end of this experiment, rats will be humanely killed under anaesthesia. Their brains will then be removed to confirm that injections of pharmacological compounds were successful.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To analyse the relationship between brain activity and behaviour, intact behaving animals are required. These experiments cannot ethically (or practically) be performed in people. The proposed experiments study the neurobiology of individual variation in motivation and addiction-like behaviour, while using translational techniques that model addiction in people. Studying such individual differences makes the research highly relevant, as people also vary in their behaviour. We choose to study rats because they are capable of learning complex sequences of behaviour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Behavioural and surgical procedures have been optimised in our previous research, thereby minimising the number of animals used. Where possible, a within-subject experimental design will be used, and this will help reduce the number of animals. Appropriate statistics will be used to minimise the number of animals used. For all studies, power analyses have been performed to help determine the number of rats required to obtain valid and reliable results.
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.	Every effort will be made to refine procedures and minimise potential sources of distress. Rats will be kept on a natural reverse light-cycle, given suitable bedding, shelter, chew blocks, and nesting material in appropriately-sized cages. Rat health will be monitored at least daily. All surgeries will be performed aseptically using combined general and local anaesthesia, according to well-established protocols. Following surgery, animals will be closely monitored for at least one week, given post-operative analgesic and anti-inflammatory agents based on Home Office practices and veterinary advice, and provided with palatable food to minimise pain and suffering. For injections, only safe volume sizes and needle gauges will be used. Individuals performing these experiments will be highly-trained and supervised. We will regularly undergo thorough reviews of our procedures with a veterinarian, helping us to improve and refine our protocols.

Project	**181. Inflammation in Arterial Disease and Co-morbidities**	
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 will study inflammation occurring in artery walls which, when higher than usual, can cause a heart attack. In 2017, it was proved for the first time that a drug blocking inflammation led to a lower number of repeat heart attacks and fewer strokes in patients. However, the specific protein blocked by the new drug is important in fighting infections so there were some unexpected patient deaths in the study from uncontrolled infections. We can now devise ways to block similar inflammatory actions in artery walls. The aims of the study are to investigate and design new and better targets/drugs to inhibit inflammation in artery	

	walls in patients with heart attacks and other related conditions.
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 research will lead to a greater understanding of the key molecules that control inflammation in artery walls. We will begin to understand how other conditions such as pneumonia, flu or low blood sugars affect inflammation in artery walls. From these studies we will be able to pinpoint events and molecules that could be new targets for treatments, or identify existing drugs used in other conditions that could also be useful in preventing heart attacks.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 10200 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?	Where we feed Western diets, the mice (like humans) become unhealthy, with greasy fur and skin irritation of mild-moderate severity. Sometimes, when we study diseased arteries alongside other conditions like pneumonia the animals may show altered behaviours or weight loss (similar to humans). If these effects are too severe, we will humanely kill the animals. At the end of the experiment, the animals will be killed and their tissues with be examined and measured.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Alongside biological mechanisms in artery walls, physiological measures e.g. blood pressure, heart contraction and brain health will be measured in adult and older adult mice. It is not possible to study these in laboratory dishes or in other non-protected animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be carefully planned and statistical calculations will be constantly checked to ensure the minimum number of animals is used. This approach will be used each time a new individual study plan is prepared.

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 still offers the best model for our studies and we have shown that our previous animal studies have helped bring new treatments to patients. We are fortunate to be able to use some of the latest techniques to measure blood pressure and to image the heart and brain enabling more and better data from one animal. General measures to minimise harms are microsampling, the use of specialised caging, individualised study plans and health and welfare recording especially for more complex procedures involving more than one condition.

Project	**182. Inflammation in the development and progression of liver diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The Objective of this study is to investigate the mechanisms by which the defence systems within the body are activated as a result of liver disease. The current evidence suggests that the loss of control of these systems may be affecting other organs and increasing the rate of disease progression. We aim to better understand these effects and to test novel therapies that may improve the lives of patients with liver disease. One example of this is where changes in neurological function occurs as a common feature of liver disease, but is often misdiagnosed. As the	

	incidence of liver disease continues to increase, the number of subjects being affected by debilitating loss of brain function is increasing, affecting memory, attention and coordination, having a major impact on quality of life. By gaining a better understanding of how liver disease affects the brain we can develop better treatments to treat the condition.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are a number of proposed mechanisms by which other organs are functionality impaired as a consequence of liver disease, these include elevated blood ammonia levels and the effects of infection/inflammation. We have already devised a new drug that reduces ammonia in the body, which appears to aid patients in the clinical trials conducted so far. By investigating the mechanisms of disease further we aim to develop and test new therapies targeting alternate approaches to just ammonia reduction.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a five year period it is expected that up to 5500 mice and 4200 rats could be produced, though with good husbandry and careful genetic testing it is likely that far fewer animals 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?	The procedures involved will not exceed moderate severity. The methods for developing liver disease are well established and have a low incidence of adverse events in our hands (less than 5% for surgical models; less than 2% for feeding models). Should any animal display signs of pain following surgery they will be treated with analgesics, should symptoms continue advice will be sought from the named veterinary officer. It is possible that animals may experience discomfort, lethargy and poor appetite as liver disease progresses, although typically these effects are minimal in most cases and are monitored closely to ensure the animals' well being. Animals will be humanely killed if they show signs of distress or if the symptoms progress overly rapidly, most likely indicating the presence of infection (though this occurs rarely). At the end of the studies the animals will be killed humanely.
Application of the 3Rs	
1. Replacement	Liver disease is complex and multi-factorial. Although the liver is the main injury site, there are profound

State why you need to use animals and why you cannot use non-animal alternatives	effects on other organs causing dysfunction and ultimately failure. Typically it is the brain, kidneys and circulatory system that are affected by loss of liver function, with a system wide modification of the immune system leading to increased risk of infection. As such, the majority of studies must be conducted in live animals. Cell culture systems and computer models are useful tools, but are currently unable to replicate the complex interactions that exist between the various body systems. The studies require the investigation of mature organs and fully functional immune systems, as such it is not possible to conduct studies on embryos or neonates. The models planned under this project provide insights into how the cross-talk between how the various organ systems work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We work extensively with statisticians and utilise tools to ensure good model design to minimise the numbers of animals involved. We will also use non-invasive imaging and neurological testing tools to use animals on more than one occasion, thereby increasing statistical power and reducing the number of animals involved. Where possible, experiments will be co-ordinated to allow overlapping projects to make use of materials (e.g. tissue samples) obtained from these studies, hence reducing the number of animals used. Where possible we will utilize animals in longitudinal behaviour and/or imaging studies. Thereby increasing statistical power and reducing the numbers of animals required. We also confirm that our studies will be conducted and recorded in accordance with the ARRIVE guidelines [https://www.nc3rs.org.uk/arrive-guidelines] and will use randomisation, blinding etc. where appropriate so as to minimise biases.
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 will be used for the majority of studies, making use of genetically modified animals to investigate the role of specific proteins in the development of liver disease. Rats will provide larger models for use with apparatus that cannot be used in the small models. Rats will also provide larger sample sizes to reduce the overall number of animals required. Careful monitoring will be conducted in all studies to minimise suffering and remove any animals

	exhibiting signs of distress.

Project	**183. Inflammation in the development and progression of liver diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The Objective of this study is to investigate the mechanisms by which the defence systems within the body are activated as a result of liver disease. The current evidence suggests that the loss of control of these systems may be affecting other organs and increasing the rate of disease progression. We aim to better understand these effects and to test novel therapies that may improve the lives of patients with liver disease. One example of this is where changes in neurological function occurs as a common feature of liver disease, but is often misdiagnosed. As the incidence of liver disease continues to increase, the number of subjects being affected by debilitating loss of brain	

	function is increasing, affecting memory, attention and coordination, having a major impact on quality of life. By gaining a better understanding of how liver disease affects the brain we can develop better treatments to treat the condition.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are a number of proposed mechanisms by which other organs are functionality impaired as a consequence of liver disease, these include elevated blood ammonia levels and the effects of infection/inflammation. We have already devised a new drug that reduces ammonia in the body, which appears to aid patients in the clinical trials conducted so far. By investigating the mechanisms of disease further we aim to develop and test new therapies targeting alternate approaches to just ammonia reduction.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a five year period it is expected that up to 5500 mice and 4200 rats could be produced, though with good husbandry and careful genetic testing it is likely that far fewer animals 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?	The procedures involved will not exceed moderate severity. The methods for developing liver disease are well established and have a low incidence of adverse events in our hands (less than 5% for surgical models; less than 2% for feeding models). Should any animal display signs of pain following surgery they will be treated with analgesics, should symptoms continue advice will be sought from the named veterinary officer. It is possible that animals may experience discomfort, lethargy and poor appetite as liver disease progresses, although typically these effects are minimal in most cases and are monitored closely to ensure the animals' well being. Animals will be humanely killed if they show signs of distress or if the symptoms progress overly rapidly, most likely indicating the presence of infection (though this occurs rarely). At the end of the studies the animals will be killed humanely.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Liver disease is complex and multi-factorial. Although the liver is the main injury site, there are profound effects on other organs causing dysfunction and ultimately failure. Typically it is the brain, kidneys and circulatory system that are affected by loss of liver function, with a system wide modification of the immune system leading to increased risk of infection. As such, the majority of studies must be conducted in live animals. Cell culture systems and computer models are useful tools, but are currently unable to replicate the complex interactions that exist between the various body systems. The studies require the investigation of mature organs and fully functional immune systems, as such it is not possible to conduct studies on embryos or neonates. The models planned under this project provide insights into how the cross-talk between how the various organ systems work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We work extensively with statisticians and utilise tools to ensure good model design to minimise the numbers of animals involved. We will also use non-invasive imaging and neurological testing tools to use animals on more than one occasion, thereby increasing statistical power and reducing the number of animals involved. Where possible, experiments will be co-ordinated to allow overlapping projects to make use of materials (e.g. tissue samples) obtained from these studies, hence reducing the number of animals used. Where possible we will utilize animals in longitudinal behaviour and/or imaging studies. Thereby increasing statistical power and reducing the numbers of animals required. We also confirm that our studies will be conducted and recorded in accordance with the ARRIVE guidelines [https://www.nc3rs.org.uk/arrive-guidelines] and will use randomisation, blinding etc. where appropriate so as to minimise biases.

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 will be used for the majority of studies, making use of genetically modified animals to investigate the role of specific proteins in the development of liver disease. Rats will provide larger models for use with apparatus that cannot be used in the small models. Rats will also provide larger sample sizes to reduce the overall number of animals required. Careful monitoring will be conducted in all studies to minimise suffering and remove any animals exhibiting signs of distress.

Project	**184. Information Processing in mammalian brain**	
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 take it for granted that we can hear a particular voice in a crowd or comprehend the same word when uttered by different people. However, certain conditions and pathologies compromise this ability. The objectives of this project are to understand how natural sounds such as speech are processed by the healthy and damaged brain.	
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 insight we will obtain from the research will be an important step towards the prospect of decoding sensory information processing in the brain. This understanding is essential for development of brain-machine interfaces (e.g., cochlear implants) and will be of great value for	

	scientists working in the field of artificial intelligence, robotics and neuroinformatics as well as for software developers making use of these approaches. By understanding how the brain operates at this level we hope to provide clues that will guide therapeutic interventions to unlock the brain's limited ability to recover after trauma, cerebrovascular accidents and tumours.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use approximately 2000 mice and 750 rats over the course of 5 years. The total number of mice and rats to be used over the entire 5 year duration of the project have been determined according to the average data yield of the experimental methodologies that will be employed to meet our scientific objectives.
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 the majority of our animals to undergo anything beyond mild suffering. However, those under protocol 2 will undergo recovery surgery, and therefore be exposed to moderate suffering. Animals will usually undergo a surgical procedure (always under general anaesthesia) and will be closely monitored and provided with pain relief before and after surgery. Animals will be constantly and closely supervised by trained individuals. All animals will be humanely killed at the end of an experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We require the use of an animal model to uncover the complex and unknown underpinnings of how natural sounds (such as speech) are processed by the brain. Any non-protected animal alternative will provide an inadequate model of these complex phenomena, because they do not have an auditory cortex (a region of brain we use to process sound).
2. Reduction Explain how you will assure the use	Where possible, we will use computer modelling to test hypotheses prior to experimentation on animals. In addition, we will use very recent methods that maximise the

of minimum numbers of animals	amount of data collected from each animal, which for example will allow us to record from many brain cells at once. Furthermore, we will also use brain slices from those animals after experiments for further analyses.
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 at all times strive to minimise harm to animals, performing experiments under terminal anaesthesia except where it is necessary to simultaneously measure brain activity and behaviour. Animals are constantly and closely supervised by trained individuals and advice is sought from the veterinary team if there is any cause for concern. Overall, we use anaesthesia, analgesia, and humane endpoints to limit suffering.

Project	**185. Injury mechanisms and therapeutic targets for perinatal brain 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
		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 work is to understand normal developmental processes in the brain, and how these processes are perturbed by inflammation or other injuries. It will identify mechanisms of injury during gestation or in the perinatal period that have long-term effects on brain function and animal behaviour. We will model mechanisms of injury observed in the clinical to identifying and test novel therapeutic agents.	
What are the potential benefits likely to derive from this project	This work will identify process of brain injury that can be targeted with drugs to improve the lives of	

(how science could be advanced or humans or animals could benefit from the project)?	significant numbers of babies and their families by reducing the severity of injury. We are also asking novel questions about how the brain develops and the processes by which it may be injured. This will be of substantial interest to all researchers in the field of brain development. In addition, we aim to identify sensitive and specific biological markers of injury (e.g. with MRI), that will aid detection of injury and selection of patients for appropriate future therapy. In the laboratory, this work will ultimately reduce the number of animals required for experiments, as each animal will be able to be imaged at multiple time point. Currently animals need to be killed at all time points so that the results of the MRI can be compared and verified by other methods.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 8000 mice will be bred for this project, both transgenic and wild type, and either the pregnant animals or the newborn pups used for experiments. Up to 1000 pregnant mice will have either treatment of the mother or foetus directly. The effect of these interventions on the foetal (and early postnatal) brain development will be assessed hours or days after the intervention. Up to 6800 of the breed animals will be used postnatally, with interventions occurring over the first three postnatal weeks, and with outcomes measured within minutes to hours through to adulthood. Preterm brain injury will be modelled by inflammation and oxygen deprivation.
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 maternal inflammation models, mothers will typically receive one or two injections and experience mild ‘flu like symptoms. Alternatively, the mothers will be anaesthetised and the foetuses will receive an injection directly to the brain. The maternal surgery is quick, and pain relievers will be used. In both cases, the mother and the pups will be killed by Schedule 1 methods either before or after birth, possibly after a second injection of a potential therapeutic agent. The overall severity of both these protocols is moderate. In newborn mice, at different times over the first week of life, mechanisms of injury and therapy will be explored in by either peripheral or direct brain administration of inflammatory agents or other

	brain signalling molecules, or by reduced blood supply to the brain. If a direct brain administration route is required, or for producing reduced blood supply, surgery will be performed under anaesthesia and with pain relief and is expected to only cause short-term pain and distress. The overall severity of this is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our project aims to understand the mechanisms involved in early life infectious/inflammatory and oxygen deprivation-induced brain injury and to test neuroprotective strategies. As such, we need models that allow us to mimic human brain injury that occurs before or at birth, in systems where we can modulate the genetic and environmental stressors of injury. In particular, our experiments take a whole body approach, considering how multiple cell types interact over development and injury, both within the brain and influenced by the rest of the body. Experiments are required that cannot be conducted in humans for ethical and scientific reasons. In addition, for pharmacological studies, distribution of the drug in whole organisms, with functioning organs, is key to inferring potential clinical use. Where possible we will replace whole animals studies with primary cell preparations or experiments in appropriate cell lines (e.g. for neuronal stem cells, microglial, oligodendrocytes, endothelial cells) for our preliminary and proof-of-concept studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our intention is always to use as few animals as possible for ethical reasons. At the same time sufficient numbers have to be used to ensure accuracy of results obtained. Through our previous work, we have optimised the conditions for the proposed experimental models, therefore ensuring the reproducibility of the injury and reducing the number of animals needed for each experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will	There are other animal models we could use, some in which the brain structure is more similar to that of a preterm or term human (foetal sheep,

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.	primates, piglets). However, we have decided to replace the use of such animals with mice, without detriment to the science. This specie is considered to be less sentient, easier to handle, breed easily and have a short generation interval. Importantly, mice share several important features with the human brain with regard to brain complexity and injury response in white and grey matter and thus can be considered a valid model in which to deliver the objectives of the project. While mice are small, and there are difficulties in performing complex surgical procedures in these animals, they have the advantage of responding well to anaesthesia and pain relief measures and recovering quickly from interventions. Our team has sufficient expertise in small animal surgery to ensure procedures are as minimally invasive and quick as possible to reduce the cost to the animals. The brain injuries produced are mild and there is no evidence (from monitoring normal behaviour and interactions in their home cage) that the mice experience distress or pain subsequent to the initial injury induction.

Project	**186. Innovative Approaches for Mucosal Vaccination**	
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)	This project is focused on the development of highly innovative new vaccines that use bacteria as the delivery system. The long-term goals are to see these vaccines used for preventing disease in humans and in animals. Their innovation is that they can provide superior and localised immune responses that are heat-stable and are safe. There exists a real and immediate need for vaccines that are heat-stable enabling simplified storage and distribution of vaccines and this is particularly so in developing countries where many people fail to receive adequate vaccination because of poor distribution and storage facilities. In developed countries non-injectable vaccine are	

	attractive and likely to improve the quality of life. In addition, many existing vaccines carry risks associated with their use and can lead to serious side effects or poor immunogenicity requiring frequent boosters. It is with this in mind that multiple strategies for new vaccination strategies need to be addressed. In this proposal bacterial spores as well as live bacteria will be assessed for their ability to confer protection in animal models of infection. Beyond this, the aim is to see the evaluation of these vaccines in human clinical trials and eventual consideration as licensed vaccines for human or animal use. All vaccines to be considered for evaluation in humans must first be assessed in animals. For this reason this project will establish the efficacy of prototype live bacterial vaccines in animal models. Our project will first design and construct prototype bacterial vaccines using existing platform technologies. Those showing the most promise with regards to their stability and capacity to express target immunogens will be evaluated for immunogenicity in animal models. We will use mice because they enable experiments to be conducted efficiently and with minimal animal suffering. In addition hamsters will be used since they are specifically recommended for evaluating vaccines to C. difficile. This project is focused on two diseases for which there exists no licenced vaccines, *Clostridium difficile* infection (CDI) and *Helicobacter pylori* infection (HPI). CDI is a disease that is acquired in hospitals and mostly with the elderly population. The disease leads to ~2,000 deaths/annum in the UK and is caused by a bacterium that has a high probability of acquiring antimicrobial resistance. HPI is a disease that mostly is of concern in developing countries particularly in SE Asia where the impact of this disease substantially increases the risk of acquiring gastric cancer.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit	Neither CDI or HPI have vaccines available. Therefore, we have no method to control or prevent infection and must resort to the use of antibiotics. This work would provide a possible

from the project)?	solution with vaccines evaluated and suitable clinical evaluation in humans and ultimately, vaccines available for humans.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will primarily use mice, hamsters for work with C. difficile and mice and gerbils for work with H. pylori. Over five years we predict 4,000 mice, 600 hamsters and 400 gerbils sufficient to conduct this work. In addition, we will use up to 40 rabbits as needed for preparation of immunological reagents.
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 dosed with prototype vaccines to determine their ability to mount an immune response. Dosing would be by injection or by a mucosal route (oral delivery or nasal delivery). In some cases, we will go on to determine whether animals that have been immunised (as described) will then be able to prevent infection by exposure to the relevant pathogen (a challenge). Some of the procedures (about one third) we will conduct will produce symptoms of disease that may cause discomfort to animals. However, procedures are designed such that first signs of animal discomfort are immediately recognised and animals will be killed using a licenced Schedule 1 procedure. Considerable effort is made with monitoring of animals and in the case of some procedures this equates to continuous monitoring such that first signs of animal discomfort are recognised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Vaccines that are to be evaluated in humans are expected to be first assessed rigorously in animal models of infection. This is a requirement of the regulatory authorities that permit clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have designed our experiments carefully and with sufficient numbers to reduce the possibility of failed experiments that ultimately must be repeated. Thus, careful experimental design ultimately reduces the numbers of animals used.

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 preferred animal model for immunological studies since they are inbred and produce the most consistent experimental data. Hamsters are required for evaluating C. difficile vaccines since they respond best to the pathogen and thus provide the most reliable data to the scientist. Only well trained and skilled staff will be used to oversee and perform animal studies. This is the single most important step in reducing animal suffering.

Project	**187. Interactions of immune cells controlling immunity and cancer progression**	
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)	Our immune system is our defence against the outside world. It is a complex system of many different cell types with specific tasks. These range from detection of infection to the detection and direct killing of infected cells. Whilst we know what each cell type is capable of, the ways in which different cells work together has remained more mysterious. For efficient protection, immune cells must patrol every inch of our body and travel enormous distances to relay messages should they detect danger. This role is carried out by	

	immune cells named dendritic cells. Dendritic cells are guided towards lymph nodes, where they can relay their message, by non-immune cells that form the structures of vessels and the architectural underpinnings of lymph nodes. It has only recently been acknowledged that the non-immune cells, broadly termed stromal cells, in fact play an important role in regulating the outcome of immune responses, and is fast becoming one of the most exciting areas of immunology research. I have recently shown an important reciprocal crosstalk between immune cells (dendritic cells) and non immune cells (fibroblastic stromal cells). This interaction which occurs as these cell types come into contact enhances both the migration of the immune cell (dendritic cell) and causes the non-immune cells (fibroblasts) to relax and elongate, which was required for our lymph nodes to swell and enlarge during any immune response. This mechanism showed a completely new role of dendritic cells in initiating immune responses and highlights the importance of looking at immune responses from the point of view of the whole tissue. In my future work I plan to expand on these findings to ask 'how does the non-immune cell stromal architecture affect immune responses?' I will undertake experiments in the immune system, looking at the changes occurring in lymph nodes during the course of an immunisation, and also look at the behaviour of the immune system in cancer. The overall aim of this work being to find ways to enhance immune responses to vaccines and anti-tumour immune responses, to benefit human health.
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 primary potential benefit relates to new knowledge about the mechanisms underlying how immune responses are regulated; both to infection and to cancers.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 500 Mice/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?	Most of the experiments described in this application require the use of genetically-modified mice that express forms of genes that can be inititated at specific times and are therefore mostly indistinguishable from any other mouse, and undergo no adverse effect as a result of their genetic mutation. Other mice express genetically labelled cells that also have no observable phenotype, but allow us to see them using microscopy. For a majority of the experiments, the animals will be bred to produce cells or tissue for in vitro experiments. Some of the experiments will involve manipulation of genes in vivo that will result in changes to gene expression in tumours or lymphoid tissue. Other experiments involve injections of tumour cells to assess tumourigenic potential and spread. These animals will be monitored regularly and any animals showing signs of distress will be humanely culled. Based on previous work, the likelihood of distress in the animals is considered low and the severity level is judged as moderate, for a minority of the animals. Animals will not be allowed to develop tumours greater than 1200mm3 and the tumour mass will not exceed 15% of bodyweight. Some experiments involve in vivo imaging of cell migration. These animals will be anaesthetised throughout each procedure and will be culled at the end of the imaging experiments, if surgical methods were used to expose the tumour site.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Complex processes such a lymph node swelling and full scale immune responses cannot be satisfactorily modelled *in vitro*, therefore it is necessary to use animal models. However, we do work *in vitro* using cell lines to investigate some of the processes involved in cell-cell interactions such as cell shape rearrangements. We also use 3D coculture systems for both immune cell/stromal cell interactions and immune cell/tumour interactions. These model systems can identify important signaling molecules and pathways of potential relevance. However, many effects measured *in vitro* are

	not fully reproduced *in vivo* due to the additional complexity. Therefore it is always critical to test our models in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experimental designs and methods of analysis have been discussed with our experts in statistics. Factorial experimental design will be used wherever appropriate to maximize the information obtained from the minimal numbers of animals required. In brief the sample size will be calculated using power analysis, using a significance level of 0.05, a practicable different between groups of at least 25% and a power of 80%. We expect that 6-8 animals will generally be required per treatment group to obtain statistically relevant datasets. In cases of tumour development, this may need to be slightly higher since not all animals may develop tumours. The *in vivo* experiments outlined in this project make up only one part of our research programme. Our extensive use of *in vitro* methods reduces the need for *in vivo*e xperiments to be carried out. Furthermore, to ensure the most efficient use of all animals bred for this research, I the licence holder will ensure: High standards of animal care, welfare and utilize most appropriate breeding methods Ensure colony sizes are monitored and adjusted according to the requirements at each stage of the project. For example, transgenic lines not expected to be required within the next 6 months will be cryopreserved for future use and not continuously bred. Breeding colonies will always be kept at their minimum size so as not to over produce. Ensure that personal licence holders working on this project are appropriately trained to ensure a high success rate and to minimize the number of experiments that require repeating.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	The mouse is the only mammalian species for which there exists a large number of transgenic models available and techniques are widely available for generation of novel transgenics. The mouse immune system is similar to

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	humans and therefore they provide a powerful tool for modelling human disease and are therefore the model animal of choice, before clinical trials can be undertaken. This project does not include protocols where the outcome is expected to be severe. However the biological resources services oversee a comprehensive health monitoring programme, and therefore where adverse effects may develop, they will be recorded, and appropriate action will be taken to minimize severity or to end the protocol.

Project	**188. Intestinal cancer development and progression**	
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)	Some of the genes involved in the development of intestinal cancer in humans are known, but there are other genes that when altered are likely to play a role in cancer formation and progression. The objectives of this project are to: (a) Identify genes that drive tumour formation. (b) Investigate how these genes interact with environmental factors to contribute to tumour development.	

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)?	Cancer is due to abnormal and uncontrolled cell proliferation that is caused by both genetic and environmental factors. Although much work has been done to improve the understanding of the molecular events that occur during tumour formation, many cancers remain incompletely understood and some are incurable. This project proposes the use of mice to better understand the genes involved and their molecular mechanisms in intestinal tumour formation and progression in humans. Mice represent the ideal model for this study, based on the similarities between humans and mice in terms of anatomical, physiological, pathological and genetic features, together with the ability to make specific changes to mouse genes. This project intends to generate and characterise mice that have genetic changes or mutations in known genes or newly identified cancer genes or combinations of genes that increase susceptibility to intestinal and other tumour formation. This will advance scientific understanding of the processes of development and progression of cancer. The work will identify and characterise new cancer genes, and how they interact with key environmental factors such as alcohol, which may open up new possible avenues for the development of anti-cancer treatments that target these genes and their pathways, or cancer prevention or modification advice that relates to exposure to the environmental factors.
What species and approximate numbers of animals do you expect to use over what period of time?	Genetically altered mouse studies allow changes to be made to specific genes in particular tissues, with study of all stages of development of cancer, including the interactions between genes, cells, tissues, and between tumour cells and the surrounding environment. Importantly, such mouse studies can be used for pre-clinical trials of potential anti-cancer therapies. It is predicted that approximately up to 11,200 mice will be used over 5 years of the programme.
In the context of what you propose to do to the animals, what are the expected adverse effects and the	The primary adverse effects on the mice in this research programme are the development of intestinal tumours. This work will use

likely/expected level of severity? What will happen to the animals at the end?	genetically altered mice produced in other laboratories. 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 tumour formation, or other causes, including intestinal inflammation (up to 24 months of age). and tThus, the expected adverse effects due to tumours will be kept to a minimum, mostly at the mild level of severity (although occasionally moderate), including some rectal bleeding causing anaemia and weight loss (in 10-20% mice). Other mice will be culled at pre-defined time points according to the experimental protocol at which time tissues will be taken for analysis. At the end of the studies the mice will be humanely killed and dissected to analyse tumour formation and progression. The work 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The cellular interactions, both cell-cell interactions and cell-environment interactions, involved in cancer formation can be studied in mouse models, whereas they can't be studied in the same way in ex vivo human cancer samples or using in vitro studies of cancer cell lines. Where possible, we will use in vitro studies of cancer cell lines and non-cancer cells from animals instead of animal studies for some functional studies of gene effects not involving cellular interactions with other different cell types or the environment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The protocols and numbers of animals used in these experiments have been statistically optimized to ensure that the minimum number of animals is used and that any adverse effects of the genetic alterations are kept to a minimum. Where possible, mice with existing abnormal genes will be imported. Where possible, we plan to use both in vitro cell line studies for functional analyses of gene effects and small pilot in vivo experiments prior to determine the final experimental design that involves the minimum

	number of animals. We propose to generate and utilise several different genetically defined mouse strains, which will all bear genetic changes known or suspected to be associated with tumour formation, and are relatively well characterised in terms of their predisposition to tumour formation, so we can utilise these data to design experiments with the minimal animal usage required to give significant data.
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 represent appropriate experimental subjects as they are of small size, have relatively short life spans and rapid reproductive cycles. Mice are prone to develop spontaneous and induced tumours. Mice are well defined genetically and their genes can be readily altered. There are in existence already, several mutant strains of mice including those with genetic alterations in cancer genes relevant to this programme of work. Hence, mice are a particularly good choice for modelling human cancer and for investigating the basic biological mechanisms involved. We already have much experience in looking after mice that develop tumours, in particular looking for the early signs of tumour formation. Our emphasis will be focussed on sound experimental design to test our hypotheses, based on experience from our own previous work, use of organ-specific or tissue-specific gene alterations, and appropriate use of statistical tests of significance. Harm will be minimized by careful daily observation of the mice for signs of illness, particularly the early signs of tumour development, with use of well designed protocols based on previous experience. We aim to gain the information we need before there is a significant negative impact on the animals welfare.

Project	**189. Intracranial drug delivery and gene therapy for the treatment of hydrocephalus**	
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)	Hydrocephalus is a condition where the amount of fluid within chambers inside the brain increases either due to fluid overproduction or a failure of normal drainage processes. The increased volume causes the pressure inside the brain to rise, which results in destruction of brain tissue. Hydrocephalus can be triggered by many brain diseases including tumours, infections or bleeding. Untreated it rapidly leads to severe disability or death. Current treatments rely on the surgical implantation of a shunt to lower the pressure within the brain by allowing the excess fluid to drain away. Complications with this	

	approach are common, requiring many patients to undergo further surgery. REDACTED This project aims to utilise animal models of hydrocephalus to determine if gene therapy to reduce CSF production within the brain could be an effective novel treatment for this complex disease. This is necessary before we can move towards development of a new treatment to potentially benefit patients
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 development of a non-surgical treatment for hydrocephalus is attractive in that it would: 1) avoid the need for patients to undergo brain surgery. 2) avoid the long-term problems associated with shunts which include infections and blockages, and which often necessitate further surgery interventions. 3) provide a safer treatment than surgery, which particularly for young children and premature babies involves significant health risks. 4) reduce the financial burden to health systems (including the NHS) by avoiding the cost incurred by repeated surgery and subsequent periods of hospitalisation. In addition, the findings of this study will advance the knowledge of fluid production in the brain, and the mechanism that control it, which in turn may generate new ideas for novel therapies for the treatment of hydrocephalus in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate using 250 mice and 250 rats over the 5 year duration of the 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?	All surgical procedures will be performed under general anaesthesia in a dedicated small animal neurosurgical theatre by experienced staff. All animals will be given post-operative pain relief. Animals are expected to recovery rapidly from the surgical procedures. Direct delivery of drugs and or fluids into the normal CSF spaces in the brain is a regular neurosurgical procedure in humans and normally very well tolerated with side effects being rare. This is the same in small experimental animals, such as mice and rats. The side effects will be rare, but include infection, bleeding in the brain (intracranial haemorrhage), and wound

	problems. The risk of general anaesthesia is low. The development of hydrocephalus is painless, and animal will be killed at an early stage in the development of the disease to avoid any suffering. Animals with hydrocephalus will become disoriented, lethargic and drowsy. This will be first evidence with poor grooming feeding. Symptoms are progressive and predictable in the models we will employ. Therefore at the beginning of any signs of hydrocephalus the animal will be terminated before deterioration. The severity limit for the licence is moderate. Hydrocephalus, when untreated, is fatal. All animals that have hydrocephalus will be monitored regularly by experienced staff during any experiment and humanely terminated when there are signs of clinical deterioration prior to the animal showing signs of distress. All animals in this study will be humanely terminated at the end of the experiment, as tissue from the brain is required to analyse the effect of treatment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Hydrocephalus is a complex disease with involves significant disturbance to normal biological functioning of the brain, which can't be modelled in a meaningful way without the use of animals. Furthermore, both the effectiveness and safety of the new approach have to be demonstrated in a living animal before permission can be granted to progress the approach into clinical trial. Consequently, *in vivo* studies are the only way of translating this novel approach into a clinical setting.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Great care will be taken when designing the experiments to ensure that only the smallest numbers of animals needed are used. The study will follow a step wise design. Progression from one step to the next will only occur if the previous step has yielded a successful outcome. The animal models to be used have been selected because they reliably produce a predictable outcome. This enables studies to be designed using minimal group sizes. Power calculations will be used to ensure animal numbers are kept to the minimum needed. Where the data need to perform power calculation cannot be obtained

	from the literature small pilot experiments will be undertaken in order to obtain the required information.
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 will be used in initial studies as they have the lowest neurophysiologic sensitivity of the species available that are suitable for these studies. In addition, the gene delivery system for the treatment is already established in mice. The mouse model of hydrocephalus is also used widely in hydrocephalus research and reliably reproduces a predictable disease pattern that will enable the efficacy of treatment to be assessed at an early point in its development, thereby minimising both suffering and the number of animals required. Work will only proceed to use rat model if the results of the mouse studies clearly demonstrate that the new treatment approach is successful. The rat hydrocephalus model has the advantage of being more representative of the clinical disease in humans. The development of hydrocephalus in the rat will be determined by detailed clinical assessment and MRI scanning which will enable the response to treatment to be determined at a point before any suffering occurs. The surgical interventions that will be used are well tolerated by both mice and rats. Surgery will be performed under general anaesthesia, with strict asepsis precautions, by researchers with extensive prior experience of the procedures. All animals will be provided with post-operative pain relief. Following induction of hydrocephalus mice will be assessed twice daily from day 4 onwards. Specific criteria have been set for assessing the development of hydrocephalus and clear end points have been established that will ensure that animals are killed at the early stage in the development before suffering occurs.

Project	**190. Investigating and modifying renal 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
	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)	Many forms of kidney inflammation that are not diagnosed early enough or treated sufficiently quickly lead to chronic kidney disease, in some cases requiring renal replacement therapy. We now have certain treatments that can be used but are associated with severe adverse events. However, we remain largely ignorant of the natural regulatory pathways that suppress inflammation and how these may be best harnessed. Our objectives are to define means of suppressing renal inflammation and understanding how certain internal or external factors can modify the inflammation and damage within the kidney.	

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 programme of work will extend our knowledge of the critical biological events that cause kidney failure in patients with autoimmunity such as small vessel vasculitis, and other forms of renal inflammation, and will potentially translate our findings to develop novel therapeutic targets that could be applied to patients with various forms of kidney disease or those at high risk of developing kidney disease (such as patients undergoing certain surgical procedures).
What species and approximate numbers of animals do you expect to use over what period of time?	The studies will be carried out in mice and rats. The primary determinant of this choice was driven by the fact that we are building upon previous work that developed these models of kidney inflammation in these species. These species are sufficiently close to human physiology, but yet sufficiently far down the sentient scale, to allow relevant conclusions to be drawn regarding what may be happening in patients with varied forms of kidney inflammation, while still avoiding use of higher animals such as non-human primates. The explosion in the availability of genetically modified mice now allows us to dissect in detail disease mechanisms that were hitherto impossible. We expect to use a total of just under 1500 animals over the 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 development of kidney failure is generally without symptoms. The models we use do not induce severe kidney failure but significant kidney inflammation, which is not noticeable to the animals. The induction of various forms of kidney inflammation requires administration of substances by injection, but these are performed by experienced operators and suffering is kept to a minimum. At the end of the protocol animals are humanely sacrificed and the extent of their kidney inflammation assessed by histology, biochemistry and immune monitoring. In the models investigating clearance of infection abscess or ulcer formation is possible and will be carefully monitored for. In the surgical models of ischaemia there are operative and anaesthetic issues that have to be taken into account, to minimise suffering. Post

	operative pain is minimised by ensuring all animals receive adequate analgesia and are carefully monitored in the post operative period for signs of distress and surgical (wound) complications, which thanks to the procedures being carried out by well trained and experienced operators, are rare. In the unilateral ischaemia model little change in kidney function is seen, but in the bilateral model we expect a mild and transient decline in kidney function, but to levels that again will not be noticeable to the animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have modelled some aspects of kidney damage in vitro, which have allowed us to replace and reduce animal use, however we are limited by what can be added to the models and may be missing critical interactions that we do not yet know about. The events that lead to blood vessel, glomerular or tubular injury in the kidney in crescentic glomerulonephritis and following ischaemia or drug induced inflammation are highly complex, involving numerous cell types, antibodies and immune system proteins, the integration of which would be impossible to achieve by complete replacement with test-tube studies. REDACTED
2. Reduction Explain how you will assure the use of minimum numbers of animals	Through thoughtful experimental design and careful articulation of the specific scientific questions to be answered, we have reduced the numbers of animals used in this programme to the smallest possible. Examples of ways that animal use can be minimised include combining 2 or more questions into one experiment, careful storage and cataloguing of all biological samples to facilitate future studies without having to repeat experiments, and accurate statistical consideration of the numbers of animals in each experiment so that the experiment has a realistic chance of answering the question being posed. The studies have been refined so that they are of minimal severity wherever possible, that anaesthetic protocols are reliable and safe, that immunisation routes cause the least amount of discomfort and that animal husbandry is of a sufficient standard to maintain the animals in an

	environment as close as possible to their natural environment.
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 studies will be carried out in mice and rats. The primary determinant of this choice was driven by the fact that we are building upon previous work that developed these models in these particular species. These species are sufficiently close to human physiology, but yet sufficiently far down the sentient scale, to allow relevant conclusions to be drawn regarding what may be happening in patients with AASV or other forms of renal inflammation under different circumstances, while still avoiding use of higher animals such as non-human primates. The significant expansion in the availability of genetically modified mice now allows us to dissect in detail disease mechanisms that were hitherto impossible. In addition, since inflammatory kidney disease is highly diverse , with some targeting the gomeruli predominantly, while others target the tubular compartment, different models are needed to adequately replicate these conditions. For example within the clinical scenarios there are various causes of tubular damage, including toxins(often nephrotoxic antimicrobials) or ischaemic insults or autoimmune mediated damage. As such we have refined a number of models that best reproduce these real life clinical scenarios. Finally, within the IRI model we have included the possibility of performing bilateral IRI(in which one kidney is removed and the other undergoes IRI) to mimic the situation in transplantation where there is a single ischaemic transplanted kidney and to allow for hard endpoints to be reached, in measurement of serum urea and creatinine, which are temporarily but significantly elevated in teh bilateral but not unilateral model. These will be used sparingly to validate the best therapies tested in the unilateral model and allow us to produce more robust data on the potential transplational aspect of defining new tubular protection therapies.

Project	**191. Investigating disease mechanisms and therapy for Friedreich's ataxia (FRDA)**	
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)	Friedreich's ataxia (FRDA) is an inherited lethal neurodegenerative disorder that affects 1 in 50,000 people of Caucasian origin. Individuals with FRDA usually present in early childhood with difficulty in walking due to poor coordination or "ataxia".The condition progressively deteriorates, leading to the development of debilitating musculoskeletal deformities and immobility, while the majority die in early adulthood due to heart failure or associated complications. There is currently no effective therapy for FRDA. Our primary objective is to develop new therapeutic strategies for FRDA.	

	To achieve this, we will investigate the disease-specific changes in the levels of metabolites and proteins in FRDA mouse models and test novel treatments targeting these changes. We will also investigate potential treatments using natural compounds with antioxidant properties and also drugs available for other neurological disorders. These drugs have already been approved for use in human and therefore, following successful pre-clinical studies, they could proceed rapidly to clinical trials in patients with FRDA. In addition, we aim to develop a novel gene therapy-based approach for FRDA that we hope will be effective in correcting the defective gene associated with the disease.
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 studies will provide new insights into the treatment of FRDA and will provide further stimulus in the scientific community opening new lines of investigation for the development of new therapies. There is currently no therapy for FRDA. Therefore, the outcome of this project by providing new compounds, therapeutic approaches and/or novel targets will provide relief from suffering and better quality of life for FRDA patients worldwide.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5-year project we expect to use maximum of 1,000 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The work outlined in this project aims to characterise mouse models for FRDA and evaluate the effects of antioxidant on reducing disease effects. The procedures undertaken in this study will include breeding and maintenance of genetically altered animals, injecting candidate drugs to ascertain how they are distributed in the body and trialling of potential therapeutic in models of FRDA disease. The efficacy of candidate therapeutics would be tested by using benign behaviour test to ascertain improvement of mobility/agility. All protocols used in this project are classified as mild or moderate. All the behavioural tests for the assessment of disease associated neurological deficits are non-invasive and are unlikely to cause any pain, distress or harm to the mice. For gene and cell therapy protocol,

	based on observations made in previous studies using similar approaches in mice, we have a good idea of the expected adverse events. These effects are typically mild and the severity limit is moderate. We anticipate that some animals may experience a moderate amount of pain and/or discomfort. We will use appropriate analgesia to minimise these effects. At the end of the studies, all animals will be killed under terminal anaesthesia or by a schedule 1 method. Any other procedures including drug administration have been widely used and are not associated with any adverse effects. All animals will be inspected regularly and any abnormal signs or symptoms will be discussed with the veterinarian. Any mice that show signs of moderate or severe pain or distress will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no currently effective therapy for FRDA. Therefore, numerous research studies are being carried out to understand FRDA disease mechanism with a view to develop a therapy that will halt or reverse the progress of the disease. Some information relevant to understanding FRDA and its treatment have been obtained from studies of bacteria, yeast, worms, fruit flies and human cells grown in a laboratory. We are currently performing studies using cells grown in our laboratory to determine potential novel FRDA treatments. We also aim to establish further FRDA mouse model cell lines as part of this project. In addition, several groups around the world are now attempting to develop heart and neuron cells from FRDA patient skin cells, which may prove useful for future investigations of FRDA. However, in order to better understand the disease and to assess treatment strategies, a living mammalian animal model with complex systems, organs and tissues similar to humans is considered necessary. Use of animals is now required to extend the initial information that has already been achieved from studies of lower organisms or cells grown in the laboratory in order to provide a more complete understanding of this human disorder and to undertake testing of

	novel drugs before progressing to human clinical trials.
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 at the same time minimising the number of animals used. Breeding will be kept to the minimum amount necessary to obtain the experimental groups of mice for disease-like characterisation and therapeutic testing. For all of the experiments, each group will contain between 4 and 16 age- and sex-matched mice, which is the number needed to obtain the essential results.
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 have been chosen as they are the lowest vertebrates to study neurodegenerative disorders such as FRDA and also to make our findings relevant in humans. In our FRDA mouse models, the genetic alteration will have little or no impact on their wellbeing. However, the animals may develop ataxia-like phenotype in old age, therefore, in this project they are used prior to the onset of these late stage disease associated symptoms. All the proposed therapeutic approaches have been previously shown to be safe and would not be expected to have any adverse effects. Suffering will be minimised by the administration of safe doses of potential therapeutic agents by the mildest available route of administration. Mice will be closely monitored after treatment and should any mice show signs of mild pain or distress, suitable supportive care measures will be taken, such as supplying food to the bottom of the cage. If mice show more than mild signs of abnormality of gait or coordination, lack of balance during walking, or movement they would be killed.

Project	**192. Investigating Energy and Glucose Homeostasis**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Energy homeostasis is the balance between energy intake and energy expenditure. Glucose homeostasis aims to maintain steady levels of blood glucose to provide fuel for tissues, and avoiding the ill effects of too high or low concentrations of glucose in the body. Both systems are regulated by a number of different organs in the body, including the gut, body fat and the brain. This project is designed to elucidate the complex mechanisms by which these different organs interact to regulate energy intake, energy expenditure and glucose levels. The systems which regulate body weight and glucose homeostasis are very similar in rodents	

	and humans, and many of the drugs currently in clinical practice for obesity and diabetes type 2 were designed based on data suggesting their utility in rodent models. The use of rodents will allow us to carry out experiments to characterise the most useful new targets for better, more effective drugs, that could not practically or ethically be carried out in humans. The sophistication of these systems, their roles in a variety of different tissues, and the complex interactions between them, mean that a significant number of rodents are required to understand how they function. The objectives of this programme of work are: **1)** To make and maintain rats and mice which have had parts of their biology regulating energy or glucose control genetically altered. **2)** To determine the effects of the short and long term administration of specific signalling molecules, for example, hormone or nutrients, on food intake, energy expenditure and glucose regulation in rodents. **3)** To investigate the mechanisms by which such substances influence food intake, body weight, glucose regulation, activity and other metabolic parameters in rodents. Many of these studies will focus on the effects of targeting particular systems through altered diet, pharmacologically or genetically, on food intake, body weight and blood glucose levels. In particular we intend to investigate how specific dietary components, such as protein and certain forms of carbohydrate, are sensed by the body in order to change food intake and glucose regulation. Such mechanisms have important effects on metabolism on a daily basis and may therefore have great potential to be targeted therapeutically. Understanding the effects of these signalling molecules and how they work to influence energy and glucose homeostasis will help determine their potential as targets for anti-obesity and anti-diabetes therapies.
What are the potential benefits	Obesity is a major medical problem in Western

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	society, and results from a long-term disturbance of energy balance, i.e. the energy taken in as food is greater than the energy expended. The environment we live in, with its easy access to high calorie, highly palatable food, can be described as 'obesogenic'. Evidence suggests that 60-80% of our variation in body weight in a given environment is genetically driven. Our genes in combination with this environment thus create a powerful drive to put on weight and develop metabolic disease which is very difficult to combat. Currently it is estimated that there are over 1 billion people overweight worldwide. It is estimated that in the UK alone, obesity causes 30,000 premature deaths a year. Obesity is linked with glucose dysregulation, and thus has driven the huge global increases in diabetes rates also observed. Studying the systems that regulate food intake, energy expenditure and glucose levels will improve our understanding of how they interact to control metabolism, and how their dysfunction can result in obesity and metabolic diseases such as diabetes. Identifying the most important signals and understanding their effect on the body will aid the development of novel effective therapies for obesity and metabolic disease.
What species and approximate numbers of animals do you expect to use over what period of time?	I expect to use 6825 mice and 2100 rats over a five year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	None of the proposed studies is aimed to result in adverse events, and none should result in events of more than moderate severity. Dietary modifications, including changes in nutrients, reduced calorie content (restriction), and the administration of specific experimental agents, may result in hunger and weight loss but this will be limited to 20% of the animal's pre-experimental body weight and periods of food withdrawal will be the minimum to meet the scientific objectives. Genetic models exhibiting any unexpected harmful abnormal effects will be killed, or in the case of individual animals of particular scientific interest, advice will be promptly sought from the local Home Office Inspector. Most genetically altered animals models used are expected to show only mild

	differences compared with unaltered animals, with fewer than 5% of animals showing a moderately severe effect. Laboratory Animal Science Association (LASA) guidelines will be followed regarding the volume of substances that can be administered. Animals showing unacceptable responses- such as hunched body posture, hair standing up for prolonged periods, abdominal tightness, head tilting, circling, lack of coordination of muscle movements, blood loss, increased sensitivity to pain, or separation from the group in group housed animals- to substances administered, treatments given, surgeries performed or genetic changes, will be monitored and killed by a schedule 1 method if they remain sick for more than 24 hours. The least invasive forms of surgery necessary to address the scientific questions being asked will be carried out, using appropriate anaesthesia and analgesia. Any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health has deteriorated, will be killed by a schedule 1 method. When work under terminal anaesthesia is involved, anaesthesia will be maintained at sufficient depth for the animal to feel no pain. All animals will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Energy and glucose homeostasis involve the interactions of multiple body organs and systems. Their study thus requires the investigation of whole animal physiology. Initially, it is neither ethical nor possible to perform these experiments on humans. There is therefore no viable alternative to the use of animals. When possible and appropriate, substances will be initially characterised using cell lines and other non-animal methods. Where tissue level mechanisms are being investigated, it will also be possible to use tissues obtained from humanely killed animals rather than using whole animals to screen agents for their effects on particular tissues. For example, we may examine the effects of agents on the release of hormones from the gut by taking and growing cells from regions of the gut and seeing if these

	agents cause hormone release from them. However, the control of such processes is very complex in a whole animal, and so it may be necessary to use animal studies to confirm such initial positive findings. We will also use computer simulation where this is practical to reduce the number of initial studies required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All animal experiments will be carefully planned. The natural variation in food intake and body weight between rodents of the same species and genotype can necessitate relatively large group sizes to detect effects. Statistical tests will be carried out to ensure only the minimum number of animals required for each study is used. Where practicable, at the end of the studies the maximum number of tissues will be used from each animal to minimise the numbers required. The maintenance of transgenic breeding colonies, the breeding of different lines together, and the need to investigate the effects of specific genes in one particular sex, can necessitate the breeding of relatively large numbers of transgenic animals. Breeding strategies for genetically altered animals will be optimised to minimise the number utilised.
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 are the species most widely used to study energy and glucose homeostasis and metabolism. The systems that regulate these functions are very similar in rodents and humans, and there is a lot of background knowledge on how they work in rodents, which reduces the number of experiments that need to be carried out. For all studies, anaesthesia and analgesia will be used where appropriate to minimise welfare costs.

Project	**193. Investigating new polymer- based bioelectronic technologies to improve neural interfaces with electronic devices**	
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)	When bioelectronic devices such as cardiac pacemakers, cochlear implants and bionic eyes are implanted into the body they induce an inflammatory response that is difficult to control. Metals used historically for these types of devices are stiff and easily recognised as foreign by the body. Consequently, these implants are often walled off in a scar-like capsule. As a result, high powered and unsafe currents may be required to activate tissues and	

	produce a therapeutic response. In this project, a range of novel conductive biomaterials will be used to either coat conventional devices or fabricated as fully organic (plastic) electrode arrays. Using animal models, we will evaluate these plastic coatings and devices to determine the interaction of nerves (specifically the sciatic nerve and brain) with the device, acceptance of the new materials by the body, and the long-term performance. These will be compared to conventional metal devices.
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)?	First we will demonstrate the effectiveness of our coating systems in improving the performance of existing metal based devices. These technologies can be applied to cochlear implants, bionic eyes and even pacemakers to improve their performance. An example may include better hearing for a cochlear implant patient. The second activity will replace the metal within the tissue contacting component of the device with a plastic alternative. By showing the safety and effectiveness of this technology, we will enable the fabrication of devices that are compatible with important diagnostics. For example, patients with these devices will be able to have common diagnostic treatments (including magnetic resonance imaging (MRI)) that patients with the existing metal based devices cannot.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 60 rats per year for 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 project will involve the implantation of “cuff”-type devices (electrode arrays) around target nerves. A connection will be made to the device through the skin. The devices will be stimulated periodically at a level that is below pain threshold but will cause a leg twitch. This is necessary to confirm that the device is working. For the majority of experiments the expected severity is classified as moderate. The expected adverse effects primarily relate to surgical procedures required to implant the cuff electrode. These experiments have been

	performed previously and the effect of the electrical stimulation can only be described as mild. At the end of the experiment animals will be euthanised by an approved humane method under anaesthesia. The nerve tissue and implant will be collected and analysed to gather data on the connection between the device and nerve.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our goal is to produce better functioning electrodes for neural-interfacing medical devices. We have performed extensive lab based cell culture work to improve the biological properties of our material systems and ensure compatibility with cells when studied in isolation. However, using cells (or even whole nerves), cannot replicate the interaction of a medical device with an intact nervous system. It is also important to understand any impact the device may have on other surrounding tissues, such as blood vessels and muscles. The only way to understand the impact the device and new materials have on the body and the quantify performance of a device, is to conduct studies within animals. We will however be working extensively with in vitro (cell culture) and ex vivo (whole nerves obtained from animal cadavers) techniques to refine our designs and minimise the number of animals needed for in vivo work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal studies will be conducted on devices that have been extensively tested, but with appropriate numbers, as is necessary to reach statistical significance. Initial study numbers have been based on our own prior research and other data published in the literature on electrode technologies. These numbers have been shown to demonstrate differences in device performance with a probability of showing these differences in over 8 out of 10 cases. We are also using a software program to support our experimental design and will routinely assess our animal numbers to ensure that we are using the correct number to obtain valid, statistically significant data, while using the minimal animal numbers required to support this research.

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 have a suitable nervous systems and anatomy for implantation that allow reliable data for comparison in humans. Size is a factor when considering the placement of electrodes. Mice would be too small to permit the implantation of the types of electrodes available for human use without causing unnecessary suffering. The use of standard electrodes which could be implanted in humans is also necessary as this work translates directly to improving the performance of such electrodes in in the clinical environment. Changing the electrode sizes to accommodate smaller animals would not be of scientific and clinical relevance as they would undoubtedly function differently. Rats have been chosen due to their historical use in assessing tissue reactions to neural implants. There is established literature on the tissue response to cuff electrodes such as those being studied in this project, and data that can be used for comparison. There is also a variety of commercial implant devices that have been specifically designed for use with rats, such that they are comfortable with implants in place. We will reduce animal suffering principally by using optimal operating technique and providing a suitable pre-emptive pain relief and good post-operative care. Pain and distress will be carefully monitored after procedures and appropriate measures will be taken if signs of pain/distress are observed. The ex vivo studies and pilot studies will be performed to ensure effective placement of devices prior to recovery surgeries. This will ensure animals are not euthanised earlier due to adverse effects of the implant placement. Our experiments will consist of predominately moderate level procedures. Where our protocols will involve stimulation to establish a threshold for nerve activation, the animal will be under anaesthesia. This level will not be exceeded when the animal is awake. Nerve stimulation once calibrated to a suitable level has been shown not to interfere with rat behaviours and no adverse effects have been observed.

Project	**194. Investigating the effects of ischaemia and ways to reduce them**	
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)	Any tissue which suffers a restriction in blood-flow (ischaemia) will show either temporary or permanent changes some which will be detrimental and, if untreated or treated inappropriately, can lead to severe damage. Some restrictions in blood-flow happen without any interventional stimulus as in stroke, heart attack, diabetes and obesity. Some restrictions happen during, and sometimes after, many forms of surgery and some happen due to accidental trauma. If blood is re-introduced inappropriately after a period of restricted blood flow, then a condition known as “ischaemia	

	reperfusion damage" (IR) can occur. By increasing our understanding of how and when such events occur we can develop and refine techniques and devices to detect them earlier and treat them more effectively.
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 advance our knowledge of how ischaemia/ischaemia reperfusion damage (IR) occurs and progresses and where are the most appropriate places along this progression that we can intervene to reduce or prevent the effects. This knowledge will be important in developing new treatments or prevention strategies for ischaemic episodes as in stroke, heart attack, seizures and diabetes. Likewise, this research will identify new procedures and other regimes which are able to prevent or reduce ischaemia/IR in a variety of surgical areas such as transplantation, implantation, and endovascular stenting using novel techniques and new instrumentation. The development of new devices to limit haemorrhage would benefit all surgical cases where there is a potential for bleeding that can result in increased ischaemia/IR and associated tissue damage. All of these advances will reduce patient morbidity and pain, increase quality-of-life, reduce dependency on the NHS and may save lives in some cases. These treatments will have uses in both human and veterinary medicine. Common situations where ischaemia occurs include accidental injuries, heart disease, stroke, diabetes, obesity, transplantation, implantation and most surgical procedures. For example, just considering ischaemic heart disease, 7% of adults in the United States, over 20 years of age, are estimated to have a coronary artery disease diagnosis. Therefore, any improvement in the prevention, detection or treatment of ischaemia could have wide reaching effects.
What species and approximate numbers of animals do you expect to use over what period of time?	Protocol 1: Adult mice 200 animals Adult rats 500 animals Adult rabbits 200 animals

	Weanling to adult pigs 240 animals Juvenile to adult sheep 200 animals Protocol 2: Adult mice 100 animals Adult rats 200 animals Adult rabbits 100 animals Weanling to adult pigs 100 animals Juvenile to adult sheep 50 animals
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Creation of small areas of reduced blood flow for assessment of potential treatments of ischaemia/ischaemia reperfusion damage(IR) under general anaesthesia will not result in lasting harm to the animals. Those who are recovered from anaesthetic for long term assessment may show transient effects of the ischaemia/IR but this will be closely monitored and if pain is suspected then this will be treated under veterinary supervision or, in the case of prolonged symptoms the animals will be humanely killed. For animals that have undergone endovascular stenting, no adverse effects are expected however all animals will be closely monitored and if pain is suspected then this will be treated under veterinary supervision or, in the case of prolonged symptoms the animals will be humanely killed. For all of the studies the severity limit will be moderate and close monitoring of all animals used will keep pain, suffering and distress to the lowest possible levels whilst keeping the animal's welfare to the highest standard possible. This is achieved by the use of analgesia, appropriate anaesthetics for species, antibiotics where relevant, attention to fluid balance and appropriate animal nursing and husbandry. All procedures will be carried out under aseptic conditions using aseptic technique and we do not expect to see any post-operative infection.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All treatments applied under this license will have to show substantial potential for the prevention or reduction of ischaemia/IR *in-vitro* and *ex-vivo* before we will take them into studies using animals. We will continue to investigate cellular culture and other *in-vitro* analyses alongside these studies which may reduce animal use by replacement or refinement during the course of this license. There are currently no non-animal alternatives for these studies as a fully functional physiological system is required to create ischaemia/IR and test the treatments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Early “proof-of-concept” studies and size translation studies will use limited numbers (between 4 and 6 animals) of least sentient animals, where possible (for some studies it is not possible to use smaller animals due to physical size restrictions e.g. stenting). Ensuring that only those procedures, materials or devices that have proven potential by way of laboratory based and cadaveric tissue assessment progress to live animal studies will keep animal use to a minimum. Only regimens with substantial evidence of success potential will be progressed through to GLP studies. Where studies are conducted according to regulatory body requirements, the minimum number of animals will be used and arguments against “controls” will be pursued where possible. For transplantation and implantation we will, where possible, use donor tissues/organs retrieved from animals under other projects at this institute, at termination. Where specific donors are needed the maximum amount of tissue/organs will be harvested per animal, to minimise donor numbers. In some studies we can argue that functional assessment of the targeted tissue can be observational rather than statistically driven which means fewer numbers will be needed. We have in the past been able to successfully argue against the use of controls in some of these studies and will continue to do this, where appropriate, in order to reduce the numbers of animals used. We will also use a statistician to make sure we use the least numbers of animals possible. Where studies allow, rodents will be utilised first and if the procedure is successful then it will be

	transferred to larger animals such as pigs and sheep for compatibility with human requirements.
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 Using an increased bank of assessments, in which we can now include non-invasive imaging, has further refined our techniques and we will endeavour to develop additional refinement as these studies progress. Where possible the lowest vertebrate group is used for each procedure. Size of an animal is an issue that has to be considered when utilising surgical techniques especially intravascular techniques e.g. stenting. The species chosen for each procedure also depends on the tractability of the animal species, statistical requirement and clinical relevance. Where a procedure has generated good results in, for example, rodents, this may then be applied to a 'higher' species, for example, pigs, to evaluate the results that may be generated in man. Some animals are chosen because their size makes them easier to use for operative procedures and because of their physiological and physical similarity to humans, for example, pigs are utilised for skin and bowel procedures and pigs, sheep and goats are used because of better surgical access, size of organs and longer length of vessels.

Project	**195. Investigating the mechanism of cooperation and socially- induced suppression of reproduction in REDACT rats REDACT**		
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)	A range of animals including mammals and birds adopt group living and cooperate in foraging for food, anti-predator behaviour and caring for young. This is often at the expense of their own reproduction, and such non-breeding “helpers” may have their reproduction suppressed by dominant breeders in the group (who are often close relatives). Some vertebrate examples resemble the societes seen in insects such as bees, ants and termites. The		

	mechanisms by which these societies are maintained are of great interest to biologists. This project aims to determine the hormonal and genomic factors that drive an extreme but natural example of cooperation and the social environment suppressing fertility. Using a highly social mammal as a model system, we will address the central question as to whether the hormone prolactin (first described for its role stimulating the production of breast milk) and its associated pathways have been co-opted to facilitate the evolution of cooperative breeding and sociality. It is well known that clinically high prolactin leads to infertility in humans and other mammals, and furthermore elevated prolactin has also been reported to be associated with cooperative care of offspring in birds and mammals (although cause and effect has not been proven). This leads to the intriguing possibility that prolactin may be involved in both social suppression of reproduction and the expression of helping and cooperative behaviour. Our research is now at a crucial stage where functional studies will confirm or dismiss a direct role for these proposed prolactin pathways, and in doing so will elucidate the mechanistic role of prolactin in the evolution of extreme sociality and cooperative breeding.
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 simple, relatively non-invasive study will produce unambiguous results and have the potential to make a major contribution to our understanding of social behaviour and cooperative breeding, and how the social environment and social stress can lead to infertility. This will provides basic knowledge that could be drawn upon for more strategic and applied projects involving the captive breeding of endangered species.
What species and approximate numbers of animals do you expect to use over what period of time?	We propose using a highly social cooperatively-breeding rodent. Approximately fifty animals may be used at various points over the three- or four-year study.
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?	Apart from the occasional collection of small blood samples, and administration by small subcutaneous injection of a common treatment for infertility in humans, our proposal includes no

What will happen to the animals at the end?	work of an invasive nature. The level of severity is mild and we do not expect any adverse effects. At the end of the study the animals will remain in our captive colonies, either in their social group, or as founders of a new colony.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project looks at the role of hormones in the evolution of cooperative behaviour and reproductive suppression in mammals. Non-animal alternatives are not available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have used statistical methods to calculate the minimum number of animals required to run the experiment robustly. We are also using methods that allow multiple data to be gathered from the same individual, which reduces the total number of individuals required.
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 methods are generally non-invasive; the most discomfort the animals should experience is from the tiny needles we will use to inject substances or take blood samples. We are using a new technique that allows to analyse gene expression in blood samples, which is a huge improvement from previous methods that required animals to be culled so tissues could be harvested. As a team, we check the wellbeing of the animals at least twice a day, every day of the year.

Project	**196. Investigating the neural basis of episodic and spatial memory**	
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)	Learning and memory are two important functions of the brain and an enormous amount of research has been carried out to find the neural mechanisms and circuits responsible for them. Memory can be sub-divided into many different psychological processes. However, the process that best sums up what people most commonly refer to as memory is episodic memory, the ability to remember specific, personal events that have happened to us in the past. Our episodic memories, to a large extent, make up who we are and they are used frequently to guide our current behaviour. The	

	study of the neural basis of episodic memory has centred on the medial temporal lobe of the brain and its associated structures, focusing on an area called the hippocampus. Studies have shown that people with damage to the hippocampus have severe anterograde amnesia, an inability to form new memories. This project will examine how the hippocampus and associated areas process episodic and spatial memory by examining the neural mechanisms involved in these psychological processes. From a systems perspective there is at least some agreement that the hippocampus has role to play in learning and memory but the specifics of how it interacts with other brain structures to process episodic memory are still unknown. At a more fundamental level the mechanisms within brain areas like the hippocampus that support episodic memory are poorly defined. This work will address these problems at the level of the single neuron, networks of neurons and systems of brain areas. It will go on to examine the genetic factors associated with these types of memory and apply this to the study of disorders of memory including dementia and Alzheimer's disease.
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 not only benefit the scientific community by furthering our understanding of learning and memory but will also provide potential therapeutic targets for disorders of memory like Alzheimer's disease. It will also test potential therapies for disorders of memory using appropriate behavioural models.
What species and approximate numbers of animals do you expect to use over what period of time?	The project uses rats and mice. We will use approximately 50-100 rats each year depending on grant funding and 0-750 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?	The majority of the experiments involve behavioural tests of animals which involve them learning to remember stimuli in their environment. A typical example would be a pot of sand in which they have to dig to find reward. The main adverse effect of this is stress caused by new environments. This will be minimised by habituating the animals to the environments in groups of their cage mates where possible.

	Some of the experiments involve either recording brain activity or manipulating the brain by infusing substances to affect brain activity. These experiments involve the animals having surgery and to minimise pain and suffering all appropriate analgesia and anaesthesia will be used. As with any surgery there will be a small amount of weight loss associated with the procedure but animals will quickly recover healthy weight gain. Some experiments cause small amounts of damage to specific portions of the brain. These may result in cognitive impairments such as deficits in memory. There may be occasional incidents when surgery does not go as planned but animals will be killed when such incidents become evident. Most experiments will only have a severity level of mild but some of them will be moderate. At the end of the study animals are given an overdose of anaesthetic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The proposed experiments aim to examine the neural mechanisms underlying learning and memory. This involves studying an organism that can demonstrate that it has learnt information and that it can remember that information for long periods of time. The available options here are humans and non-human animals. Human subjects are used whenever possible to better define memory processes, however in order to examine how the brain processes memory we need to carry out experiments that monitor brain function or manipulate the system in some way (through lesions or drugs). Monitoring brain function can be done in humans using methods like fMRI. However, these methods do not allow us to examine very specific parts of the brain as activity patterns have to be averaged across relatively large areas that include millions of neurons. In order to gain a real understanding of how the brain performs psychological functions we must examine the activity of individual neurons. This is not usually possible in human subjects. Other alternatives include cell cultures, which are clearly unsuitable for examining psychological processes, and computational

	models. Models can be very useful for generating hypotheses and where possible these are used. However, current models are necessarily simplistic and so cannot yet simulate many memory processes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to carry out good research we must use enough animals to make meaningful conclusions. This means that the number of animals in each group (sample size) is large enough so that the results can be generalised to all rats and potentially humans. To calculate how many animals we need we use a method called statistical power which tells us how many animals we need to use in our studies to make meaningful conclusions from the statistical tests of the data. To reduce the numbers of animals we use whilst maintaining statistical power we use methods that maximise the data we can obtain from individual animals. As an example, single unit recording studies use neurons rather than animals as the unit of analysis. In this case increased statistical power is gained not by increasing the number of animals but by increasing the number of neurons from which we can record. We will still need to use multiple animals as there are individual differences between animals but by using methods such as this, we can reduce the number of animals used by refining the method to allow us to record from more neurons in each animal. Where possible we use behavioural experimental designs that allow us to increase statistical power and so reduce animal numbers. For example, we will be employing a procedure that allows us to run multiple memory trials in one day for each animal. This will reduce variance, increase power and allow us to reduce sample size.
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 species used in the current application are rats and mice. These are used for a number of reasons. We require a species that shows the same type of memory process as humans and has a memory network similar in structure. This ensures that findings from these studies can be used to make conclusions about memory

the animals.	processes in humans. This rules out fish, insects and birds. The rodent brain is highly analogous to the human brain with the hippocampus being very well preserved across species. Rodents have also been shown to have largely similar memory processes making them a good model for the current set of studies. We need to use adult rodents as juvenile rodents do not show the same type of memory processes. For these reasons adult rats and mice are the most appropriate species for this application. We will make use of procedures with the lowest levels of pain and suffering for the animals. Where surgery is necessary this will be done aseptically based on local operating guidelines which are based on the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery and have been approved by the Animal Welfare and Ethics Committee which includes the NVS and HOI. Analgesia is given both peri and post-operatively. Soft bedding, wet mash and supplementary heat will be provided to help mitigate the pain. Pain is assessed in post-operative animals using the grimace scale and in consultation with the NACWO. Where possible we will use non-recovery surgeries, but this will be very rare as the majority of our research involves examining memory processes which require an awake behaving animal. We will consult with the NVS to ensure that we are using the best possible anaesthesia and analgesia. We will also keep up to date with current methods for recording brain activity and manipulating neural networks and adopt new methods that will result in lower levels of pain and distress wherever possible. As an example of this we are currently transitioning from using neurotoxins to create lesions within the memory network to using genetic methods to manipulate the network. While these still require surgery they do not result in neurons being destroyed and so have fewer long term impacts on the welfare of the animals. When using genetically modified mice we will not be developing new strains and will only use those with well specified phenotypes. The majority of these will involve no adverse effects. We may use mice with genetically altered

	signalling of AD linked proteins such as the *ob/ob* mouse. In these cases we will source the model with the least adverse effects that allow us to address our scientific question and consult with the NACWO and NVS to ensure that specific phenotypes are taken into account when designing experimental protocols to minimise pain and suffering.

Project	**197. Investigating the role of humoral immunity in tissue-specific immune responses 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 immune system is the body's defence system, protecting it from infections and cancers, and helping to repair damage to tissues. It was previously thought that immune cells mostly lived in the blood and 'professional immune organs' such as the lymph node and spleen. However, recent studies show that some immune cells permanently live in different organs, like the kidney. Our aim is to investigate one part of the immune system – 'humoral immunity'. This includes	

	immune cells called B cells, which become plasma cells and produce soluble defence molecules called antibodies. We want to find out how many and what sort of B and plasma cells live in different tissues, whether they play a part in responses to local infection or tissue damage, and if this changes with age. The tissues we are particularly interested in are the intestine, the kidneys and bladder, and the brain, including the membranes that line the brain, the meninges. We also want to look at how these cells change when a tissue becomes cancerous.
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 our understanding of the role of tissue B cells and plasma cells in infection and inflammation in different parts of the body. This includes the intestine, providing information that can be used to develop treatments for gut inflammation (conditions like inflammatory bowel disease). These diseases are currently increasing in prevalence and some patients do not respond to standard therapy. Our work on kidney and bladder immunity will help identify strategies for the prevention and treatment for urinary tract infection, kidney injury and kidney transplant rejection. This will help prevent people from getting kidney failure and make kidney transplants last longer, with important benefits; dialysis accounts for 2% of total NHS spending and transplantation restores patient independence and allows return to work. There is currently little information about the cells in the brain linings, and our work will be relevant to understanding and finding treatments across a broad range of brain disorders, including meningitis, mood disorders and neurodegenerative diseases such as Parkinson's disease. Finally, there is an increasing appreciation that immune responses can be harnessed to help fight cancers, but little is known about the part of the immune system that we are studying. Our project will begin to address this knowledge gap.
What species and approximate numbers of animals do you expect to use over what period of time?	Species- Mouse. 10000 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 undergo an immunisation – this is just like receiving a vaccine, and then tissue immune cells will be assessed by taking organs following killing by a humane method. In other cases we will use infection models where mice are given a kidney or bladder infection or a gut infection to determine how B cells and antibodies affect these infections. For some experiments we will place some tumour cells underneath the skin and assess whether B cells and antibodies affect the ability of tumour cells to grow. In all cases, the mice will be carefully monitored for adverse effects such as weight loss, diarrhoea, abnormal kidney function or the tumour size measured regularly, so that we can make sure that experiments are terminated before the disease becomes too severe. Blood, urine, and faecal samples may be collected and we may also perform scans using special microscopes whilst the animal is anaesthetised. In these cases the mouse is put under deep anaesthesia and carefully monitored. In all cases, animals will be humanely killed at the end of their breeding lifespan or at the end of experiments and following death tissues will be further processed for detailed investigations.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will carry out many parts of our research using cells grown outside of the animal in tissue culture to minimise animal use. We have obtained ethical permission to use human cells for some studies and cells from human organ donors that have given consent for their tissues to be used for research, so we can investigate immune cells in multiple organs including spleen, kidney, bladder and liver. However, although these approaches can provide some useful information, they cannot accurately model an immune response in a body, which is complex and requires a coordinated action from many different immune cell types. This response takes place within specific tissues, and is influenced by these differing environments. The complex interactions between immune cells, the environment of

	different organs, and a whole body immune response cannot be recapitulated using tissue culture methods hence the need for animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing the experiments statistical analyses ensure the use of minimum number of mice per group. We have obtained mouse strains that have fluorescent immune cells that we can scan, something that is difficult to achieve using labelling techniques. This will limit the number of mice required for these studies. In addition, numerous cells can be imaged at once, generating a large amount of data and information per animal, which will also limit the overall number of mice required. To avoid breeding new mouse strains we will investigate specific immune cells that are labelled. We will provide any excess mice generated by our breeding programme to a number of other researchers avoiding duplication where other groups breed the same strain.
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 genetically modified mice that have been bred for our research, and have alterations in the amount of B cells, the immune cells which we are interested in. This maximises the quality of experimental data generated, minimising the numbers of animal required. In order to carry out imaging studies, we have imported fluorescent reporter mice made by expert imaging laboratories. These provide the best possible way of seeing specific immune cells as they have fluorescent markers which tag specific cells on our microscopes. This means there is a large amount of data that can be generated from a single experiment. The mice are kept deeply anaesthetised for the full duration of the imaging, but this technique allows us to magnify and image in real time the interactions of individual cells in the animal. We minimise suffering via careful and skilful handling of the animals, use of the smallest possible size of needles, minimal numbers and

	frequency of repeated steps of the experiments. We constantly refine our protocols to ensure that the least amount of harm is caused to the animals during protocols whilst trying to gather the most amount of scientific data. Where possible we store samples to archive for future use. All animals undergoing a procedure will be carefully monitored for signs of distress thereafter, and treated appropriately or humanely killed by an authorised method to minimise any suffering.

Project	**198. Investigation into drug and vaccine delivery**	
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)	We will investigate whether and in what way, an animal's sex, age and feeding status (i.e. fed or fasted) influences the fate of a medicine or vaccine that is given to the animal. For example, is a medicine more active in females? Or when an animal is fed (rather than fasted)? Or is a medicine less active as we get older? We will also develop new medicines and vaccines, with specific properties, for example, those that can be applied to different sites in the body, e.g. skin or those which are processed in different parts of the gut, or those which are more stable and therefore more effective, for	
	example, at extremes of temperature .	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work will generate new knowledge which can, within the next 10 years, be used to produce medicines and vaccines which are more effective, less toxic, more acceptable to people and cheaper. We will also show whether it is important that females as well as males are routinely included in all or most animal experiments. We will also show whether the fed or fasted status of an animal is important when conducting animal experiments. We will show whether the efficacy of medicines and vaccines change as people get older.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a period of 5 years, up to 1872 rats and 1488 mice will 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?	It is expected that the maximum level of severity will be moderate. While each individual procedure (fasting, administration or collection of a sample) is expected to have a mild severity, with expected adverse effects ranging from discomfort and irritation, the cumulative severity, for example, following fasting, multiple drug/vaccine administration and sample collection events is deemed to be moderate. Experiments will last for a maximum of 3 months, and at the end of the experiments, animals will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are necessary to determine what happens to a living body (eventually the human body) when medicines and vaccines are administered to it and what the body does to the medicines and vaccines. Mammals such as rats and mice will be used in our work to develop medicines and vaccines for humans. These animals have a long history of use as models, and there is extensive knowledge about their properties such as, gut pH and bacteria. Such knowledge will enable us to develop and test formulations that are responsive to stimuli such as pH and gut bacteria. Although the body's parameters in rats and mice may not be exactly

	the same as in the human, these animal models will enable us to show proof of concept. Rats and mice will also be used as their faeces, urine, blood, saliva are similar to their human equivalents, and the possibility of collecting such samples from rats and mice will enable us to measure the effects of our drugs and vaccines. It would not be possible to obtain such samples from 'lower' species, such as invertebrates (e.g. nematode worms) or zebrafish or microbes, to test for efficacy.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Prior to animal work, a large number of experiments will be conducted in vitro i.e. in test tubes, in order to optimise the vaccine and medicine formulations that are given to the animal. The conditions within the body, such as acidity, temperature will be simulated in vitro to make the in vitro experiments more reliable. In addition, a number of experiments using animal samples (e.g. faeces) and tissues (e.g. pieces of gut that is obtained after killing an animal) will also be conducted prior to animal work. As far as possible, experiments will be conducted in parallel, so that the same group of animals can be used as the 'control' group for several experimental 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.	To minimise harm to the animals, the experimenter will have adequate training and help by experienced people, such as the technicians in the Animal House. Animals will be handled with care, appropriate speed and confidence, and the animals will be observed at suitable intervals and durations, for any signs of distress. Sufficient anaesthetics and painkillers will be used when required.

Project	**199. Investigation into the mechanisms causing neuromuscular pathogenesis and development of related therapies**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our research is focused on developing treatments for the childhood disease called spinal muscular atrophy (SMA). Approximately 650-1300 people have SMA in the UK at any one time. SMA is caused when a gene called SMN stops working and an obvious cure is to replace or fix the SMN gene. Many of these treatments, called SMN gene therapies, have been tested in animal models and the better ones are being tested in patients. However, it has become more and	

	more evident that SMN gene therapies may not be able to repair all of the SMA symptoms. We and others have already demonstrated the benefit of additional treatments that are not SMN gene therapies in animal models of the disease, some of which are currently being tested in patients. Our aim is to test the benefit of the drug called mifepristone in SMA mice to improve their symptoms. The ultimate goal is to develop treatments that are suitable for patients and can eventually be combined with SMN gene therapies.
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 will provide new accessible treatments for SMA, a childhood disease that is currently uncured.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use an average of 165 mice over the course of a 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?	The most clinically relevant experimental animal models for the fatal childhood disease spinal muscular atrophy (SMA) shows adverse effects due to the loss of nerve cells in the spinal cord and muscle function, leading to reduced movement, decrease in body weight and early death. These adverse effects are minimised by very close monitoring of the disease progression and selecting appropriate humane endpoints. However, most animals are likely to experience a moderate level of severity. These animal models are particularly important for the discovery of new treatments as they accurately mimic many of the clinical features found within SMA patients. The SMA mice in particular have proven beneficial for the development of a SMN gene therapy that has recently been approved by the FDA and EMA for all SMA patients.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The activity and safety of mifepristone and NR will firstly be evaluated in cell culture systems in the laboratory. The cell culture systems used (including cell model systems derived from patients) will allow us to understand how the drugs work, enabling us to refine the design of new treatments, guide future drug development and provide early insight into safety and efficacy. The goal of our work is to develop a new treatment for the currently incurable childhood disease SMA. We will therefore eventually have to understand drug activity and efficacy in a whole-body system such as a representative mouse model. However, evaluating mifepristone and NR in high quality cell culture systems prior to evaluations in mice increases our chance of success. In addition, only in the animal are we truly able to test and understand the safety profile of any new treatment. Therefore, without the information acquired from well- designed animal experiments, we would not be able to enhance the efficacy and understand the safety of new treatments, a critical requirement for any new treatment aimed at benefiting patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We take precautions to only take mifepristone and NR forward in animal experiments once it has been successfully tested in cell culture systems or been validated in the literature in similar mouse models. Matching age, sex and strain for all treatment groups helps to reduce the number of animals required per treatment group. All studies begin with a small pilot group. Only treatments that are successfully active and efficient in the small pilot group will be used for larger 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.	In most cases we will use genetically altered mice which mimic the human disease at a behavioural and molecular level. The first time mifepristone and NR is used in animals, it is given at an amount previously published and tested in similar animal models. We may then incrementally increase or decrease the amount given to reach the best activity and efficiency. Should animals begin to show intolerance to a certain amount of the drug, that particular treatment is no longer used or only used at a tolerated lower amount.

Project	**200. Investigation of a novel harmless delivery system for targeted gene therapy of cancer.**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	This project aims at addressing major limitations that gene therapy has faced for many decades. Therefore, we propose to investigate the efficacy of a newly designed gene therapy vector in tumour-bearing mice.	
Why is it important to undertake this work?	Gene therapy, or therapy with genes, uses vectors or delivery systems to introduce genes in cells in a diseased tissue to achieve treatment because genes need vehicles to transport and deliver them. Once inside the diseased cells, the gene will produce a therapeutic protein that will initiate treatment, such as a toxic protein that will start destroying cancer cells. Gene therapy has been around for many decades but has not met the expectations because gene therapy has	

	faced major problems including the cost, safety, etc...One major problem has also been lack of efficacy when gene therapy is injected to patients through the systemic circulation since real clinical benefit can only happen with systemic gene therapy. These challenges that have delayed efficacy of gene therapy to treat incurable human diseases are associated with vectors of gene therapy, such as human viruses which have mostly been used as vectors of gene therapy because they can enter human cells and deliver therapeutic genes as part of their natural infection process. Our previous work shows that the harmless and non-pathogenic bacteriophage or phage, viruses that infect bacteria only, can deliver genes to human cells if they are modified. An important advantage is that these viruses can be modified to be selective to the disease while leaving healthy organs unharmed. Our first generation of these phage vectors showed safety and efficacy against cancer in large animals with natural tumours after intravenous administration. We have spent the last 10 years to improve these vectors and generate systems that can overcome limitations in human since bacteriophage has evolved to infect bacteria only with no strategies to deliver therapeutic genes to human. Indeed efficacy of gene therapy depends on the ability of vectors to deliver genes at therapeutic levels in the diseased tissues. Importantly, very recently our efforts have yielded a bacteriophage vector that could present a major advance in systemic gene therapy of cancer. It is important to undertake this work because the vector shows ability to overcome most of the limitations that gene therapy has faced for many decades and could bring to fruition the promise of gene therapy to save the lives of patients with deadly cancers.
What outputs do you think you will see at the end of this project?	This project will allow assessment of the efficacy of the new vector in gene therapy of cancer. The project will also establish and identify the vector treatment plan that achieves the highest effect against tumours while preserving tumour selectivity of the treatment and its safety. The proposed research is original, novel and aims at solving problems of gene therapy against cancer. We have provided a comprehensive plan and design of the studies that should result in outcomes ready for submission to high impact publications. Moreover, we will file a patent application toward the end of the studies.

	The proposed studies will help to acquire sufficient evidence in animals and generate a product ready to initiate further safety studies in larger animals, or a phase I trial in human (first stage of testing in human, that assesses toxicity of a new treatment). The outcome of these studies should also strengthen our future discussions with the regulator for a phase I trial. Our comprehensive investigation in mice should result in establishing the most efficient therapeutic plan, and help devise a plan for clinical testing in human. We will have a product suitable for clinical application upon successful completion of our research. We also will engage with commercial collaborators to push the product further along the drug development pipeline and into clinical trials, and in the longer term into the clinic with direct benefit to patients and healthcare providers.
Who or what will benefit from these outputs, and how?	Researchers using gene therapy for human diseases other than cancer will be able to use our vector to deliver their therapeutic genes in their animal models of human diseases. This should be possible within 2 years of the start of this project. If successful our strategy can be used to treat cancer patients, in particular adults and children with gliomas which are the most aggressive and deadly brain tumours with very disappointing prognosis. Patients with metastatic tumours (tumours that have spread to form additional tumours in other organs throughout the body) should also benefit, as most cancer patients die because of metastases. Indeed, our gene therapy approach is administered intravenously, a clinical route, applicable in localized tumours and metastases. Thus, at the end of this project, 5 years, we will have a product ready for clinical trials in cancer patients. Yet, we will have to obtain approval from the regulator and produce our vector at Good Manufacturing Practice, for patient use; which should take an additional 2 years. Finally, pharmaceutical companies have expressed interest in our vectors; we will enter into negotiations and collaboration with them for the best way forward to develop our therapeutic approach for clinical use. Negotiations could start within 2 years, after we show proof-of- efficacy in subcutaneous tumours.
Will this work be offered as a service to others?	No

How will you look to maximise the outputs of this work?	The data will be accessible to the scientific community after publication, but we can share data any time before publication under a confidentiality agreement. We will publish in Open Access journals, our website and at major conferences (i.e. gene therapy), which will give other researchers ability to access the data. Our policy is to publish in Open Access journals, which will allow a wider scientific community access to our publications without charges. Our institution has continuously covered our Open Access charges. We also will aim at presenting unsuccessful approaches, as negative results, at conferences through posters and oral presentations. Material for research can be shared with other researchers, for academic purposes, under Material Transfer Agreements. No limits to data sharing and dissemination are required because the proposed work will be protected by patent applications. We will identify potential users of the scientific and technical outcomes of our research outside university. We will have institutional support in this area in terms of advice and funding. We also will promote our research findings on the Institutional website. We will aim at transferring technical and career development skills to new generations of scientists. This will occur either directly (undergraduate, Master and Ph.D. students, or staff) or indirectly for health professionals who come into contact with our research.
Explain why you are using these types of animals and your choice of life stages.	Mice are the most commonly used species for the type of procedures detailed within this application. Mice have been, and continue to be used extensively in cancer research because they can be used to model human tumours. These rodents are relatively low order sentient animals; however these species are accepted by the scientific community as the standard models for the establishment of cancer models and for the assessment of the therapeutic efficacy of gene therapy vectors, which covers the kind of research work we intend to carry out. We have proposed to use juvenile or adult mice. It is not practical for us to use animals at a more immature life stage. Indeed, our studies are performed over a long time period involving tumour cell implantation into mice followed by awaiting time which could take a few weeks until the tumours are clearly visible. Next the tumour-bearing mice are administered with our phage vectors followed by monitoring therapy response over a few weeks or months depending on the efficacy of the
	therapeutic approach being tested.

Typically, what will be done to an animal used in your project?	Animals will be implanted with tumours cells in the back, in the breast or in the brain. Phage vectors will be administered to mice when the tumours are visible. Injections of phage vectors can be repeated up to four times. Other substances such chemotherapeutic drugs, TMZ, might also be given to mice in combination with the phage vectors. Durations of the experiments will depend on the growth rate of the tumours and efficacy of the treatment regimen.
What are the expected impacts and/or adverse effects for the animals during your project?	Side effects can be caused by large tumour size and burden. Mice can show adverse effects which are expected to start from around 3-4 weeks after cell implantation, mostly in control groups that won't receive any treatments. However, mice will be observed twice a day and the tumour size measured daily to avoid the tumours growing beyond 12.5mm, which should avoid lasting adverse effects. To further avoid any lasting adverse effects, the animals will be observed twice a day to detect any clinical sign or indicators of pain and suffering that could be associated with the tumour burden, such as body weight loss with poor body condition, or body weight loss maintained for 48 hours in mice with brain tumours growing inside the brains of mice.
What are the expected severities and the proportion of animals in each category (per animal type)?	The severities won't exceed **Moderate** in each category and for each animal. 55% of mice with s.c., mammary or brain tumours, are expected to reach a moderate severity, while 45% should reach mild.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	While cancer cells can be used, they do not approximate the clinical condition as well as animals. Indeed, primary and metastatic tumours have various interactions with other cells of the body in which they are growing, as well as with the immune system and contain, in addition to tumour cells, various other types

	of cells which each have a major function in tumour growth. Tumours have a blood supply and inflammatory/immune responses that also play pivotal roles in tumour growth and tumour invasion. In brain tumours, the blood-brain barrier and the environment of the brain cannot be duplicated by other methodologies. Animal experiments to gain greater insight into the human condition are therefore crucial.
Which non-animal alternatives did you consider for use in this project?	Occasionally, we have used three-dimensional tumour spheres to mimic the three dimension form of solid tumours to assess efficacy of our phage vectors in the lab. These three-dimensional tumour spheres grow in a dish to form a three-dimensional structure resembling solid tumours and are considered as valid models to replicate some features of solid tumours.
Why were they not suitable?	However, the three-dimensional tumour spheres couldn't be used to recapitulate all the features of tumours. Unlike tumours established in animals, the three-dimensional tumour spheres do not contain blood vessels (blood supply), while in our project most of the treatments are injected to mice intravenous. Moreover, we intend to assess the tumour targeting potential of our vector, which can only be investigated by quantifying the accumulation of the vector in tumours and compare to vector accumulation in the normal healthy tissues in individual animals.
Enter the estimated number of animals of each type used in this project.	mice: Immunodeficient mice (600); Balb/c (400); C57Black (400).
How have you estimated the numbers of animals you will use?	We have outlined the experiments in a logical and succinct manner that allows the minimal number of animals to be used. In our recent publications, we reported studies in tumour-bearing mice in which we used 5 animals per group, all the published data had statistical analyses. Therefore, we will continue to use 5 mice per group as the minimal number to perform an experiment, and we will increase this number in rare circumstances where the animal-to-animal variation is larger than expected. Five animals per group should be enough to detect statistically significant differences between treated groups of animals and non-treated animals.
What steps did you take	Well accepted statistical methods were used at the

during the experimental design phase to reduce the number of animals being used in this project?	design stage in order to ensure that the research data obtained is adequate, but at the same time ensuring that the minimum numbers of animals are used. After consultation with colleagues with expertise in statistics, this minimum number of animals to perform the experiments is 5 mice per group.
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 maximise the amount of data generated from a single experiment by combining imaging of the tumour-bearing mice with therapy. Indeed, imaging allows i) tumour detection, ii) measures tumour viability and size, and iii) monitors tumour response to therapy in individual animals. This strategy allowed us, in our previous studies to generate maximum amount of publishable data from each experiment, thus reducing the need to repeat experiments several times, and allowing the use of minimum numbers of animals. As we previously did, in some experiments we will perform pilot studies with lower number of animals to gain insight into the efficacy of newly generated vectors. If the data are promising, then we will plan experiments with larger numbers of animals.
Which animal models and methods will you use during this project?	Mice are the most commonly used species for the type of procedures detailed within this application. Mice have been, and continue to be used extensively in cancer research because they can be used to model human tumours. These rodents are relatively low order sentient animals; however these species are accepted by the scientific community as the standard models for the establishment of cancer models and for the assessment of the therapeutic efficacy of gene therapy vectors, which covers the kind of translation research we intend to carry out. Phage have a historic safety profile as they have safely been administered to human over many years to treat bacterial infections. Moreover, safety studies of our phage vectors have already been carried out by my team and independent groups both in small and larger animals, all these studies proved that our phage vectors are safe. In combination experiments, we will aim at enhancing the phage efficacy, we will use drugs at lower and no-toxic doses that should not cause any harm to the animals. We will maximize the amount of data generated at early

	stages of tumour growth before the tumours reach advanced large sizes that could cause pain or distress to the mice.
Why can't you use animals that are less sentient?	It is not practical for us to use animals at a more immature life stage or animals terminally anaesthetised. Indeed, our studies are performed over a long time period involving tumour cell implantation into mice followed by awaiting time which could take a few weeks until the tumours are clearly detectable. Next the tumour-bearing mice are administered with our phage vectors followed by monitoring therapy response over a few weeks or months depending on the efficacy of the therapeutic approach being tested.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	I will attend training courses such as "A Guide to the 3Rs & Responsible Animal Research". I will attend seminars organised by the CBS 3Rs Advisory Group. I will attend the Annual Animal Research Forum. I will attend the Laboratory Animal Science Association "LASA" 3Rs Section Annual Conference. I am in the email list of the CBS 3Rs Advisory Group , which keeps me updated on any new developments on the 3Rs, seminars, workshops etc...
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	We will take every measure possible to avoid unnecessary animal suffering and we will continue to do frequent monitoring, twice a day, as soon as tumours are established in mice. Mice with tumours could experience weight loss, change of body condition or reduced ability to groom. We will use imaging methods of the whole living tumour-bearing mice to allow tumour detection, monitoring tumour response to therapy and generation of maximum amount of data, subsequently allowing early termination of our experiments while the tumours haven't reached large size. Moreover, using more than one imaging method will help to obtain consistent and conclusive data that show proof of efficacy during a short period of time, without the need to extend the therapy experiments for longer times to obtain conclusive findings. This should minimise animal suffering that could result from large size tumours. We also will approach companies that have recently developed a new system to measure subcutaneous tumours (tumours growing under the skin, in the back of

	animals) which improves the traceability, accuracy and reproducibility of data while ensuring greater animal welfare in adherence with the 3Rs. We will continuously seek advice from our Vets for any necessary pain management, particularly in mice with established intracranial tumours (tumours growing inside the brain of mice). Objective#1 will provide the necessary information regarding the anticancer gene candidates to be tested further in mice with intracranial tumours; thus we will use the minimum number of mice to achieve objective#2 and subsequently to minimise animal suffering that could result from intracranial tumours.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We will adhere to the NCRI guidelines for the welfare and use of animals in Cancer Research (Workman et al. BJC (2010) 102, 1555-1577). We will also adhere to the LASA guidelines of administration of substances.

Project	**201. Investigation of Cryptosporidium Host-Pathogen Interactions**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	To understand how the host immune system recognizes and responds to a Cryptosporidium infection, and how the parasite has evolved to evade this process.	
Why is it important to undertake this work?	Cryptosporidium is a major cause of diarrheal disease and causes significant mortality, morbidity, and developmental stunting in children around the world. Annually, there are an estimated 200,000 deaths and over 10 million disability adjusted life years attributable to Cryptosporidium infection. Despite this significant impact of public health, there are no fully effective drugs or vaccines available and the basic knowledge to drive their development is scant.	
What outputs do you think you will see at the end of	The goal of this project is to develop a better understanding of the host-pathogen interactions of the	

this project?	Cryptosporidium parasite. From the perspective of the host, we hope to identify the genetic factors that play a role in resistance and immune response. From the perspective of the parasite, we hope to identify the virulence factors that the parasite uses to evade host immunity and cause infection. Overall, we hope to broaden the foundation of knowledge that we can use to develop better therapies to treat and prevent infection. We will communicate our research as frequently as possible through public engagement and presentation, scientific conferences, and publications.
Who or what will benefit from these outputs, and how?	The Cryptosporidium research community will greatly benefit from the research we will perform, and to a much lesser extent, the scientific community at large, as we hope that our research will uncover immunity and virulence mechanisms relevant to other fields. Whenever possible, we will explore the translational aspects of this research to benefit infectious disease clinicians and their patients. Cryptosporidiosis is a serious public health concern and we will do our best to advance the science, train new researchers, and educate our community about the disease.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	During the project we will encourage visiting scientists and students to come and learn techniques that we use and develop. We will also make these methods and protocols available through publications whenever possible. Data will be published in a timely manner and presented often.
Explain why you are using these types of animals and your choice of life stages.	We use a mouse model of cryptosporidiosis for two important reasons: 1) To mimic human disease and 2) to propagate Cryptosporidium parasites. The ability to mimic human disease in a rodent model is an invaluable tool that will help us to understand disease pathology and develop better therapeutics. Human disease typically occurs in the first 10 years of life and thus juvenile and adult mice are infected for this research (1 week to 2 months of age). There is no cell culture system to propagate Cryptosporidium, therefore, to maintain the parasite lines

	that we use for both in vivo and in vitro research we must use mice. Some species of Cryptosporidium, especially those that naturally infect humans, do not reproduce well in healthy mice, thus immunocompromised mice are required for propagation.
Typically, what will be done to an animal used in your project?	Mice are required for comparative infections and propagation of Cryptosporidium parasite strains. For comparative infections, mice will be inoculated with Cryptosporidium parasites by oral gavage. Infection will then be monitored indirectly via collection of faecal material or directly via whole animal imaging. For propagation of parasites, mice will also be inoculated with Cryptosporidium through an oral gavage. Faecal material will then be collected for 2-3 weeks during the peak parasite shedding period of infection.
What are the expected impacts and/or adverse effects for the animals during your project?	Most mice do not develop overt symptoms and recover from infection in 2-3 weeks. Some will experience bloating, gastrointestinal discomfort, and loose stools.
What are the expected severities and the proportion of animals in each category (per animal type)?	Highly immunocompromised mice occasionally show more severe illness and symptoms such as bloating and gastrointestinal discomfort; if any of those symptoms appears animal will be humanely killed.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	The use of animals for project is required for two main reasons: 1) Mice serve as a naturally infected host in which we can study both parasite virulence and host susceptibility. To develop new therapeutics for this organism we require a better understanding of parasite biology and host immunity. Mouse models of infection allow for both. 2) There are no reliable and reproducible methods to propagate Cryptosporidium strains in vitro. Therefore,

	mice are required to maintain wild-type and transgenic strains required for all research.
Which non-animal alternatives did you consider for use in this project?	There are no non-animal alternatives for the propagation of Cryptosporidium. However, intestinal organoids were considered to replace mouse models to study the infection biology of epithelial cells.
Why were they not suitable?	Until there are reliable and reproducible methods to propagate Cryptosporidium in vitro, mice will be required to maintain and produce the parasite strains required for this research. Intestinal organoids, in contrast, are a suitable replacement for animals for the study of epithelial cell biology during infection.
Enter the estimated number of animals of each type used in this project.	mice: 5000
How have you estimated the numbers of animals you will use?	For genetic screens, sample sizes have been determined using power calculations based on a pilot screen of the founder populations of mice. The number of mice required for confirmatory follow up screening has been estimated based on previous comparative infection studies. For mice required for propagation of Cryptosporidium parasite lines, the number of mice has been estimated based on the number of lab members and the annual usage rate of labs that perform similar research.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Pilot screens were performed to determine the means and standard deviation of the parasite burden in the founder strains of mice. Sample sizes were then determined using power calculations to minimize the number of mice for each experimental group.
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 continue to perform pilot studies to determine samples sizes for mouse experiments with quantitative data. And for large experiments we will collect as much information (including faecal and tissue samples) as time allows to avoid repeat experiments performed to look at new parameters from a previous study.
Which animal models and methods will you use	In this protocol mice will be used to model Cryptosporidium infection and to propagate parasite strains. Each step has been optimized to reduce stress

during this project?	and suffering and mice are monitored closely during infection.
Why can't you use animals that are less sentient?	The mouse model allows for control over host genetic factors, which is crucial to studying the pathogenesis of the disease. With this model we can investigate how the immune system recognizes and responds to infection, which can lead to new avenues of treatment and prevention.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We will keep up to date on the latest Cryptosporidium scientific literature. Should there be an advancement that allows for us to improve our protocols, in respect to the 3Rs, we will gladly do so.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	We will work closely with the veterinary staff to ensure that we are always refining our protocols to minimize harms for the animals we work with. Where applicable we may minimise use of wire mesh cage floors replacing them with any suitable bedding.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We will stay up to date with the best practice guidelines developed by the National Centre for the Replacement, Refinement, & Reduction of Animals in Research, and the scientific literature for estimation of sample sizes based on power calculations.

Project	**202. Investigation of metformin action**	
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)	Metformin is currently among the best first-line drugs for type 2 diabetes, because it also reduces risk of cardiac events like heart attack. Unfortunately, because we don't know enough about how it works, we can't develop newer agents based on these properties. These are needed because up to one third of patients refuse to take metformin because of side-effects. Our research is aimed towards understanding the basic mechanisms by which metformin exerts its protective effects.	
What are the potential benefits likely to derive from this project	Through this work, we may develop a better understanding of which targets in cells are the	

(how science could be advanced or humans or animals could benefit from the project)?	best ones to pursue with design of new drugs with 'metformin-like' properties. Such agents could benefit those diabetes patients who cannot take metformin itself. It is also possible that they could help in the control of cardiovascular disease in people who do not have diabetes (we have evidence that metformin can have anti-inflammatory effects in the absence of diabetes).
What species and approximate numbers of animals do you expect to use over what period of time?	We intend to use mice that bear genetic alterations that will allow us to study the role of AMP, a molecule that has been implicated in the mechanism of action of metformin. We expect to use about 1500 animals over three years in establishing and then maintaining these 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?	There are unlikely to be significant adverse effects in any of the mice. At various developmental stages, animals will be killed humanely and their tissues analysed
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We already replace mouse studies, wherever this is possible, with cell-based pharmacological assays. When studying effects of metformin on inflammation, we can study cells, animals and humans. *In vitro,* we can investigate effects of metformin on cells grown in petri dishes. In human studies, we can give metformin and then measure effects of this on inflammation in the blood. Animal studies fill the gap between these two models, where neither in vitro (wild-type) nor human studies are possible. When studying effects of metformin on inflammation for example, it is sometimes important to use tissues obtained from animal models with a gene missing or altered, as this can be a useful way of deducing whether or not that gene is required for metformin to work- there is rarely an equivalent approach that can be carried out in humans and pharmacological tools which can be used on wild-

	type cells almost always affect more than one target, making interpretation difficult. The animal models we will grow are necessary in such situations, to validate pharmacological data.
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 this project we will not only accumulate basic knowledge about the pathways that control gene expression to match oxygen supply and demand but we will also learn the effects of controlled (genetic) manipulation of the activity of individual parts of this pathway on health and in disease models of relevance to both human and veterinary medicine. This work will provide a background to decide which components to target when with drugs which are now becoming available to modify disease models and what side effects we might anticipate from such treatments. The pharmaceutical industry is already making drugs that target these pathways. However, the drugs produced to date are not particularly specific and have mainly been produced for one use – to increase red blood cell production in people with kidney failure. The work we are undertaking will identify which components need to be targeted when to maximise benefits (whilst minimising harm) in a number of other conditions – specifically including cancer, vascular disease, wound healing, autoimmunity and transplantation, as well as protective and/or therapeutic immunisation. Hopefully our results will stimulate the pharmaceutical industry to produce more accurately targeted drugs that have the relevant beneficial effects.
What species and approximate numbers of animals do you expect to use over what period of time?	We will mainly use mice. We estimate that over the five year course of the experiments proposed in this project we may need to breed up to 33,000 mice to produce the ~5,800 mice with the necessary characteristics for us to use ourselves in our more detailed experiments. We anticipate being able to supply some mice from our breeding protocols with these special characteristics to collaborating scientists who have specialist experience which we lack (e.g. relating to heart surgery or models of infectious diseases). We will also use a small number of rats for some experiments where they provide technical advantages. We currently estimate use
	of 50 rats over the five year 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?	The vast majority of mice produced will not be expected to have any adverse effects. Despite using very carefully designed breeding plans it is not possible to generate the mice we need for our experiments without producing others that do not carry the necessary genetic modifications. These mice will have a very minor procedure performed that causes only transient discomfort to establish what genes they are carrying and once it is established that they are not of use for our experiments they will be killed using an approved humane technique. Approximately 18% of the mice produced will be suitable for use in direct experiments. Individual mice will only be exposed to one protocol. Protocols include treatments that equate to acclimatising to an altitude of 5000 metres (either by actually reducing the concentration of oxygen in the air breathed or by treating with a low controlled amount of carbon monoxide which causes a stable and defined reduction in the amount of oxygen the blood can carry), developing a tumour of limited size that does not interfere with normal function / behaviour, interruption of the blood supply to one limb that causes a degree of limping that improves over time, interruption of the blood supply to one kidney (which causes damage to that kidney but not kidney failure because the undamaged kidney works adequately), receiving a defined wound or skin graft to examine effects on healing or receiving a series of inoculations to stimulate an immune response and tests of how effective that immune response is in protecting against diseases like influenza and tuberculosis. Some mice will also be given bone marrow transplants. Some rats will have treatments that at the most severe equate to acclimatising to an altitude of 5000 metres. No animals will be allowed to experience effects of more than moderate severity. All animals will be killed humanely at the end of the experiments or if they experience any untoward adverse effects.
Application of the 3Rs	
1. Replacement	The approaches we will be taking have been tested and shown to have the intended outcomes

State why you need to use animals and why you cannot use non-animal alternatives	on pathways that mediate the response to altered oxygen levels in cell based systems. However, we need to use animals for the experiments covered by this licence because we are investigating effects on integrative physiology that cannot yet be modelled in vitro or in silico. Whilst the pathways we are investigating are conserved in lower organisms such as fruit flies, nematode worms and fish these species are too distant from mammals to provide good models of the disease processes we wish to study. The ability to translate physiological, and then pharmacological findings, relevant to all the models we wish to explore across these species difference is limited.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before starting any experiment we will examine data from any relevant previous studies we have undertaken and the literature to help us design our experiments in such a way as to minimise the number of animals we need to use to obtain a decisive scientific result. Where no prior experience is available we will perform pilot experiments to assess the likely size and variability of effects to allow power calculations to be performed to design the definitive experiments. The inducible system we need for our experiments requires mice that have inherited multiple transgenes; unfortunately despite careful design of breeding programmes it is inevitable that quite large numbers of animals that do not have the relevant genotype (or any associated adverse effects) are produced. Our breeding programme is highly regulated to ensure its effectiveness but also avoid levels of in-breeding within our colony of mice that might lead to misleading results occurring as a result of background mutations. Some of our procedures are better performed on male mice (preferred by our collaborators on tumour immunity) whereas others are better performed on female mice (preferred by our collaborators on BCG immunisation); one advantage of having this variety of applications is that it reduces wastage of mice that are not of the appropriate gender for a particular procedure.

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 generally the most appropriate animal model for the experiments we wish to undertake because of the knowledge base around murine physiology, murine experimental disease models and the ability to manipulate its genome. An exception to this is that rats are better than mice for electrophysiological studies on the type 1 cells of the carotid body that sense oxygen in the bloodstream and so we propose to use rats for a limited number of experiments relating to ventilatory control and the use of compounds that effect oxygen sensitive pathways. No lower organism satisfies these requirements. To improve our experimental techniques we collaborate with expert scientists who have complementary skills to our own. To perform the experiments we wish to undertake we have developed systems that allow reversible temporal control of transgene activity. This means that we can limit the period any animal experiences the consequence of the genetic manipulations we wish to test. The animal models of disease that we are proposing to use are all based on previous literature and in each case we have access to relevant expertise to perform them optimally and minimise suffering e.g. by use of anaesthetics and analgesia. Examples of specific experimental refinements that we routinely use include: ● using ear clips undertaken to mark mice for genotyping ● scoping doses to seek the minimum dose required to produce the desired effects ● administering tamoxifen by gavage which has been better tolerated than parenteral routes. ● when creating bone marrow chimaeras we generally administer radiation in two fractions rather than a single dose. ● using pre-tested agents and previously established dosing schedules to define

	physiological processes e.g. pimonidazole (a bioreductive drug that produces immunologically detectable adducts in hypoxia) to define areas of tissue in which oxygen tensions are below 10 mmHg; BRdU to detect DNA synthesis. ● when using adjuvants to improve the efficiency of immunisation we initially use Titremax Gold as a lower severity adjuvant, reserving use of other adjuvants only for circumstances in which this proves inadequate. ● the appropriate use of anaesthetics and analgesia, humane endpoints, restrictions on sampled blood volumes, volumes of substances to be administered, frequencies of administration by particular routes and the duration of time that individual animals will be used in individual protocols. ● cancer related experiments will be consistent with the guidelines in Workman et al. (Br. J. Cancer (2010) 102, 1555 – 1577). For example, we limit the maximum size tumours are allowed to grow to that is commensurate with the scientific aims of the experiment. ● using appropriate conditional models to assess effects of enzyme deficiency on physiology, circumventing the risk of animals suffering ill effects from life-long widespread enzyme deficiency. ● when reducing oxygen levels we use a purpose built apparatus developed with input from a previous Home Office Inspector to acclimatise the animals to hypoxia and then maintain them in the required environment. The apparatus includes a number of safeguards to protect the mice which are accommodated in their normal cages. ● one of the physiological effects of hypoxia is that it induces an increase in ventilation which in turn tends to cause a fall in carbon dioxide levels. We have found that supplementation of the atmosphere with

	3% carbon dioxide mitigates this effect and any disadvantageous consequences. ● the hindlimb ischaemia model chosen provides an adequate ischaemic stimulus with a lower risk of distal necrosis than models previously described. ● the renal injury models chosen only involve injury to one kidney; the function of the uninjured kidney is sufficient to protect the mice from developing kidney failure. ● in several experiments we duplicate the intervention on a single mouse allowing comparisons to be made within the animal as well as between groups of animal. ● using elastic bandages to cover skin transplants has reduced the number of times dressings need to be adjusted since their flexibility means they seldom impede animals breathing or mobility. ● on the occasions when it is necessary to house animals individually we provide environmental enrichment (e.g. cardboard or plastic tubes / egg boxes and where scientifically appropriate running wheels) to mitigate the social isolation. If the indication for individual housing is transient we return animals to group housing wherever possible. ● where appropriate we perform phenotyping under terminal anaesthesia to minimise suffering whilst maximizing the scientific yield. We continuously re-appraise our approaches in response to the outcomes of our experiments, the 3R's newsletters, peer-reviewed publications and information circulated by our establishment's Animal Welfare systems.

	carbon dioxide levels. We have found that supplementation of the atmosphere with 3% carbon dioxide mitigates this effect and any disadvantageous consequences. ● the hindlimb ischaemia model chosen provides an adequate ischaemic stimulus with a lower risk of distal necrosis than models previously described. ● the renal injury models chosen only involve injury to one kidney; the function of the uninjured kidney is sufficient to protect the mice from developing kidney failure. ● in several experiments we duplicate the intervention on a single mouse allowing comparisons to be made within the animal as well as between groups of animal. ● using elastic bandages to cover skin transplants has reduced the number of times dressings need to be adjusted since their flexibility means they seldom impede animals breathing or mobility. ● on the occasions when it is necessary to house animals individually we provide environmental enrichment (e.g. cardboard or plastic tubes / egg boxes and where scientifically appropriate running wheels) to mitigate the social isolation. If the indication for individual housing is transient we return animals to group housing wherever possible. ● where appropriate we perform phenotyping under terminal anaesthesia to minimise suffering whilst maximizing the scientific yield. We continuously re-appraise our approaches in response to the outcomes of our experiments, the 3R's newsletters, peer-reviewed publications and information circulated by our establishment's Animal Welfare systems.

Project	**203. Investigation of pathological mechanisms in ocular models of inflammation – autoimmune and infective uveitis**	
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)	Uveitis is an inflammatory condition which can lead to blindness. The project uses mouse uveitis to provide information on how uveitis is caused and how it can be better treated. Uveitis can develop in patients with an underlying infection (approximately in 50% of cases) or by a faulty immune system in which the patients' immune cells attack ocular tissues (approximately	

	50% of cases). We think that the cellular mechanisms for ocular damage might be different in these cases; however as the treatment may be similar we hope to shed light on both. This work is needed as it can indicate better treatments for patients who are at the moment treated with potentially toxic medications since otherwise they may have severely impaired vision or complete blindness. If customised specific treatment such as cell therapy can be shown effective in preclinical animal studies, this approach can be brought to the clinic and would provide patient-specific treatment for ocular inflammation patients as well as lead the way for treatment of patients with other autoimmune or immune-mediated conditions.
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 aims to identify and understand the mechanistic background of eye inflammation (i.e. how it develops in humans). This specific knowledge is crucially needed to ultimately improve treatment options for patients with eye inflammation (uveitis), as current therapies are rather unspecific and risky. Predicted novel approaches arising from this work include case-specific cell treatment (i.e. injection of specific cells of the immune system). It is likely that these mechanistic clarifications will also prove beneficial for other auto-/immune diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using mice for our studies as they develop clinically identical disease to humans. We are planning to use genetically modified/transgenic mice, which do not always develop uveitis, hence the estimation of 6000 mice during the duration of the project (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 will be kept group-housed in cages of 4 or 10 animals at maximum (depending on cage type/size), with constant access to fresh drinking water, adequate diet, and enrichment. Animals will be continuously monitored and handled by well-trained personnel. Cages will be kept in ventilated, temperature- and humidity controlled rooms. Procedures that will be performed include: 1. General anaesthesia: Anaesthesia will be performed intraperitoneally (i.p.; into the abdomen) in full agreement with the LASA good practice guidelines for the administration of substances. Animals are expected to fully recover within a short

	period of time (~30 min) following interventions, will be kept in a warm environment with facilitated access to a food and water (mushy diet), and monitored throughout. Side-effects of this procedure include very minor, self-limiting bleeding at the site of injection. 2. Surgery: Surgery will be done under general anaesthesia (see above). Surgical procedures will be performed under an operating microscope using aseptic technique, and include removal of the spleen and lymph nodes. First, respective small areas of the skin will be shaved, and incisions made at the neck (for lymph nodes), or at the abdomen (for spleen), respectively. Incisions will be 1 cm long to expose lymph nodes, and 2 cm long to expose the spleen. Lymph nodes can be removed without further preparation; as for the spleen, associated vessels will be closed with sutures prior to organ removal. Skin will be stitched with skin sutures. The procedures will take 10 min for lymph nodes, and 15 min for spleen removal. Complications that might occur include bleeding and infection as a result of suture loosening through scratching, leading to prolonged wound-healing. 3. Immunisations: Immunisations will be performed to induce eye inflammation (uveitis). This will be done by injecting small volumes of Complete Freund's adjuvant (CFA; contains a heat-killed bacterium), together with a peptide underneath the skin (s.c.) at both rear legs. Adverse effects of this procedure include minor skin dry scabs at the sites of injection which are likely to occur in 50 % of all animals injected. A large proportion of the mice will develop eye disease which in itself is painless (as in humans), and does not exhibit any external signs or symptoms. 4. Fundoscopy: The procedure of fundoscopy (i.e. taking photographs of the background of the eye to assess severity of inflammation) will be performed under general anaesthesia (see 1. General anaesthesia), using a small endoscope connected to a digital camera. Pupils will be dilated using eye drops (mydriatic agent), and eyes kept lubricated throughout the procedure. Fundoscopy is non-invasive and completely pain- and adverse-effect free. 5. Viral infection: Following general anaesthesia (see 1. General anaesthesia), mice will be immunised with herpes simplex virus applied directly onto the eye surface (cornea), after

	scratching it. Herpes infection might become visible on the skin surrounding the eyes which is painless and settle without any further intervention. The procedure further holds the potential risk of viral spread into the brain of the animals where it could induce inflammation. 6. Cell treatment: Cell treatment will be performed to administer different cell types of the immune system to animals. Specific cells will be dosed intravenously (i.v.; into a vein) into the tail of the animals, using temporary restraint in a suitable mouse restrainer. This procedure bears the risk of pulmonary embolism, but this risk is small. For administration of cells also the subcutaneous route will be chosen (s.c.; underneath the skin). This procedure might lead to very minor, self-limiting bleeding at the site of injection. All of the above procedures have the potential to induce moderate suffering, despite the fact that some of them in themselves are mild in nature. The moderate classification is based on the fact that all procedures will be performed on genetically modified animals. All animals are expected to fully recover after all procedures in a timely manner. If deemed necessary based on animal behaviour (facial expressions according to the Mouse Grimace Scale), adequate pain relief will be given. Other medications (e.g. antibiotics) will be administered as advised by vets (NVS). At the end of the study, all animals will be humanely killed, and after confirmation of death, tissues taken for further examination. As all animals will be used for experiments, keeping them alive, setting them free and/or re-homing are therefore not applicable.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Experimental autoimmune uveitis (EAU) is an animal model of human disease for which there is no *in vitro* (in lab) model. Johns Hopkins ALTWEB (alternatives to animal testing) was searched for reduction strategies for the animal model of uveitis. The search returned no results. The same was true for our Medline and PubMed searches. It is ethically impossible to sample human eyes during the flares of inflammation and as the interaction of the whole immune system (eye related as well as lymphoid organs in the rest of the body) is needed for understanding of the processes which would

	lead to inflammation in the eye, only a living organism can be used to investigate this. Understanding the mechanisms of inflammation and translating them into therapeutic opportunities for medical practice has already reaped benefit for patients and our further work with these models should in the long term provide safer, individualised treatments which would also prevent visual loss in affected patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible (approximately 10% of the work) *in vitro* (in lab) techniques will be used which will reduce the number of animals used. Also the preparation of the cells from animals will be done with the techniques that allow us to expand retrieved cells in the lab, thereby reducing the number of animals needed. Experiments are planned to obtain powered, statistically strong data, which would take into consideration also biologic variance. This includes meticulous planning of a mouse breeding programme and regular checks of the breeding colonies, in order to meet the experimental requirement with minimal mouse numbers. Once particular experiments have been completed certain genetically modified lines may no longer be required and will be frozen-down.
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 with known (specific) genetic background for our studies that are known to develop clinically identical disease to humans, and therefore represent the most refined model available. The protocols involving surgical procedures will be performed under general anaesthesia, so animals will not be aware of these procedures and therefore not experience pain. All procedures and general animal handling will be done by trained and experienced personnel in eye surgery. With regard to method refinement, scoring systems will be developed over time and used after consulting with vets (NVS). Principles of aseptic surgery will be used to reduce the risk of infection, and accelerate wound-healing. Specific attention will be paid to appropriate care before, during, and after surgery. These means include body heat control, pain relief, and facilitated access to food and water. Additional cage enrichment will be provided to animals.

Project	**204. In-vivo assessment of new coatings for joint replacement implants**	
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)	Joint replacements are commonly performed on people who have severe joint pain due to conditions like arthritis. Joint replacements describe the process of replacing a damaged joint with an artificial implant. To keep the implant more securely in the bone most implants have a coating that encourages the bone to stick to the implant. In this project we are investigating whether a new generation of coatings, based on glass technology, can stick the implant to the bone better than the coating that is currently used in human surgery and to investigate the claim that the glass coatings	

	may help prevent infection at the site of surgery. The latter aim - whether the glass coatings are able to prevent infection will be studied in the laboratory and not in the animal models.
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)?	Joint replacements are usually successful, however 12% fail due to loosening of the joint replacement implant within the remaining bone or infection. If successful this project will have discovered a new coating that can be used to stick implants into bones more securely and with a reduced risk of infection to the patient. This will have significant implications for human joint replacements. It will reduce the time that the patient takes to get back to normal daily life, reduce the likelihood of loosening of the implant (making a second surgery less likely) and reduce the likelihood of infection from the joint replacement.
What species and approximate numbers of animals do you expect to use over what period of time?	96 sheep 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?	There are two protocols within the project. In the first protocol (80 sheep) the sheep will have a general anaesthetic and have small (6mm diameter) pins placed across one of the bones in one hind leg (all the pins will be placed during one anaesthetic). The number of pins that can be safely implanted will be determined in a pilot study but will be no more than 4. The purpose of this protocol is to identify which pins provide the best interface between the bone and the pin (bone integration and growth). The expected adverse effects of this protocol are pain lasting a short time associated with the placement of the pins and all animals will receive drugs to prevent pain after surgery. There is also a theoretical (but very small chance) of fracture of the bone after pin placement. In the second protocol (16 sheep) the animals will undergo a hip replacement surgery. The possible adverse effects of this protocol are hip dislocation and fracture of the bone associated with the joint replacement. To help prevent this, all animals will be weight supported by a body sling for the first day after surgery and then be kept in a

	small pen (approximately 9m2) for 3wks after surgery to minimise exercise movement that may predispose to hip dislocation before being kept together in a larger pen. At all times, sheep will be kept together with at least one companion to avoid separation anxiety. All animals will receive drugs to prevent pain after surgery including the use of an 'epidural' – a long-acting injection that reduces pain from the pelvis and back legs. At the end of the study the animals will be killed humanely so that we are able to collect information from the tissues to verify whether the study has been successful.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Developing new technology to improve surgical implants takes many years. The materials that will be used as coatings on the transcortical pins and hip implants in this study have been tested thoroughly in the laboratory prior to be used in animals and many possible materials that are not suitable for this purpose have been abandoned. We now need to use animals in the final part of this study so that we can show that the proposed coating materials fit well with bone (in a transcortical pin mode)and that they are successful in fixing a hip replacement into position in the animal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will always use the least number of animals necessary to achieve the aims of the project whilst getting meaningful results. For this study we will use 6 per group in the bone pin model and 8 animals per group in the hip replacement study. These numbers have been worked out statistically from previous studies. In our research we randomise our experiments – for example we randomise which animals get which treatment and in what order they undergo surgery. In some instances we ask researchers we work with outside our laboratory to randomly allocate animals to experimental groups. We believe that these methods contribute to the robustness of our data interpretation by removing bias. . In addition we use 'pilot' studies of a small number of animals to check what we plan to do is well tolerated by the animals before we apply the experiment to
	larger groups of animals.

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 sheep as our model for a number of reasons. Whilst sheep do walk on 4 legs, not 2 like humans, the structure of the hip joint is similar to humans and so provides a good model in which to perform this work. If successful, the new coatings could be used in human patients without further experiments in animals being undertaken. We will minimise animal suffering by ensuring that animals receive anti-inflammatory medication, similar to ibuprofen, during and after surgery and that the animals having the joint replacement receive a long-acting injection that reduces pain from the pelvis and back legs.For the majority of this work, of our animals are kept in groups and are kept as naturally as possible out in a grass field. A small number of animals will be kept in indoor pens immediately after their surgery (first 1-2 weeks), but will always be kept in close proximity with and within sight of other sheep to avoid separation stress during this time. . We monitor our animals movement continuously through the experiment using measures of how much weight they are placing on their operated legs using clinical scoring and a force plate and a 'Fitbit tracker' [(this is a tracker placed on a collar around the neck of the sheep that records the activity of the sheep). Any animal that is showing abnormal movement or behaviour (as assessed by clinical examination, weight bearing and Fitbit activity monitoring can be quickly identified, examinedand any necessary treatment given.

Project	**205. Livestock vaccine development**	
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)	Vaccines are one of the most effective methods of controlling infectious disease. This project aims to develop vaccines to combat diseases in livestock species for which vaccines are either unavailable or lack efficacy. Diseases targeted in this project cause continuing and significant welfare and economic problems in livestock and some are also a threat to human health. This work seeks to identify mechanisms by which animals become immune to such diseases, and mimicking these immune responses through development of appropriate immune-stimulating compounds (i.e. adjuvants). Once vaccines have been developed they will be tested in animals to determine how effective they are in	

	preventing infection. The project will also determine what types of diet are most effective in improving vaccine performance in livestock.
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 is in response to national and international needs, contributes to biological, veterinary and medical knowledge and is in the public interest. The development of vaccines will reduce the disease burden of our livestock species, thus improving the health and wellbeing of farmer livestock, and in the case of diseases carried by livestock which affects humans, improve human health. It will reduce the reliance on chemical treatments including antimicrobials to control disease, resulting in reduced contamination of the environment and slowing the development of antimicrobial resistance.
What species and approximate numbers of animals do you expect to use over what period of time?	The animals used in this work (cattle and sheep) are the natural hosts for the diseases being studied, with the exception of mice which will be used in some instances to refine vaccine prototypes prior to testing in sheep. The numbers used are restricted to those expected to produce statistically significant answers to questions posed, using a number of statistical methods based on previous work and experience in conjunction with experts in the field. Over the 5 year project, up to 400 sheep, 350 cattle and 200 mice will be used, although wherever possible the use of non-animal systems will be employed to address research objectives.
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 experimental models used in this work have been developed over a number of years with great care and attention in order to minimise suffering by the animal. Work with cattle and sheep is not expected to be of greater than moderate severity. It is not anticipated that the infection protocols used in the studies will result in clinical disease, with the animals remaining apparently healthy. Experienced observers, with access to veterinary advice and care at all times, monitor clinical signs of all experimental animals at regular intervals in order to quickly identify any animal requiring veterinary treatment. Any animal failing to respond to treatment will be killed humanely. By necessity,

	the majority of experimental animals will be killed at the end of procedures although some animals may be re-used for other studies provided they are fit and healthy.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible, the use of non-animal models will be employed throughout this project. However, the development of vaccines requires firstly, an understanding of the mechanisms by which an animal becomes immune to disease, secondly, which parts of the disease causing organism (the pathogen) to target through vaccination, and thirdly, the characterisation of the immune response generated following vaccination. To address these questions, we need to study the immune response as a whole (i.e. *in vivo*), as many different components of the immune system interact to generate the final immune response. Furthermore, some of the pathogens under investigation cannot survive outside the animals and we will need to infect animals to obtain pathogen material to identify new vaccine targets. The use of animals is an absolute requirement for the assessment of the efficacy or effectiveness and safety of any new vaccine.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The careful refinement of experimental models ensures that only the minimal number of animals required to obtain statistically significant and biologically relevant outcomes will be used. Independent advice on the experimental design is provided by trained statisticians in advance of any experimental work being conducted. In addition, proposed experiments are reviewed by an ethical review committee to ensure that the minimal number of animals is used. Wherever possible, experiments will be conducted *in vitro* to minimise animal usage.
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	REDACTED We have developed relevant, reliable and reproducible disease models in conventional cattle and sheep, which have been refined to be the least severe necessary for valid results to be obtained. Considerable care and attention has gone into refining the techniques

general measures you will take to minimise welfare costs (harms) to the animals.	employed to monitor the immune responses during animal studies in order to reduce the degree and duration of any suffering to a minimum. Trained teams of observers monitor animals at regular intervals, accurately evaluating the responses of individual animals and seeking veterinary intervention where necessary.

Project	**206. Loss and recovery of exocrine gland function**	
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)	There are some diseases in man that cause an irreversible loss of function of the glands that provide saliva and tears, which keep the mouth and eyes moist and healthy.Long-term dryness of the mouth and eyes leads to infection and reduces our ability to eat food and undertake normal everyday activities. We still do not fully understand the mechanisms causing glands to stop secreting nor do we fully understand how damaged glands can repair themselves and begin to function again. The aims of this project are to study the biological mechanisms causing loss of gland function and the mechanisms, which	
	can lead to recovery of function.	

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)?	Firstly, the knowledge gained in this project may enable us to develop drugs that can help to prevent loss of glandular function by blocking the chemical signals in glands that inhibit the secretion of saliva and tears. Secondly, by increasing our understanding of the ways in which glands are able to repair themselves following damage we can develop drugs that can enhance the repair mechanism in glands which remain damaged.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 2200 mice and 640 rats during the 5 years 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?	A substantial part of the project will cause minimal suffering (mild) but the overall severity of this project is moderate. The most common adverse effect is a transient period of post-operative pain following recovery from anaesthesia. Some animals might experience an adverse reaction to specific drugs used or wound healing may sometimes be a problem following surgery. At the end of the experimental protocols, animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our research projects use samples collected under ethical approval from human subjects attending hospital clinics and suffering from diseases of the glands supplying saliva and/or tears. We will continue to use cell culture methods in order to investigate aspects of the way in which glandular cells function. However, there remains a need to undertake animal experiments in order to study gland function since these complex organs do not exist outside of the body.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals used will be reduced by: (1) undertaking some experiments on animal and human cell lines, (2) taking pieces of human and animal glands and using them to

	prepare cells that can be grown in the laboratory, (3) because each animal has 2 of each gland one gland can sometimes serve as a control, which means that a second animal does not need to be used.
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 in our studies since their saliva and tear producing glands function in a very similar way to human glands. There are genetically altered mice that can be used for studying gland function and disease.We have refined many of our protocols in order reduce the number of animals used and in particular many fewer rats are now used in our protocols.All of our surgical procedures are conducted under anaesthesia.When needed appropriate analgesia is given for 24 hr following recovery from anaesthesia in order to reduce the possibility of post-operative pain.

Project	**207. Lymphatics and cell trafficking in organ transplantation**	
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)	Organ transplantation is the best treatment for patients with end stage organ failure. However, rejection remains the main cause of graft failure. The lymphatic system, which forms a crucial part of the body's immune system to reject transplanted organ has received very little attention. It drains excessive fluid from all parts of the body back into the blood circulation. It also facilitates the movements of various immune cells responsible for fighting infections and rejection of foreign materials (such as transplanted organs). Here, we will study the changes in the lymphatic system during organ transplantation and explore treatment strategies targeted at lymphatics to see if they can prolong graft survival. Organs such as	

	heart, kidneys and tissues such as pancreatic islet cells are routinely transplanted in the clinic. We plan to use these models in mice to study various aspects of the body's defence system in order to devise ways to improve treatment for recipients of organ transplants. Donor cells that traffic in the lymphatic system are crucial in the rejection process. We have developed a specific toxin that can kill mouse cells that express certain human molecules crucial in the immune response and will be testing its effectiveness in prolonging graft survival in mouse models of heart, kidney or pancreatic islets cells transplantation. We will use mice that express the relevant human molecules so that our toxin can be tested.
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)?	Better understanding of the lymphatic system in organ transplantation may lead to better treatment to combat rejection. If rejection and therefore, failure of the transplanted organ can be reduced, the morbidity and mortality of organ recipients would be reduced. In addition, if patients survive longer on the transplanted organs, there is a reduced need for them to return to the organ transplant waiting list, thereby, allowing more potential recipients to have organ transplants, not to mention the improved quality of life. In many cases, having an organ transplant is cheaper for the health service than the alternative treatment. For example, in kidney transplant, although the first year of transplant cost approximately 50% more than staying on dialysis, in the subsequent years, it is over 60% cheaper. The toxin that we have developed is specific for human cells that are responsible for triggering the rejection response. Killing these cells does not affect the normal functioning of the transplanted organs, but would dampen the rejection response. Therefore, if successful, it can be applied to the clinic to prevent rejection without the need to re-design its molecular structure for

	use in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used, typically up to 1500 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?	Animals will undergo organ transplantation which involves surgery. The type of procedures they undergo are very similar to humans who are undergoing organ transplants. Heart, kidney and pancreatic islet cells will be used. Animals will be observed closely after surgery and post-operative care such as analgesia will be similar to that received by humans. They may also be given various types of treatments to observe their effects, such as injection of drugs or antibodies. Complications can occur, such as hernia, infection or bleeding. Care will be taken to minimise the risk of these. Animals will be observed closely until they fully recover from surgery. Veterinary advice will be sought if in doubt and animals will be killed humanely if they show sign of distress beyond the expected level, such as poor mobility, hunched posture or poor skin condition. The level of severity is the same as humans would undergo when having an organ transplant. Adequate anaesthesia will be given during all operations. Analgesia will be administered after each operation to ensure that pain and discomfort are kept to a minimum . At the end of the experiment, animals will be culled using a recognised method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune response is the result of complex interactions between many different elements within the body. This cannot be recreated in a test tube. However, whenever possible, for example, when examining a very specific aspect of cell behaviour, we will use cell cultures instead. For example, as this project studies the rejection response in organ transplantation, cells from the donor strain of mice can be isolated, for examples from lymph nodes and mixed with immune cells from the recipient strain of mice in test tubes to

	study their interaction, thereby, avoiding the need to use live animals for organ transplantation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical modelling would be used to predict the minimum number of animals in each group which will answer the scientific questions being asked. A major output of this project is the survival of the transplanted organ. There are specialist statistical programmes designed to estimate the minimum number of animals required in each experiment to make it valid. This will be used to ensure that excess numbers of animals are not used.
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 mice as they have immune systems similar to that of humans. Animals lower down in the evolutionary tree do not satisfy this criteria. Established standards on how to perform the procedures will be observed. For example, to minimize harm to the animals, aseptic technique is applied throughout the procedure to reduce the risk of infection. Pain relief will be provided as advised by the Named Veterinary Surgeon (NVS), which is standard for all surgical procedures. Animals are transferred for 24 hours to a temperature-controlled heating box to keep animals warm during recovery. Close monitoring occurs after surgery to ensure good recovery from the anaesthetic. In the unlikely event that animals show adverse effects following surgery, they may receive supportive care and treatment as advised by the NVS or Named Animal Care & Welfare Officer (NACWO)or be humanely killed as appropriate. Any animals that are not fully recovered from the surgical procedure within 24 hours will be humanely killed. REDACTED

Project	**208. Maintenance and breeding of genetically altered 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
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	To provide a centralised breeding services programme with stringent colony management by highly specialised technicians to reduce wastage via the use cryopreservation services and transport of embryos or sperm whenever possible as opposed to live animals.	
Why is it important to undertake this work?	The (REDACTED identifying acronym)-unit has over 95 genetically altered (GA) colonies that are maintained for the production of GA rodents and Zebrafish for use in procedures under approximately 30 project licences with the authority to use animals of this type. This project licence is for the(REDACTED - identifies type of establishment)establishment to offer a centralised service for the provision of GA animals, embryos and biological products (for	

	example urine and blood samples) to support the research projects that require them but also to minimise the numbers of animals bred and wastage. Genetically altered animals are important in helping to discover what causes disease. For example they may be disease models with a genetic change that mimics a disease like cystic fibrosis in man, or enable the study of implanted human tumours for testing new anti cancer medicines. Knockout mice allow the function of a single gene to be studied in a particular disease and their use has provided a wealth of knowledge on the regulation and expression of genes and how this can impact the health of humans and animals alike when normal mechanisms don't function properly. In addition, the use of reporter models has allowed imaging of live animals which has reduced the number of animals required, particularly in cancer research studies. This centralised service licence for breeding and supply of mice to scientific projects is administratively efficient, with breeding controlled to produce the numbers of animals as needed and any spare can be made available for use by several different scientific projects.
What outputs do you think you will see at the end of this project?	An efficient service provision to breed and maintain genetically altered (GA) animal models for use in research programmes for scientific discovery and development of new medicines and knowledge to benefit human and animals alike in the treatment of disease.
Who or what will benefit from these outputs, and how?	The researchers within the (REDACTED-identifies type of establishment)establishment and their collaborators both inside and outside of the UK will benefit from a centralised service provision for the breeding and maintenance of GA animals. The health and welfare of the animals will be the top priority under this project licence. The high standard of housing and care and considerable expertise of the REDACTED team will ensure minimum wastage of animals and bio-banking of any tissues in order to reduce the numbers of animals required in the future. The movement of live animals will be avoided at all costs and the ability to transport sperm, embryos and fish eggs as opposed to adult animals will be another benefit from this project licence.

Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	With regular meetings, colony management and a quick genotyping service we can ensure the required numbers of animals are produced with minimum wastage. We will further contribute to the reduction of animals used by bio-banking tissues to replace the need for more animals to be bred. Through this project we will ensure that we keep our animals in optimum health by using rederivation services to keep our colonies of animals clean and free from any opportunistic pathogens. We will encourage the sharing of resources to ensure that animal colonies are not duplicated and also provide assistance with any exports to help future collaborations and the advancement of scientific knowledge.
Explain why you are using these types of animals and your choice of life stages.	The REDACTED unit currently has over 95 separate colonies of GA mice, occasionally we have GA rats in but currently do not breed these in house. We are expanding our Zebra fish facility and capabilities, it is hoped to encourage researchers to consider this species as an alternative to mammalian work. Mice represent our biggest species and the majority of these are GA lines, we need the different life stages of these animals in order to supply them to our project licence holders for the research work they do.
Typically, what will be done to an animal used in your project?	Only breeding and maintenance, other procedures are to keep the animals clean and free from opportunistic pathogens and to cryopreserve lines in order to reduce the numbers of animals bred and used.
What are the expected impacts and/or adverse effects for the animals during your project?	The adverse effects for the vast majority (approximately 90%) will be non existent or mild at the most. For the few that may experience moderate adverse effects these will be associated with the disorder they are modelling and humane end points are in place to ensure no animals will suffer.

What are the expected severities and the proportion of animals in each category (per animal type)?	For the mice, 90% will have no severity consequences or mild at the most. 10% may have some effects as a result of their genotype but typically this will be stunted growth, immune suppression, dermatitis due to reporter gene activity, accelerated ageing but none will suffer welfare issues beyond mild to moderate. For the rats, all of them will experience either no consequences of their genotype or mild at the most, typically this will be immune suppression but our housing conditions will protect them from infections. For the fish, all of them will experience either no consequences of their genotype or mild at the most, typically this will be stunted growth or reduce ability to reproduce and husbandry procedures will be in place to manage these.
What will happen to animals at the end of this project?	used-in-other-projects
Why do you need to use animals to achieve the aim of your project?	In vitro work is an important preliminary step for assessing the biological function of a gene or protein and expression studies can be performed using cell lines, however, the interaction of transcription factors and other genes and the dynamic regulation that goes on via multiple systems will be absent in vitro. Therefore, our researchers sometimes need to proceed from these initial in vitro experiments to test the effects in vivo in a fully functioning biological system. In order to investigate the interaction between all the individual cells, growth factors, molecules etc involved, this work must be performed in animal models and GA animals in particular can be very useful and powerful models to help with this research. It is hoped that the results of the in vivo studies that our researchers do will also contribute to the refinement and development of in vitro models that will be useful in further experiments as opposed to live animals.

Which non-animal alternatives did you consider for use in this project?	Our researchers are always looking for alternatives and the use of human cell lines, mathematical modelling and invertebrates is active and promoted throughout the REDACTED establishment. We run workshops that look at alternatives to the use of animals in research and these also encourage and enable collaborations with researchers using non-animal alternatives.
Why were they not suitable?	The researchers using the GA animals bred in this licence require the fully functioning biological systems that currently can only be replicated in a live animal but they are always looking for alternatives to this work.
Enter the estimated number of animals of each type used in this project.	zebra-fish: 5000 mice: 56000 rats: 200
How have you estimated the numbers of animals you will use?	The numbers are over 5 years, some animals will be used to establish clean colonies, some animals will be used to freeze down and preserve colonies to reduce numbers bred, others will be for the maintenance of colonies. Numbers estimated are based on the numbers of the previous project licences for breeding and maintenance of animals and on the future predictions. Currently, there are approximately 95 colonies of GA animals in house, the numbers estimated represent a usage of 10 mice per colony per month over 5 years.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	This service licence is intended to centralise our breeding and supply of GA animals to our research community, no experiments will be done under this licence.

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 adhere to all the recommendations on "assessing the welfare of GA mice (April 2006)" or any other Home Office endorsed guidelines superseding these to ensure that the welfare of animals used is at the highest levels and numbers of animals used and bred are optimised to reduce wastage and unnecessary breeding.
Which animal models and methods will you use during this project?	Mice are the recognised species for work involving genetically altered animals. There are standard protocols that are utilised in this Project Licence and none are expected to cause pain, suffering, distress or lasting harm. Rats are used more rarely than mice but are needed for some specific projects e.g. Nude rats (rnu/rnu) are used in cancer projects as their immunocompromised status allows them to grow subcutaneously implanted tumours but no tumours will be produced under this project licence. GA fish are being used more and more as alternatives to the use of mammalian models. They represent a lower neurophysiological species to rats and mice and their use on the licence is to provide an alternative species for use in future research projects as opposed to mammals.
Why can’t you use animals that are less sentient?	The actual procedural and experimental work on the animals produced under this licence will be done in other project licences that we supply to. All the project licences minimise the harm done to animals and whenever possible immature life stages are used and work is non-recovery with terminal anaesthesia involved. The Zebra fish on this project licence will be used to produce eggs and embryonic stages of fish for use in preliminary studies as an alternative to more developed and sentient animals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We run regular 3Rs workshops, we are signed up to the NC3Rs newsletters and have a dedicated NC3Rs regional manager to help keep us up to date on advances and in the 3Rs. The project licence holder is a member of a management group with considerable expertise in the breeding, maintenance and management of GA animals and publications and peer reviews are shared and promoted. We are kept up to date with regular publications and advice notes from the Home Office and follow any recommendations that come out of this regulatory body. The project licence holder is an active member of the AWERB and the wider community AWERB HUBs. Regular attendance at workshops, conferences and CPD training will keep the holder up to date on advances in the 3Rs. All of these resources will be used to keep informed about advances in the 3Rs and implementation of them throughout the lifespan of the project licence.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	Our team have extensive experience and expertise in the breeding and maintenance of GA animals and this will be drawn upon to maximise the welfare of the animals in our care. We use tunnel handling for all our mice and they are well trained for the technique which further reduces the anxiety in this species. We have a standardised 7 day post operative monitoring in place and always employ a regime of post operative analgesia for any procedures done.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	APCs (Animal Procedures Committee) recommendations on “Assessing the welfare of GA mice” (April 2006) or any Home Office endorsed guidelines superseding these.

209. Mammalian Erythrocyte Micronucleus Test Laboratory Proficiency and Validation Project

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants.
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)
- Protection of the natural environment in the interests of the health or welfare of man or animals.

Key words

Erythrocyte, Micronuclei, Genotoxic, DNA, Reticulocyte

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to set up the Erythrocyte Micronucleus Test using rats and mice in this laboratory so that it can be performed to understand whether or not chemicals are safe for human health and the environment or if they cause damage to DNA and are said to be genotoxic. Damage to DNA is potentially very serious and can lead to cancer.
In this test, animals are dosed with a chemical and then the red blood cells in the bone marrow (immature erythrocytes) or blood stream (reticulocytes) can be sampled and inspected for fragments of DNA; these are called micronuclei. As red blood cells don't contain a nucleus, the presence of micronuclei in these cells is an indicator of DNA damage.

This project has three objectives; the first is to show that DNA damage can be detected using the methods used in this laboratory and that the results can be repeated using at least 2 known genotoxic chemicals. The second objective is to build a database of a minimum of 10 experiments worth of data, showing micronucleus levels in groups of animals which have been treated with either water or saline (vehicle control) or a known

genotoxic chemical (positive control). This database will be used for the interpretation of results from individual experiments testing the safety of potential new chemicals. The third objective is to implement a further labelling/staining technique to be able to identify the type of DNA damage a micronuclei contains and to build a database with at least 2 experiments worth of data.

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?

New chemicals must be tested by law to make sure they are safe to be taken by humans in the case of medicines, food additives and flavours or used in the environment in the case of weed killers and fertilizers.

By providing this test as a service, chemicals will be tested to see if they are likely to cause damage to DNA and any which are found to be toxic may be stopped in their development or not approved for sale. This will help protect people and the environment from potentially dangerous chemicals which could cause cancer.

Species and numbers of animals expected to be used

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

The period of the licence is 5 years.

The project will be conducted in two parts; the first part will be performed in the rat and will require approximately 1750 rats (875 per sex) and the second part will be performed in the mouse and will require approximately 750 mice (375 per sex).

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 severity level for this project is set at moderate. However, most animals (over 95%) will not experience any adverse effects, but a few may experience, at worst, mild adverse effects. After dosing of the vehicle and positive control chemicals, the rats and mice will be humanely killed and samples of either bone marrow or blood will be taken for processing, (preparing bone marrow smears on glass microscope slides or running the blood samples through the flow cytometer) and counting the micronuclei in the immature erythrocytes or reticulocytes.

Replacement

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

There are several in vitro assays which are also performed to assess a chemicals potential to cause DNA damage. They use genetically altered bacteria or mammalian cell cultures in the case of the Ames test and Mouse Lymphoma Thymidine Kinase assay. Whilst these tests are very useful and provide a good indicator of DNA damage they do not have the ability to fully mimic what happens to a chemical in a live animal system in terms of absorption, distribution, metabolism and excretion by the animals organs and tissues. Therefore, it is necessary to conduct the erythrocyte micronucleus test in order to fully assess a chemicals potential to cause DNA damage before giving to man or exposing the environment.

Reduction

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

The experiments will be designed in line with the regulatory test guidelines which have been developed over the past 30 years to explain how to conduct the test in the best possible way and using the minimum of animals to conduct the test properly.

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 test will be conducted in the Han Wistar rat and the CD-1 mouse as these are two widely used rodent species and strains used in safety assessment of chemicals.
For experiments in both rats and mice, animals will be dosed twice, 24 hours apart and sampled once 24 or 48 hours after the second dose. This dosing and sampling schedule requires the least number of animals.

Micronuclei will be assessed in the rat in two tissues and by two methods; the bone marrow by microscopic analysis and in the blood by flow cytometry. For the mouse, only bone marrow samples will be assessed by microscopic analysis.

Three dose routes will be used in this project; oral, intravenous and intraperitoneal injection. The oral and intravenous routes are the most commonly used dose routes in genotoxicity testing and the intraperitoneal injection route used only for administering the aneugen positive control substance.

Animals will be humanely killed and bone marrow samples will be removed at post mortem. Blood samples will be collected on anaesthetised animals which are then humanely killed. Blood sampling in this way is a more refined method than taking samples using conscious animals from peripheral tissues and reduces any pain, suffering or distress potentially experienced by the animal.

Although the experimental design is highly prescribed in the guideline and literature a review will be conducted after each experiment in order to identify and refine any

procedures or processes which could be improved up on.

Project	**210. Mammalian Erythrocyte Micronucleus Test**	
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 aim of this licence is to conduct the Mammalian Erythrocyte Micronucleus Test using the rat. The test is performed in order to determine whether a chemical is safe in relation to human health and exposure in the environment or if it induces damage to DNA and is said to be genotoxic. Chemicals which damage to DNA are potentially very serious and can lead to cancer. The basis of the micronucleus test is the examination of the red blood cells in the bone marrow or blood stream for the presence of small fragments of DNA inside the cells following dosing the animal with the test chemical.These	

	small fragments of DNA contained within the red blood cells are called micronuclei and are formed as a result of DNA damage. Red blood cells do not contain a nucleus as it is ejected during red cell development, therefore the presence of micronuclei in these cells is a good indicator of DNA damage. The experiments conducted under this licence have two objectives; the first is to determine a maximum tolerated dose (MTD) for a test chemical from which a suitable dose range for the micronucleus test can be set. The MTD is defined as the highest dose that will be tolerated without evidence of toxicity, relative to the duration of the study, but not death or evidence of pain, suffering or distress necessitating humane killing. The second objective is the conduct of the micronucleus test using a range of at least 3 dose levels covering a range from the highest dose (MTD) down to a low dose in which little or no toxicity will be observed.
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 conduct of this test is a requirement by law and all new chemicals must be tested to ensure that they are not toxic to DNA and are safe to be taken by humans in the case of new medicines, or consumed in food for example food additives and flavours or not toxic to the environment, in the case of weed killers and fertilizers. By offering this service, test chemicals will be assessed to see if they have the potential to cause DNA damage. If a new chemical is found to cause DNA damage it may not be given approval to be sold and its development halted. This is to protect humans and the environment from chemicals which are potentially harmful to human health, which may lead to the development of cancer, or be highly toxic in ecosystems.
What species and approximate numbers of animals do you expect to use over what period of time?	The period of the licence is 5 years. The experiments will be conducted in two phases; the first phase will be a dose range finding experiment in small groups of animals in order to establish the highest dose that will be tolerated. The dose range finding experiments will require approximately 1200 rats (of either sex). The

	second phase will be the conduct of the Mammalian Erythrocyte Micronucleus Test and will require approximately 3000 rats (of either sex).
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 severity level for this licence is set at moderate. In the dose range finding phase, small groups of animals will be treated with the test chemical in order to establish the highest concentration for the micronucleus test. The test chemical will be given either orally, by injection in to either the blood stream or abdomen. These animals may experience adverse effects for example; changes to breathing, body weight loss, pain or discomfort, but which should not exceed a moderate degree of suffering. Blood samples may also be taken from these animals which result in no more than minor pain or discomfort. In the main micronucleus test greater than 60% of animals will not experience any adverse effects, but some in the highest dose group may experience, at worst, moderate adverse effects for a short duration as described above. At the end of the study animals will be killed humanely and bone marrow or blood samples taken for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Prior to animal testing, computer programs which analyse the chemical structure are used to evaluate the molecules interaction with DNA.Several cell based tests are also performed to assess a chemicals potential to cause DNA damage. These tests are very useful and provide a good indicator of DNA damage, however, they do not have the ability to fully represent how a chemical is altered in a live animal system in terms of absorption, distribution, metabolism and excretion by the animal's organs and tissues. This is the reason why it is necessary to conduct the micronucleus test in order to fully assess a chemicals potential to cause damage to DNA before giving to humans or exposing the environment. At the moment there is no non animal test available that the regulators will accept for this evaluation. However over the course of the licence I will regularly review all avaialable literature to ensure that if any aspect

	of the work can be achieved using a non-animal alterative it will be imediately adopted.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be designed in accordance with the regulatory test guidelines which has been developed over the past 30 years and updated in 2016. The updated guideline includes all the latest advancements in technology and ethical practices to make sure the tests are being conducted properly and using the least number of animals. The dosing schedule for these tests will be that the animals are given two doses 24 hours apart and sampled once 24 or 48 hours after the second dose.This dosing and sampling schedule requires the least number of animals.
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 test will be conducted in the Han Wistar rat as this rodent species and strain is widely used in safety assessment of chemicals. Micronuclei can be assessed in the rat in bone marrow and in blood. Animals will be humanely killed and bone marrow samples will be removed at post mortem.Blood samples will be collected on anaesthetised animals which are then humanely killed.Blood sampling in this way is a more refined method than taking samples using conscious animals from peripheral tissues and reduces any pain, suffering or distress potentially experienced by the animal. Blood micro-sampling for determining blood levels of the test chemical will be conducted where possible in the dose range finding animals in preference to additional groups of animals for this sole purpose. Although the experimental design is highly prescribed in the guideline and literature a review will be conducted annually in order to identify and refine any procedures or processes which could be improved up on.

Project	**211. Management of genetically altered rodent lines**

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
What's the aim of this project?	Import, generation, rederivation, breeding and cryopreservation of genetically altered mouse lines	
Why is it important to undertake this work?	Because of its small size, rapid reproductive cycle and extensively understood genetic background, the mouse is a very useful and productive model system in which to study mammalian biochemistry, physiology and molecular biology. This licence covers the necessary procedures to generate or import new lines of genetically altered mice for use by scientists in a number of important research areas, e.g., the immune system, diabetes, cancer, and neurodegenerative disease. It also covers the techniques to preserve these lines as frozen sperm or embryos.	
What outputs do you think you will see at the end of this project?	Well-managed supply of commonly used GA mouse lines Imports and creation of new GA mouse lines to underpin existing and new research programmes, resulting in new information, publications and	

	funding opportunities
Who or what will benefit from these outputs, and how?	Researchers in local (REDACTED read as 'establishments') and their collaborators.
Will this work be offered as a service to others?	Yes
How will you look to maximise the outputs of this work?	Close collaboration with other specialist centres, REDACTED and the world-wide community respresented by the International Society for Transgenic Technologies (ISTT).
Explain why you are using these types of animals and your choice of life stages.	Genetically altered mouse lines are widely used in biomedical research. The activity of a particular gene may be disrupted (the so- called "knock-out mouse") so that its contribution to normal physiology and the development of certain disease states can be studied in detail. Or a very subtle change may be introduced, perhaps to make the gene product more similar to its human counterpart and thus improve the predictive value of the mouse.
Typically, what will be done to an animal used in your project?	We shall breed and maintain lines of genetically altered mice in which no deviation from normal welfare is expected. We shall harvest embryos or sperm from these animals in order to have a frozen stock (and thereby avoid the need to continue to breed a line that is temporarily not needed). We shall send frozen embryos or sperm to other centres in order to distribute the lines we have and we shall import lines from other laboratories as embryos or sperm too, for "rederivation" into live mice here. Occasionally we may generate entirely new genetically altered mouse lines ourselves, by manipulating embryos or embryonic stem cells before implanting them into adult female mice. Breeding and maintenance are not expected to cause any significant adverse welfare effects.

	Hormones are used to promote the yield of embryos in some cases, but their administration is also highly unlikely to cause harm. Embryos are usually implanted into pseudo-pregnant
	females (i.e. mice whose physiological systems have been “fooled” by mating them with vasectomised male animals). This implantation, and the vasectomies, are surgical procedures conducted under general anaesthesia. The procedures are routine, and excellent rates of recovery are expected. All animals undergoing them will receive pain relief after surgery. At the end of a procedures, animals will be killed (some of them for the harvest of embryos or sperm for rederivation or frozen storage) or retained for further breeding under the authority of this licence or other project licences to which the mice might be transferred.
What are the expected impacts and/or adverse effects for the animals during your project?	Those animals that undergo surgery will be expected to show some limited mobility for a few hours after recovery from the anaesthetic. They will be given sufficient analgesia to control any post-operative pain.
What are the expected severities and the proportion of animals in each category (per animal type)?	The great majority of animals are expected to experience "sub- threshold" or "mild" severities. Some, particularly those that have undergone a surgical procedure may experience "moderate" severity. No animal is expected to experience a "severe" event.

What will happen to animals at the end of this project?	kept-alive, used-in-other-projects
Why do you need to use animals to achieve the aim of your project?	Precise alteration of the activity of a specific gene in mammalian cells still often requires the production of a whole animal carrying the alteration of choice. So, even if most of the scientific effort is devoted to laboratory work on cell cultures, the mouse is still required as a source. Other genes express their activity in a number of tissues and organs and therefore can only be studied properly in the three-dimensional whole organism.
Which non- animal alternatives did you consider for use in this project?	Modern genetic alteration methods for direct intervention in cultured cells (e.g. CRISPR/Cas9). These are already in widespread use, so only those projects requiring live GA animals need be considered here.
Why were they not suitable?	Alterations of cell cultures are rarely 100%, resulting in mixed responses to experimental interventions that can be difficult to interpret. Sometimes the relevant scientific "outputs" are in terms of the functioning of complex biological mechanisms, such as the immune and nervous systems, that cannot yet be recapitulated in non- animal systems.
Enter the estimated number of animals of each type used in this project.	mice: About 15000
How have you estimated the numbers of animals you will use?	Exact figures will depend on the demand, but are based on the most efficient breeding schemes.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	n/a
What measures, apart from good experimental design, will you use to optimise the number of animals you	"Inputs" (such as gametes, nucleic acids or stem cell cultures) of the highest biological quality. Efficient breeding and superovulation.
plan to use in your project?	
Which animal models and methods will you use during this project?	Mice, as these are well-understood in genetic terms, have a rapid reproductive cycle and are regarded as good model systems for mammalian, and specifically human, normal physiology and disease states.
Why can't you use animals that are less sentient?	In many, but not all, regards, less sentient species such as flies and worms are not sufficiently similar to humans to generate translatable results. For example, their defensive mechanisms are very different from our immune systems and they have much simpler nervous networks.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Close collaboration with other specialist centres in the field, subscription to NC3Rs newsletters and to professional journals in the field.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	The major interventional procedure is the surgical re-implantation of embryos into female mice in order to carry them to the normal term and birth. This is a routine procedure and its centralisation in skilled hands ensures that the success rate is very high and that the animals receive the best possible post-surgical care. Non-surgical alternatives have been proposed and we shall continue to evaluate their efficiency, with a view to adopting them wherever possible.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	Home Office guidance on breeding in general.

Project	**212. Mechanism and functional role of calcium signals**	
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)	Our general aim in this project is to show how calcium signals regulate diverse bodily processes. Identifying and characterizing calcium signalling pathways could lead to development of new drugs and therapies. To fulfill this aim, our specific objectives will be: *1. To determine how PLCζ is expressed during sperm development, and its location in the sperm.* *2. To elucidate how mutations in PLCζ may affect its functional properties and thus lead to infertility, using mice to explore the link between PLCζ and infertility in humans.*	

	3. To determine how the chemical messengers cyclic ADP ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) regulate calcium release in cells, by studying the ADP-ribosyl cyclase enzymes that generate them in response to physiological agents. *4. To test the theory that a novel family of calcium channels, the TPCs, are putative receptors for NAADP and assess their general role in calcium signalling*. *5. To characterise the role of TPCs, and other proteins that affect cADPR and NAADP mediated calcium signals, as mediators of key physiological events.*
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 major contribution to our understanding of fundamental bodily processes and may lead to the development of new drugs for both humans and animals. It could open up the possibility of creating genetically modified animals that are a source of valuable therapeutic proteins. In particular, this project will increase our understanding of the molecular mechanisms underlying infertility, brain disorders, diabetes, abnormalities of muscle development, heart disorders, and cancer, and could lead to new ways of diagnosing and treating these disorders. It could also aid the development of new types of contraceptives.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice are key model organisms for study of mammalian physiological processes. Maximum numbers to be used annually are 2400 mice. However, numbers should be substantially less, as some strategies may not be employed or rarely employed once optimum strategies are determined. Most animals will be used for breeding and maintenance.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of procedures will involve breeding and maintenance of mice whose genetic alteration is such that it does not cause significant adverse effects to their welfare. Some other procedures involve slightly higher severity, because of use of surgery. We will terminate animals at the end of experimental

	protocols once sufficient data has been collected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although much can be learned about cellular processes by studying gene function *in vitro*, this approach has major limitations for understanding how genes work *in vivo*. Animal models will complement human studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use careful assessment on animal numbers in our breeding colonies to keep numbers as low as possible, as well as use of power calculations to determine minimum mice numbers required for 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.	The use of transgenic, knockout and knockin mice has revolutionised biological research by allowing the dissection of gene function in a living mammal. As such, mice will be the species being studied in this project. Pain following surgery will be minimised by application of analgesics, and adverse effects in genetically modified mice will be carefully monitored by means of distress scoring sheets.

Project	**213. Mechanisms and therapies 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)	The aim of this project is to understand why nerve cells in the brain, eye and spinal cord die and find new ways to prevent this. Inherited genetic mutations or cellular stress can lead to proteins, the building blocks of cells, to be lost, or lose their function, and this causes the nerve cells to die. We do not fully understand why nerve cells die and here we want to understand that process better. Here we will make genetically altered animals that have the same genetic faults as human patients so we can study the disease better. We also want to understand how the body's natural protective machinery works to combat this normally and if we can use this machinery to protect against diseases like Retinitis Pigmentosa and Motor Neuron	
	Disease.	

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)?	Through this research we hope to gain a better understanding of how nerve cells function and why they die and discover ways to prolong their function and survival. The understanding and discovery might highlight ways to prevent or slow the progression of these currently untreatable diseases. In particular, we are trying to find ways to prevent people going blind because of an inherited condition.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using approximately 3000 mice and 500 rats over the 5 years period of this project licence. The animals will be of a variety of ages.
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 animals will be genetically modified to model human disease. Most of these will cause a mild phenotype, such as blindness, which does not affect a rodent very much, because laboratory rodents do not use their eyes as much as their other senses and it is not painful. We will use a range of treatments to try and ameliorate disease. This will be mainly drug treatments, which involve injections or oral feeding. Sometimes we will use surgery to introduce genes, viruses or cells into their eyes to modify their gene expression. We will reduce the adverse effects of these treatments wherever possible, and any suffering, with appropriate analgesia and anaesthetic. We will assess the vision of the animals and their ability to walk using behavioural tests that do not require anaesthetic and are not traumatic. We will also use non-invasive physiological tests of their vision and look into the backs of their eyes to monitor disease, and for this they will need to be immobilised by anaesthetic. Animals will be humanely killed at the end of the protocols and their tissues used to understand the disease processes and how our interventions have affected this.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The complex mammalian nervous system, and in particular the light sensitive retina, cannot be modelled in the lab and there is no alternative but to use animals to studying these vital organs. We use

use non-animal alternatives	cell models, including a mini-retina in a dish we can make from patient cells, to do as much background work as possible before considering animal use and to minimise our use of animals. Only when these studies suggest animal experiments are justified (or if we cannot study this in cells at all) do we go ahead and use animals. We constantly check the published literature for potential alternative technologies and ways to improve our animal experiments and use online platforms such as those provided by the NC3Rs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use our experience of these genetic models to ensure that the minimum number of animals is used to get reproducible and meaningful data. We will also use sensitive measurements for changes in function and nerve cell survival that also allow us to follow a single animal over a period of time rather than using multiple animals. We have access to biostatisticians and online tools (e.g. NC3Rs) to aid in experimental design and ensure our experiments are powered at the right level to deliver meaningful results, without unnecessary waste. We will also use techniques to directly modify gene expression in the target tissue and reduce the number of animals that might be required by other approaches, such as genetically modifying the germline that requires more animals to produce and also additional breeding of the genetically altered lines.
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 rodents (rats and mice) because they are the most easily genetically tractable mammals that share a similar eye and brain with man. We will mainly use mice, but in some instances transgenic rats offer a better model and their larger size makes some manipulations more refined and more likely to succeed. We can also refine our experiments by only targeting the tissues in question and reducing undesirable side effects in other organs. The models we are using have a ‘mild’ or ‘sub-threshold’ phenotype, because to the naked eye they look no different to animals without the genetic alteration. Many of the rodents we will investigate will only have a visual problem and because rodents rely more heavily on their other senses than vision, this should not cause them significant stress. Nevertheless, we will consult with professional staff and vets to ensure that animal welfare is maintained throughout experiments and

	build upon each experiment to refine our work to reduce animal suffering at the same time as meeting our objectives.

Project	**214. Mechanisms and Treatments for Neurodevelopmental and Neurodegenerative Diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	The aims of this project are to understand how genetic and environmental risk factors for neurodevelopmental and neurodegenerative disease impact on brain functioning and behaviour, and to develop preclinical rodent models that can help in the development of improved treatments for these diseases.	
Why is it important to undertake this work?	Neurodevelopmental and neurodegenerative diseases place a huge burden on the NHS and collectively feature top of the World Health Agenda in terms of Disability Affected Life Years (DALYs). The causes of these diseases remain poorly understood and current treatments are only partially effective. We urgently need to develop new drugs to help	

	people with these disorders. Gaining a better understanding of how
	genetic and environmental factors contribute to causing these diseases is crucial to helping patients lead a more normal life. Ultimately drugs need to be validated in relevant animal models before their efficacy can be tested clinically in patients with these diseases.
What outputs do you think you will see at the end of this project?	This work will provide new insight into how genetic and environmental risk factors increase the risk of developing neurodevelopmental and neurodegenerative disorders. The information generated will also give insight into drugs that may be useful in the treatment of these disorders. A major benefit of this work will involve the dissemination of these novel insights to the wider scientific community, including academics and colleagues in the pharmaceutical industry, through the publication of scientific papers and by presentation are relevant scientific meetings. As a primary aim of this work is the validation of new rodent models for these disorders, in addition to our own work, in the future these models may be used in the pharmaceutical industry for drug validation studies. This work also contributes to the increasing scientific knowledge of these disorders, and towards the more rapid development of effective drugs for their treatment. In addition, these new insights may be disseminated more publicly, to improve societal knowledge of these disorders.

Who or what will benefit from these outputs, and how?	The academic research community, interested in understanding the mechanistic basis of neurodevelopmental and neurodegenerative disorders, will be primary beneficiaries of this work in the short-term. Researcher's within the pharmaceutic industry may also benefit in the short-term, as this project validates relevant animals models and experimental approaches for the drug development process for these disorders. In the longer term the ultimate beneficaries of this work may be patients themselves, as we aim to validate drugs that can in future studies be tested clinically for the treatment of neurodegenerative and neurodevelopmental disorders. In the shorter term these patients and the general public may also benefit from the information this project reveals regarding the mechanistic basis of these disorders.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs	We will actively collaborate with other researchers using complementary approaches to maximise the outputs
of this work?	generated in this project, including through the sharing of available experimental data and animal tissue. We will disseminate the new information produced in this to academic and industry colleagues through the publication of scientific papers and by presentation are relevant scientific meetings. We will also engage with the public through press releases and relevant public engagement events. We will also share our developing knowledge with other *in vivo* researchers through presentations, training sessions and research collaboration.
Explain why you are using these types of animals and your choice of life stages.	We are using animals that have genetic changes, or have experienced environmental influences, that increase the risk of developing psychiatric disorders (such as schizophrenia), neurodevelopmental disorders (such as autism) or neurodegenerative diseases (such as Alzheimer's). We are interested in mapping the developmental trajectories of these disorders, so we characterise animals at a number of different life stages.

Typically, what will be done to an animal used in your project?	We will generate animals with genetic alterations that are associated with the development of psychiatric, neurodevelopmental and neurodegenerative diseases in humans. We will also expose animals to environmental manipulations that are associated with these disease, for example chemotherapeutic agents that induce neurodegeneration and cognitive problems in cancer patients. We will observe the behaviour of animals, to determine if they show disease-relevant changes in behaviour that relate to the key symptoms seen in neurodevelopmental and neurodegenerative disorders. For example, we will look at their social behaviour and their ability to complete tasks that look at learning and memory. We will also image the animal's brains to see if they show functional changes similar to those seen in patients. Once these alterations have been identified we will test the ability drugs to reverse these alterations, to determine whether the drugs may be useful for the treatment of these diseases.
What are the expected impacts and/or adverse effects for the animals during your project?	The genetic alterations associated with these disease can have negative consequences for the animals, but these generally have very little impact on animal health. The genetic and environmental manipulations we employ generally have subtle effects on behaviour that must be revealed through the application of specific behavioural tests, to reveal abnormal behaviour in the animals. In some behavioural tests we have to restrict the animals access to food, so that they are motivated to work for a reward. This means that animal may lose weight, but we ensure that this is limited in magnitude and duration. We closely monitor animals to make sure they don't lose too much weight and remain healthy. Many of the behavioural tests we employ involve the characterisation of behaviours that are naturally expressed by animals, and these involve subthreshold levels of suffering. However, some of the tests we employ to characterise learning and memory that involve swimming (water maze) are moderately stressful and can transiently impact on the animals body temperature. Therefore, we ensure that the exposure of

	animals to these tests is limited to ensure that they do not develop hypothermia. The administration of substances by subcutaneous injection can result in transient mild pain and discomfort, while dosing intraperitoneally carries the risk of organ puncture and peritonitis. Administration of substances by oral gavage carries the risk of installation to the lung and irritation of the throat. Administration of substances by highly trained and competent individuals substantially reduces the risk of these adverse events occurring, but if they do arise we will ensure that they are limited in their duration. The substances we administer to animals can also have negative consequences. However the substances we will use are generally well characterised and we will employ the lowest dose and duration of administration to limit the impact on animal welfare and to avoid any potential toxic effects. Substances we will administer include immunogenic compounds (including PolyIC) and chemotherapeutic agents that can induce transient (less that 24 hours) symptoms of illness. Chemotherapeutic agents can also induce weight loss, so we employ a dosing regimen to ensure that this is limited in the animals. The acute administration of substances that modify the function of the brains neurotransmitter systems can induce short-term changes in behaviour and body temperature (normally less than 6 hours in duration). While chronic administration of substances, such as antipsychotics, can induce changes in body weight (both increases and decreases), we set strict limits in terms of the decrease in body weight and the impact on general animal health that is allowed.
What are the expected severities and the proportion of animals in each category (per animal type)?	For genetically modified animals we will use in this project the expected severity is mild, and we expect 100% of animals to experience this severity, due to the need to collect a tissue sample for genetic testing. This causes on transient mild discomfort.

	For animals that undergo the administration of substance in this project the expected severity limit is moderate and we expect around 10% of the animals to experience this severity. For animals that undergo the behavioural testing paradigms detailed in this project the majority of the tests are within the mild severity limit, but some tasks involving dietary restriction involve a moderate severity limit. We expect 10% of animals undergoing these protocols to experience this severity. For animals undergoing the brain imaging protocol the expected severity limit is moderate and we expect 5% of the animals to experience this severity limit.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Neurodevelopmental and Neurodegenerative disorders are complex diseases, often characterised by very subtle dysfunction in the interconnections that exist between different brain regions. Rats and mice are the lowest vertebrate groups that can be used to reproduce the complex neurobiological and behavioural deficits, particularly the high- level cognitive deficits, of the neurodevelopmental and neurodegenerative disorders. Assessment of the ability of drugs to successfully restore the abnormal patterns of neurochemistry, brain functioning and behaviour also necessitates the use of rodents, as the well-defined functional organisation of the brain and neurochemistry in rodents is closely aligned with that in higher order vertebrates and humans. Work validating novel compounds for the treatment of neurodevelopmental and neurodegenerative diseases ultimately need to be tested in rodent models prior to their assessment in clinical trials.
Which non-animal alternatives did you consider for use in this project?	*In vitro* model systems (e.g. cell culture assays) and non- regulated experimental animal studies (e.g. fruit flies and worms) can be informative in the context of this project, but ultimately the aims of this project require the use of rodent models.

Why were they not suitable?	The subtle and complex alterations in brain connectivity and functioning seen in neurodevelopmental and neurodegenerative disorders can't be adequately replicated in cellular models or in non-mammalian experimental animals (e.g. *Drosophila melanogaster*). Non-mammalian experimental animals often lack the genes/genetic fragments of interest in our studies and lack the complex cognitive abilities that are of primary interest in our studies.
Enter the estimated number of animals of each type used in this project.	mice: 2500 rats: 500
How have you estimated the numbers of animals you will use?	The proposed experiments and methods of analysis of the results have been considered and will be discussed with an independent statistician. For most of the experiments sample sizes will be set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 25%. Otherwise, we will use the least number of animals to provide an adequate description, generally on the basis of previous experience and published work. *Post-hoc* power analysis will be utilised during the course of these studies to update our knowledge of appropriate group sizes when the data becomes available. In terms of the numbers of animals required, we expect that 6-8 animals per treatment group should be sufficient to obtain the required results in *ex vivo* neurochemical studies. For behavioural studies the variability between animals can be greater, so group sizes are typically 10-20, as are group sizes for the brain imaging studies. To minimise animal useage and maximumise the value of the data gained from each animal we will regularly undertake behavioural, brain imaging and neurochemcial analysis studies in the same animal.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	The proposed experiments and methods of analysis of the results have been considered and will be discussed with an independent local statistician. We regularly use the NC3Rs Experimental Design Assistant when formulating our studies.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	Wherever possible, preliminary drug evaluation tests are conducted using *in vitro* assays. Only those compounds that show significant activity *in vitro*, or that have proven efficacy in the published literature, will be investigated *in vivo*. Wherever possible, behavioural, brain imaging and neurochemical studies will be carried out using the same animals. This will both increase the value of the information gained and reduce the total number of animals required for these studies. Experimental variability is minimised through the implementation of standard housing conditions, standardized breeding and animal handling procedures and through the use of experimental stand-ard operating procedures. Breeding of genetically modified mice can involve relatively large numbers of animals. The num-bers used will be minimised by breeding separate transgenic/knockout and wild-type lines wherever possible. New lines will only be initiated where there is strong evidence that the gene concerned is linked to neurodevelopmental or neurodegenerative disease. My lab has an ongoing commitment to the reduction of animal use in the context of behavioural longitudinal experiments, with work being undertaken REDACTED aimed at developing novel statistical approaches to reduce animal usage in these types of studies REDACTED We regularly share tissues that we can not use in our studies with other researcher groups to minimise animal use and maximise the experimental insight gained from each animal.

Which animal models and methods will you use during this project?	The genetically modified mouse strains we have used in the past do not have overt behavioural phenotypes and exhibit only subtle behavioural deficits evident only under specific testing conditions. The precise adverse effects of novel genetic alterations that may be investigated in the future are not known at present. Some knockout lines may be embryonic lethal or lethal before adulthood, and such lines will be utilised as conditional knockouts if available, or maintained as heterozygotes. As a general rule we employ heterozygous mouse models in our experiments as the majority of genetic alterations associated with neurodevelopmental and neurodegenerative disorders tend to be heterozygous in nature. Thus we employ heterozygous models, when most appropriate, to maximise translational relevance and these tend to display phenotypes only under specific testing conditions. We will also employ conditional knock-down models to mitigate unwanted neurodevelopment effects, when relevant. We have found that repeated PCP treatment in rats produces a pattern of metabolic imaging, biochemical and behavioural changes that mirror those observed in the brains of schizophrenic patients. We have recently observed similar changes in the brains of mice with a targeted mutation in genes strongly associated with neurodevelopmental and psychiatric disorders. Hence the use of rats and mice canproduce phenotypes with a high degree of translational value. To ensure translational validity we will employ models with a strong rationale in relation to disease aetiology, using models based on established genetic and environmental risk, or established neurochemical dysfunction. The models of neurodevelopmental and neurodegenerative disease employed in these studies are such that only relatively subtle behavioural phenotypes are anticipated. Thus the models themselves are unlikely to cause overt signs of discomfort.
Why can’t you use animals that are less sentient?	As we are assessing animal behavioural performance in complex cognitive tasks , that have translational relevance to the cognitive problems experienced by patients, we can not use species that are less sentient. Due to the developmental onset of neurodegenerative diseases and the importance of ageing in the manifestation of neurodegenerative diseases we can not use animals at more immature life stages. Terminal anaesthesia would impair brain function in the animals, and would not allow for behavioural testing.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We regularly monitor the NC3Rs website (https://www.nc3rs.org.uk/) for any news regarding advances in animal welfare and for any relevant events being held. We will also regularly attend scientific meetings with a strong 3Rs focus, including meetings held by the British Association of Psychopharmacology. We will also share best 3Rs practice with University colleagues through participation in the Universities Animal Welfare and Ethics Review Board (AWERB).
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	The doses of drugs employed will be selected to be at the lowest dose possible to cause measurable effects without causing toxicity and will be based on published data, where available. This will minimise any potential discomfort. In any case where there is little available information on the *in vivo* effects of a compound pilot studies will be employed using incremental doses and very small group sizes prior to the initiation of a full experiment. In our studies we employ a refined, updated method of functional brain imaging that reduces the suffering of animals, as the protocol used does not require the surgery (intravenous cannulation) or prolonged restraint necessary in the original protocol.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We will follow best practice guidance as provided by the nC3Rs to ensure that experiments are conducted in the most refined way, including guidance provided as part of the Experimental Design resources (https://www.nc3rs.org.uk/experimental-design) and by reporting our experimental data in accordance with the ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines). For pain assessment we refer to the nC3Rs mouse and rat grimmace scales.

Project	**215. Mechanisms by which lung and pancreatic cancers evade the immune system**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Mutations in a family of cancer genes (RAS) cause about 20% of all human cancers and lead to 1.4 million deaths per year worldwide. So far attempts at blocking the function of RAS genes have been unsuccessful. There is therefore a need to develop new treatments for cancer patients where this pathway is important, in particular in major killers such as lung cancer, colon cancer and pancreatic cancer. Work carried out in this laboratory in the past has aimed to identify novel ways of killing cancer cells with mutations in RAS using both cell culture work and mouse cancer models. We	

	have found combinations of drugs that are able to cause major regressions of cancers in these mice, several of which are being taken forward into clinical testing in human patients. However, while this is very encouraging, we also know that tumours in these animals are not completely eliminated and will regrow after therapy is discontinued. We wish to continue these studies to address how we can turn short-term tumour regressions into long-term cures. In order to do this, we wish to investigate the interaction of the immune system with tumour cells as they die in response to our new drug combination therapies. We plan to explore what parts of the immune system recognise the dying tumour cells and what is preventing the immune system from then fully rejecting the tumour. We will test whether precision targeting of some of the brakes that the tumours apply to the immune system might be able to work together with the drug combinations we have already tested to cause complete cancer cure.
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 of this work lie both in advancing basic scientific understanding of cancer as a disease and in providing insights into new clinical strategies for the therapy of common cancers. In our work on therapeutic drug combinations and promoting the ability of the immune system to work together with these to eliminate tumours, we hope to provide clear rationales for the design of new clinical trials in cancer patients that optimally combine the very latest targeted drug combinations with immunomodulatory drugs. These should have implications in terms of improvement in therapy for large numbers of cancer patients, particularly the 450,000 patients diagnosed each year worldwide with RAS mutant lung tumours and 300,000 patients diagnosed each year with RAS mutant pancreatic cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use exclusively mice and will run over five years. During that period, we expect to use up to 76,750 animals.
In the context of what you propose to do to the animals, what are the	This project focuses on the study of lung and pancreatic cancer in mice that are genetically

expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	prone to developing these diseases. The major adverse effects that we expect to see are therefore those associated with the development of these cancers. If the disease is allowed to progress unchecked then the mice would ultimately die from its effects, which could involve unpredictable levels of suffering. We will therefore take great care to closely monitor the progress of the disease and will humanely kill the mice before the cancer progresses to a point where it can cause suffering. As these cancers occur in internal organs, this requires the use of scanning technology similar to what would be used for cancer patients in hospital – computerised tomography (CT) for lung cancer and ultrasound for pancreatic cancer. Experiments will be designed so that mice would be treated over a period when the cancers would not be expected to have progressed to an extent that would cause suffering to the animal. Another possible source of adverse effects is the use of drugs to treat the cancers, including experimental agents not yet used in a clinical setting; as with the treatment of cancer in human patients, these drugs can sometimes have serious side effects. To avoid potential suffering from the side effects of drug treatments, mice will be monitored very closely when on treatment. If they show any signs of suffering, such as significant weight loss or changes in behaviour or condition, they will be humanely killed. Rare instances may occur where rapid progression of the cancer could result in death from the disease, or its treatment, before any signs of suffering were detectable, despite stringent monitoring. It is expected that the rate at which this would occur would be no more than 5% of the animals bearing tumours in internal organs in experimental drug treatment studies, and considerably lower for other animals. Animals will be humanely killed at the end of the defined experimental period or at the first detectable sign of suffering caused by the cancer or its treatment. Other possible sources of adverse effects could involve the use of anaesthetics and restraints, for example during scanning procedures. These procedures are only carried out by very experienced practitioners and problems are extremely rare; again, animals are

	monitored very closely and will be humanely killed if any signs of suffering are seen.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have extensively used cultured cancer cells in the run up to this project. We have also used bioinformatic analysis of publicly available data from cancer genome sequencing studies. However, various aspects of the cancer disease process can only be addressed in living animals. The development and function of the immune system, which is a focus of most of our work, involves many different cell types that cannot be mimicked in vitro. The interaction of the tumour with the immune system and how it responds to immunotherapy can only be accurately studied in live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have used in vitro cell culture systems to define a limited set of hypotheses that merit testing in animal models. Mouse breeding experiments have been planned in detail in consultation with experts in statistics and animal breeding. We will ensure that the minimum numbers of animals are used to obtain statistically meaningful results. In practice, for therapeutic studies this involves performing pilot experiments conducted with cohorts of five mice each; these preliminary data are then used to plan appropriately powered experiments using mouse numbers that should give statistically significant results. Mouse colonies will be actively managed to ensure that the basic principles of mouse breeding will be adhered to and only the minimum number of animals required for the experiment are generated. In addition, the use of in vivo imaging methodologies such as micro CT scanning greatly reduces the number of animals needed compared with end point assays as each mouse can be followed over time and inter-mouse variability is internally controlled for.
3. Refinement Explain the choice of species and why the animal model(s) you will	The mouse closely resembles humans in its susceptibility to cancer. Mice can be genetically altered and have been extensively used for the topics of our investigation. We aim to develop

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.	mouse cancer models that accurately mimic the human disease, with relevance to some 20% of human cancers with mutations in RAS oncogenes. Only by allowing tumours to develop in these mice can we address the potential of targeting the molecular interactions that we are studying for treating human disease. The severity of the procedure will be limited by ensuring that animals are killed as soon as overt signs of the disease can be seen, and that mice are rigorously monitored for signs of suffering or distress at all times. These studies address the response of tumours to experimental therapies, but have been designed to focus on mice with early stage disease, in which setting the impact of the tumour on the overall health of the animal should be small. Drug treatment will involve only the use of agents that have already be tested on mice, so will not involve chemicals where unexpected toxicities are likely to occur.

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Project	**216. Mechanisms of Aortic Stenosis**	
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 build up of caclified deposits on the valves in the heart, that control of the direction of the flow of blood it as leaves the heart on its way to the rest of the body, can cause the valve to narrow and to stop it opening fully. This puts an extra strain on the heart as it need to generate greater force to push the blood through the narrowed valve, which eventually leads to heart failure. If it is left untreated, the patients will normally die within 5 years of the symptoms first appearing. The only current treatment for this condition is to surgically replace the valve. While currently available valve replacements have good mid-term results, they either eventually degenerate and need to be replaced	

	(for bioprosethetic valves) or require the patient to take life-long medication to prevent the blood from clotting (metal valves). Since we know that the calcification of heart valves is an active process that involves the cells within the valve, and the effects of biological messengers, it should be possible to find drugs that can slow down, or even prevent the calcifiaction process. This project aims to understand which messengers are involved in the development of valve disease and thereby identify new and existing drugs that could be used to prevent or slow down the progression of the disease.
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 development of a medicine to treat heart valve disease will delay or even prevent patients with the disease undergoing heart surgery. Since heart valve substitutes either degenerate over time (and need to be replacement) or require the patients to take blood thinners (which put the patient at risk of bleeding), being able to offer an alternative non-surgical treatment will be of great benefit to patients with valve disease. By delaying the need for surgery will mean that patients can undergo valve surgery at a time in their life when it would be expected that a valve replacements will last for their remaining years (10-15 years), rather than facing the prospect of having to undergo multiple surgeries as valve replacements degenerate.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will examine a number of different potential mediators of valve disease using mice that have been genetically altered. This will involve breeding mice together in order to combine specific genetic changes. For each experimental protocol we will need around 60 mice (30 test and 30 control), giving a total of approximately 400 experimental mice. However, the breeding programme needed to generate the specific gene mutations in sufficient mice we require the generation of up to 2500 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	This study requires no surgical interventions. The severity of any condition induced via the expression of gene mutations is expected to be no greater than moderate. We expect that some mice may experience the symptoms of heart

the end?	failure, with signs of fatigue and lethargy. Any animals that appear to be in distress will be euthanised. The majority of the animals will be killed at the end of the experiments, but some of the mice that in which specific combinations of gene mutations are expressed will be kept as stock animals and offered to other groups who may be interested in these types of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We and others have previously completed a significant amount of work using isolated human and animal valve cells to define the reasons why valves become diseased. However these cell based models lack the complexity of a living system where the cells in the valve are exposed to flow pressure of the blood. It is not possible to accurately replicate these conditions in test tube. We therefore wish to assess the role of specific mediators on the development of aortic stenosis in a whole animal using genetically altered mice that will develop valve disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Mice that develop valve disease have already been made and require no further development. We will breed these mice with other mice with known genetic mutations to produce new lines with a combination of mutations. These breeding programmes will be performed by qualified personnel and will be planned so to avoid any unnecessary animal generation. A mathematical calculation will be made to predict the number of mice we need, based on the expected magnitude of the effect we predict to see. This will allow us to calculate the minimum number of mice we will require and avoid the breeding of any unnecessary mice .
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	Genetic modification is easiest to perform in mice. All the genetic modifications we will use have already been made and the different mice exist and they can be purchased or gifted from other laboratories. The only interventions that this study require are changes in diet and administration of drugs. No surgical intervention
the animals.	are required for these experiments.

Project	**217. Mechanisms of Bacterial Cancer Therapy: Investigation of the effects of Salmonella enterica spp. on intestinal cancer suppression**	
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)	Colorectal cancer (CRC) is the 2nd most common cause of cancer death in the UK with around 60% survival rates beyond 5 years. That still leaves a significant gap, and significant number of people per year (around 16,000) dying from CRC. Thus improved cancer treatments are still sought after. Surgical resection, radiotherapy and chemotherapy remain the main treatment options.	

	Immune-based therapies have shown very promising results in other types of cancer, but are only approved in a fraction of CRC, due to low levels of immune activation in most CRCs. We aim to develop immune targeting therapies that can improve immune cell infiltration into tumours enabling efficient immune-mediated tumour destruction. The concept of bacterial cancer therapies has been around for over 100 years, with one currently in clinical use, yet the mechanisms are very poorly understood. My research over the past 4 years has been to determine if use of an attenuated Salmonella typhimurium strain could alleviate tumour development in models of intestinal cancer. Data thus far shows promising results, thus now we aim to understand the mechanisms of this bacterial therapy as well as determine optimal bacterial attenuations, and treatment timing and efficacy alongside other types of cancer therapy.
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)?	Through completing this project we will have a greater understanding of how bacterial cancer therapy works in the context of CRC. This understanding will better inform us of how such therapy can be applied to patients in the clinic. Furthermore, the aims to understand dosing, timing of administration, and co-therapy effects will further increase the flow into clinical trials. In addition to aiming for clinical application, this project will also help us to understand the basic biology around involvement of the immune system in controlling cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	All studies proposed will use mice. The maximum numbers of mice we will use in the next 5 years are: 8000 mice with either induced or spontaneous intestinal tumours. 7000 GA or APCmin/+ mice will be bred and further WT mice will be obtained from designated suppliers.
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 adverse effects experienced by the mice will depend on the protocol. Mice that develop spontaneous tumours appear healthy until around 14 weeks of age when they may start to exhibit weight loss and at some point lethargy.

the end?	Mice are culled once syptoms appear moderate (weight loss, grooming, lethargy taken into account). Mice that will have induced CRC will experience inflammation in the bowel which is accompanied by weight loss, diahroea and rectal bleeding, which resolves, but during that time mice experience moderate severity. Once tumours are developed mice appear normal and protocols are not continued long enough for the tumours to affect mouse behaviour. During the protocols (spontaneous or induced CRC) these mice will experience interventions including: oral gavage, intraperitoneal or subcutaneous injections, anaesthesia for imaging. These will have transient discomfort. Some mice will undergo colonoscopy imaging which may result in a transient discomfort upon awakening from anaesthesia. At defined times, mice will be killed by a schedule 1 method and tissues collected for microbiological and immunological analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The tumour microenvironment is complex and involves multiple cell types, metabolites and secreted factors, all of which strongly influence the immune response. In order to really understand the mechanisms of a treatment we therefore need to use in vivo models to fully recapitulate the whole tumour environment. Lower order animals cannot recapitulate tumour development nor an adaptive immune response. Mice are able to recapitulate many aspects of human immune system and can model human tumour development. Our lab does, however, actively use tumour organoid culture systems to carry out as much work as possible to understand the mechanisms of bacterial cancer therapy. I have been able to show that this system does recapitulate aspects of tumour development and response to a bacterial therapy – thus making it a good model for us to dissect very particular interactions in the absence of the other cell types that would usually be present in a tumour in vivo, making it a good reductionist system. We will further expand this system to co-culture with immune cells, thus we will be actively reducing the

	number of in vivo experiments that need running Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to ensure that high quality, reliable and valid data is generated from the minimum number of experiments we will use established guidelines such as the PREPARE guidelines alongside using the NC3Rs website to aid experimental design and statistical analysis. Each experiment requires a written protocol giving full details of the experimental aims, a description of each group, including numbers, treatments and possible risks associated with the procedures used. This allows others to share experiment tissues etc post-mortem, reducing experimental numbers or permitting use of the same experiment to answer multiple objectives. We will limit the risk of bias by inclusion of approved randomisation procedures where possible and ensuring the reproducibility of our findings. Group sizes will be guided by power calculations based on the expected degree of difference estimated between groups (informed partly by previous experimental studies or by pilot studies) to generate a study with a power of at least 80% and where $p<0.05$. Once acquired, data will be assessed by the most applicable statistical test and we will seek statistical advice to guide us as necessary. Studies will be published to conform to the NC3Rs ARRIVE guidelines
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	Spontaneous tumour models and a inflammation-associated tumour models more closely recapitulate tumour development in humans than other systems, such as xenograft models. We have rationally chosen Salmonella spp. as bacterial cancer agents for gastrointestinal cancers since it is a natural

the animals.	pathogen of the gut. We will utilise in vivo imaging to get response data throughout the protocols, which, subject to pilot studies, has the potential to greatly decrease the number of mice used (reduction) and also give a more detailed analysis on an individual tumour level. Interventions, such as oral gavage, will only be carried out by competent individuals. The project will be aiming to determine the least number of treatment doses needed for an effective response

Project	**218. Mechanisms of body weight regulation in the European starling**	
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)	Humankind is facing an obesity pandemic. However, there is still a lot unknown about the fundamental biology of fatness, in any species, especially how changing environments lead individuals to store more fat. In many species, individuals respond to food insecurity—limited or uncertain access to food—by becoming fatter. However, we don't currently know whether they do this by eating more, by digesting their food more efficiently, or by reducing their energy expenditure. In this project, we aim to answer this question using European starlings, a wild bird species that is an	

	ideal model for studying body weight regulation in the lab, but in an ecologically valid context. In starlings, we can manipulate food insecurity in simple and reversible ways (making food sometimes harder to obtain, sometimes easier) that mirror the variations in food supply in their natural environments. The birds respond rapidly by gaining and losing weight. We can ask a series of follow-up questions about how and why these weight changes happen, and why they might be greater in some individuals than others. We have pioneered automatic methods for measuring body weight multiple times per day without having to handle the birds or remove them from their flocks. We can track their activity and energy expenditure with technologies that have minimal impact on the birds' welfare.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are benefits in terms of our understanding of the basic biology of energy and fat in birds. Regulating body weight is essential for birds, since being too heavy affects flight. We already know that starling body weight varies markedly across seasons, habitats and even the course of the day. There are suggestions that they can change the functioning of their digestive system substantially depending on the environment. This project will help us understand exactly how the birds' system of weight regulation works; and hence how they manage to survive in challenging environments. We also believe our work will produce benefits for understanding obesity in humans. The weight changes in response to food insecurity in starlings are particularly clear, but their logic may be the same as those seen in humans. Inspired by our findings on the birds, we have already shown that food insecurity predicts obesity in humans in Western countries (especially women), and that this does not appear to be because they eat more calories. The understanding we build up using the birds will allow us to develop hypotheses we can then test in humans. This will, in the end, shed new light on questions such as whether obesity is more related to food consumption or physical inactivity, and will inform the development of

	environmental interventions to combat obesity.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 200 European starlings will be used over a period of 5 years. Up to 120 of these may be set free to the wild following our studies.
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 severity of our experiments will be moderate at most; but, many of them will be mild or below the threshold defined under law, since they only involve monitoring behaviour. Birds will experience periods of easier and harder access to food whilst they live in an aviary in a flock, but these manipulations will all be within the range they would naturally experience in the wild and consistent with them getting enough to eat over the whole course of the day. Their weight and feeding behaviour will be measured automatically through smart perches that both identify the bird (via a microchip on its leg ring) and weigh it. In some experiments, they will be fitted with a small accelerometer that measures activity; or be captured to take small blood samples; or be implanted with a small logger to measure body temperature and/or heart rate. There could be adverse effects from surgery, including pain and bleeding. These will be minimised by choosing the least invasive methods and providing the birds with adequate anaesthesia and pain relief. Birds will be of two types: wild-caught as adults, and hand-reared. Netting adult birds entails some small risks of injury, but birds wild-caught as adults will usually be re-released close to where they were netted following experiments. Hand-reared birds cannot be released as they would not be competent to feed themselves in the wild. Some birds will be euthanized at the end of the experiments in order to gather tissue samples. The hand-rearing may involve manipulating the amount of food or the amount of begging the nestlings have to do. These manipulations will never be worse than the conditions experienced by the smallest nestlings in natural nests, and will never be so severe that

	the bird cannot grow and fledge. We have hand-reared birds in this way before with no mortality.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our questions concern how vertebrate animals regulate their body weight. There is no alternative to using a live vertebrate animal to answer these questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Food insecurity is reversible, and the body weight response is seen within one week. Thus, over the course of an experiment, the same individual can go from food security to food insecurity and back again multiple times. We can then measure changes (and changes back again) repeatedly in the very same individuals, meaning that the total number of individuals required is quite small. Using this approach we have been able to get clear results with as few as 6 birds per experiment in the past.
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.	Birds must minimise their body weight due to its impact on flight. Their body weight and activity responses to environmental triggers are thus particularly quick and clear, making them a better model than a mouse or rat for this project. This means we can keep the numbers of individuals and the extremity of the food insecurity manipulations low. We have pioneered automatic measurement of body weight and feeding in starlings. Instead of our having to catch the bird, immobilise it and put in on a balance, the bird merely lands on a special perch, in the course of its daily activity, and its body weight and identity are recorded. Thus, we can complete all of our experiments without the need for small cages or individual isolation, and with the amount of catching and handling kept to a minimum. We have set conservative limits on the number of different procedures a bird can undergo during an experiment to avoid cumulative effects from multiple procedures.

	All birds will be kept in social groups in large aviaries provided with a natural floor substrate, baths and environmental enrichment. We have kept starlings in captivity for many years, and they generally thrive. Birds caught as adults have been successfully re-released in the past.

Project	**219. Mechanisms of breast development and tumourigenesis**	
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 the most common cancer in the UK with over 55,000 cases registered every year and the third most common cause of cancer-related mortality claiming 12,000 deaths annually. Although currently available therapies are often successful in achieving tumour regression, more than one in every three patients suffers from tumour recurrence within 10 years. Furthermore, these tumours are usually incurable as they acquire therapy resistance. Experimental evidence suggests that tumour recurrence is likely caused by the breast cancer stem cells (Br-CSCs), which represent a unique subset of	

	tumour cells that are not eliminated by currently available therapies and may cause tumour regrowth, therapy resistance and metastatic spread of tumours to other organs in the body. A 'true cure' for breast cancer might be achieved if a therapy could eliminate Br-CSCs within breast tumours. Our earlier findings suggest that inhibiting Rac1 signalling, a physiological process regulating cell motility and growth in normal tissues, changes Br-CSC properties in cultured tumour cells and ultimately prevents them from forming tumours. The aim of this project is to confirm these initial findings in animal models that more closely reflect the true biology of cancer. We plan to assess how genetic deletion of different factors that are involved in this signalling process affects tumour growth in an animal model that is genetically prone to developing breast tumours. Because these factors are present in normal breast and other tissues, we will also assess the general effect of genetic deletion of these factors on breast development and the whole organism.
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)?	Br-CSCs are likely to be responsible for metastatic spread of tumours to other organs and for tumour regrowth following therapy. Because currently available therapies are frequently ineffective against Br-CSCs, there is no 'true cure' available for breast cancer. Demonstrating that a permanent deletion of certain components of the Rac1 signalling process which inhibits or abolishes Br-CSCs but does not adversely affect the animal's health, would confirm that they could serve as targets of new therapies. This would open a way to developing new breakthrough therapies against breast cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 25,900 mice will be produced and 6,150 mice will 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	25,900 animals will be produced via breeding and 3,750 of these will be used for organ analysis; none of these will experience any adverse effects during their lifetime. 2,000 of produced animals will be used for the analysis of tumour growth; these animals may experience a 'moderate'

the end?	discomfort because of growing tumours. The animals will be closely monitored and humanely killed before the tumours reach a size that compromises the animal's welfare. 400 animals will undergo surgery, during which existing mammary tissue will be removed and normal breast or tumour cells will be transplanted into their breasts. The level of suffering from this type of surgery is considered moderate and transient. These animals will be closely monitored for signs of ill health and humanely killed if they experience any adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have performed our initial analyses in breast tumour cells grown in a culture dish with promising results. However, to establish these as clinically relevant it is necessary to demonstrate whether the functionality of Br-CSCs relies on the same factors in the intact breast tumours (in a live animal) as in the culture dish and whether interfering with these factors cause no adverse effects for the whole organism. Therefore, the clinical relevance of our earlier findings can only be assessed by using a mammalian animal model, and the most appropriate species is the mouse.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully considered all aspects of mouse colony management and optimized experimental designs to minimize the number of animals required. The estimated numbers of animals required for each experiment were calculated with the help of professional biostatisticians and are in agreement with the published literature.
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 plan to use mice because it is the most suitable animal model to study tumours of the breast. A mouse is a mammal and therefore has breasts. Mouse breast development and physiology have been extensively studied in the past, resulting in a great amount of knowledge and multiple experimental techniques available for this animal model in the field of breast cancer. All animals will be kept in groups, but not too

	crowded in any cage, to enhance social behaviour. They will be closely and frequently monitored for their wellbeing. Surgical operations will be performed under general anaesthesia, and the animals will be given pain relief medication before and after the surgery.

Project	**220. Mechanisms of cancer development**	
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 the study is to understand what makes cells behave in an abnormal manner in cancer, with the ultimate goal of using this knowledge to develop novel strategies to treat the disease. Cancer starts because cells “forget” what they are and what they should do in the organ where they reside. This “amnesia” is accompanied by acquisition of novel features that make cells capable of dividing without control, hide from the immune system, create an environment in which they can keep growing and spread to other organs. At the heart of this cellular transformation there are both genetic changes - mutations that alter the DNA sequence - and non-genetic alterations that	

	make cells use their DNA inappropriately. We study the non-genetic processes that drive cancer development, and try to understand how we can interfere with these processes to benefit patients. While mutations cannot be erased, recently developed drugs allow us to change non-genetic alterations of cancer cells, providing new hope for more effective treatments. By focusing on non-genetic process that make cancer cells misuse their genes, we are interested in addressing two major issues: 1) Understanding how loss of cell memory favours cancer initiation. Numerous cancer-driving mutations have been found in normal tissues that do not contain any cancer, indicating that, by themselves, mutations are not enough to start a tumour. By understanding what additional non-genetic events are needed to initiate the disease, we hope to generate knowledge that will help early cancer detection. 2) Understanding what determines which cancer cells are truly immortal and can drive disease progression. Tumours are made up of highly diverse cells. Even neighbouring cells within a tumour may have distinct shapes and behave differently. Most importantly, not all cancer cells can divide in same way and in most cancers only a subset of cells is truly immortal. These cells, known as cancer stem cells (CSCs), are those that make cancers grow and invade healthy organs. Often, these cells are resistant to conventional chemotherapy and are responsible for disease relapse, which in many cases leads to patient death. Understanding how these cells function and what makes them different from the chemotherapy-sensitive cancer cells will allow us to design more effective strategies to treat the disease. Very little is known about CSC characteristics and we aim at casting light on the non-genetic processes that regulate their function within a tumour.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit	The primary potential benefit of the proposed study is to increase our knowledge of how a tumour grows and identify new ways in which cancer cells can be killed or made harmless.

from the project)?	The information is likely to be directly relevant for studies focused on designing novel anti-cancer treatments. Because non-genetic processes are intrinsically reversible, and several drugs interfering with this processes are available, the path to developing novel treatments may be relatively quick. In addition, our findings may assist oncologists in cancer diagnosis and prognosis.
What species and approximate numbers of animals do you expect to use over what period of time?	For our study we will use mice, the best model system to study cancer biology. Based on the work carried out in the past 5 years by my laboratory under our previous licence, the number of mice expected to be used over the 5-year period will be approximately 30,000.
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?	In this project we will mainly create tumours in mice where specific conditions are altered, trying to understand how CSCs are formed and make tumours grow.. To achieve these aims, we will use a variety of well-established experimental techniques, including breeding of mice which will develop tumours spontaneously, induction of tumour growth by injecting tumour cells or exposing animals to chemical or biological agents. In most cases (~70%), tumours will be superficial and will not affect organ function, impacting only minimally on the animal's overall condition. On rare occasions only, minor surgical procedures, such as skin biopsies or resection of small tumours will be carried out. Non-invasive imaging techniques, similar to those performed on patients, will also be performed under general anaesthesia to monitor tumour growth. At the end of the experiment, animals will be humanely killed and tumours harvested. Overall, we expect mild or moderate adverse effects associated with the protocols used in this project. To be able to achieve our scientific objective, about 50% of the animals used will need to grow tumours to a size larger than that recommended by the NCRI guidelines. This is necessary because one of the main purposes is to induce tumour formation with the goal of producing, in a controlled fashion, high numbers of CSCs which will be then studied after tumour collection post-mortem. To allow proper diversification of

	cancer cells and ensure that enough cells will be available for analysis after tumour removal, it is necessary that tumours grow to 2 cm in diameter over a 2-month period. However, we have previously seen that such large tumours cause no more than moderate pain or distress to animals, since they are typically very superficial and do not affect organ function. Due to superficial nature of the induced tumours, up to 30-40% of the animals may develop small ulcerations. We have extensive previous experience with similar experiments and have seen that small ulcerations most of the times do not affect animals' well-being. The use of suitable analgesia will help minimizing animal suffering. To be able to achieve our scientific goal reducing the number of animals needed to obtain statistically significant results, animals developing ulcerated tumours will not be euthanized. For most of the animals we expect only mild adverse effects, but if signs of ill health are observed, animals will be culled immediately. During the previous licence, no animal had to be killed due to the presence of ulcerated tumours. All animals will be humanely euthanised at the end of the experiment, or earlier if the humane endpoints are reached first.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The experiments using animals will tightly interconnect with experiments performed using cells (*in vitro*). To partially replace the use of animals, we have found a way to mimic the early phases of cancer development by growing cells in a plate. Most of the work investigating CSC formation will be done using this system. However, to fully understand how CSC function within established tumours and how they drive disease relapse we will necessarily have to perform experiments in mice (*in vivo*), since we need to analyse tumours in their natural context. Nevertheless, most of the experimental measurements and the analysis of tumours will be performed after tumour removal post mortem.
2. Reduction	Our use of *in-vitro* approaches limit the numbers of animals required for the *in-vivo* investigation

Explain how you will assure the use of minimum numbers of animals	stage. In addition, when dissociated tumour cells are not in use, they will be stored in a frozen state. This minimises the numbers of animals required for maintaining live tumour cells. When animals are needed, we employ several strategies to try to limit the number of mice in the study. Firstly, we always aim to maximise the amount of data we get from each mouse, for example by injecting cancer cells in multiple sites to induce two tumours/mouse. Also, we will limit the use of genetic models (that often require many generations breeding) using transplants of cells and treating the mice with chemical agents to generate tumours. We also use the minimal amount of mice needed for statistical significance when testing the experimental hypothesis. Furthermore, we will use in vivo imaging, which allows us to use the same animal for repeated measurements and reduces the overall number of animals needed. Finally, by careful monitoring of our mouse colonies we try to breed as few mice as possible. We will also collaborate with other groups, sharing data and animal tissues, in order to minimize the overall number of animal used.
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 our studies we will use mice, because tumours grown in mice are very similar to human tumours and discoveries made using mouse cancer models can thus inform us on the human disease. Furthermore, many experimental model are already available and refined techniques have been developed. The first approach used in the project will mainly use mice lacking a functional immune system, which allow tumour formation by injection of human cells, or standard laboratory mice that will be injected with mouse cells. Animals will be housed in highly clean facilities to minimize the chance of infections. A second approach will use animals with genetic alterations that make them prone to cancer development or mouse that have been treated with chemical agents using refined and widely-used protocols. To minimize animal distress, in many cases we use genetic alterations that we can activate only when needed to perform the experiments. For all manipulations and procedures, we follow

	national guidelines that aim to minimise suffering. We have extensive experience with most procedures described in the licence. When performing procedures in which we are not fully competent, we will seek the help of other groups in the institute that have optimized those procedures. To minimise any possible adverse effects of the experimental procedure, we closely monitor the animal's reaction to specific experimental procedures and pay attention to any sign of suffering. The use of specific treatments (when possible) or methods of humane killing will be used depending on need. Surgical procedures, when needed, will be done with suitable anaesthesia and animals monitored post-surgery to ensure that they recover well. We will also use suitable analgesia according to the procedure.

Project	**221. Mechanisms of gene expression regulation during erythropoiesis**	
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 fact that disease can run in families has been recognised for centuries, yet we had limited power to understand the genetics of human disease until the publication of the human genome. For the past two decades, many scientists have been working hard to identify genetic variants that predispose people to disease. Unexpectedly, the vast majority of the variants identified to date do not appear to affect the structure of genes themselves. We now believe that many of these variants instead affect when, where and to what extent genes are switched on and off.	

	These sequence variants are often found within eEnhancers, areregions of DNA elements that switch genes onactivate genes in specific cell types or tissues and thereby orchestrate the development of complex, multicellular organisms. Some human diseases are known to be caused by mutations in enhancers. Ultimately, these types of mutation may explain more than 50% of genetic predisposition to disease in humans. Despite their central role in biology, we still do not understand how exactly enhancers act on their target genesthe mechanisms of enhancer action are poorly understood. We study a genetic disease called alpha-thalassaemia, in which mutations in the alpha-globin genes (or the enhancers that normally switch them on) lead to a deficiency in haemoglobin, the molecule responsible for transporting oxygen around the body. Alpha-thalassaemia is one of the most common single gene disorders in the world, affecting around one million people. The aim of this project is to increase our understanding of the processes by which enhancers switch the alpha-globin genes on and off duringthroughnormalearly development and how they direct differentiation into different cell types and tissues. We can then try to understand how these processes are perturbed by mutations that occur in human genetic diseases. By reproducing mutations we observe in human patients in mice, we aim to study how and why these mutations lead to a deficiency in alpha-globin protein. Studying human conditions in which acquired mutations occur has already identified many genes affecting blood cell production. However, curative treatments are not yet available. Genetic blood diseases, such as thalassaemia and sickle cell disease, are among the most common single gene disorders in the world. 340,000 children are diagnosed every year, leading to a global population of millions of sufferers.
What are the potential benefits likely to derive from this project (how science could be advanced	This project aims to define the proteins that bind to the enhancers of the alpha-globin genes, and to understand their mode of action. Alpha-globin has

or humans or animals could benefit from the project)?	historically been an invaluable model for understanding the general principles of how genes are switched on. In the short term, if we can identify how the binding of a particular protein at the enhancer region leads to activation of the nearby alpha-globin gene, this will represent an important advance in our understanding of the regulation of gene expression in general. In the medium term, this project might help to identify new candidate genes for anaemias of unknown origin. Whilst most inherited anaemias can be attributed to one of a handful of common genetic causes, no cause can yet be identified in a substantial minority of cases (estimated at 40-50 cases per year in the UK). These patients often suffer from lifelong chronic anaemia. For families with affected children there is uncertainty over the risk to future children, the long-term prognosis and the best form of treatment. In addition, repeated investigation to try to identify the cause is expensive and optimising treatment and/or development of new treatments is extremely difficult. If we can identify new genes responsible for switching on alpha-globin, these genes may well be involved in activating other genes important for red blood cell function and could be included in screening panels used to identify the genetic causes of inherited anaemias. Deletion of the alpha-globin genes or their enhancers are the most common cause of alpha-thalassaemia. Alpha-thalassaemia is responsible for a huge burden of morbidity and mortality. Many patients have a poor quality of life due to their continued dependence on frequent blood transfusions, which can lead to iron overload, multiple organ failure and ultimately shortened life expectancy. The only other treatment is bone marrow transplant but there is a shortage of suitable donors and it is not a cost-effective option for developing nations where these diseases are most prevalent. In the long term, we hope that a better understanding of how the affected genes are normally regulated may inform new avenues of investigation for novel treatment options.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will use in the region of 5300 mice over the 5-year life span of this PPL based on our previous experience.

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?	Harmful adverse effects are rare and unpredictable in the production of new GM lines. Any animal showing more than a temporary deviation from normal behaviour that is not of scientific interest will be humanely killed. The majority of mice will be used for breeding and will experience no more than transient discomfort from 2 or 3 injections prior to being humanely killed for tissue analysis. We do not expect to see any adverse effects as a result of any drugs administered in the doses and timescales to be used. We expect some mice to be mildly anaemic but we do not expect these animals to show any deviation from normal behaviour, nor to display any overt clinical signs of anaemia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although Zebrafish and lower vertebrates may be appropriate model systems for studying many developmental processes, particularly at the early stages of research, a mammalian model still remains necessary in order to fully understand the effects of many human genes and their disease associated mutants in the context ofand other complex mammalian physiology.physiological systems that mammals share. In order to fully understand the effects of human genes and their disease-associated mutants, there is no substitute for a mammalian model. This will allow the analysis Animal models are the only way to study of the effect of a gene at early developmental stages in different tissues. Similarly, there are no suitable non-animal alternatives to study the interactions between different tissues.The study of interactions between factors requires a whole animal model. There are no suitable non-animal alternatives. Although whole-animal models are necessary for understanding how human genes function in the context of normal development, much can still be achieved with cell lines. Our lab uses cell culture systems that mimic red blood cell production to generate and test hypotheses and new scientific methods, whilst reserving our use of animal models only for those experiments where animals are strictly required, where we are confident the

	results will be of scientific interest and where we have validated that the experimental techniques we plan to use are appropriate. We also make a concerted effort to read newly published papers and attend conferences in order keep up to date with the latest developments and identify new cell lines that could replace animals in any aspect of our work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will only breed mice as required. We will cryopreserve mouse lines when they are not actively required to reduce maintenance breeding. Instead of embryo freezing, we will cryopreserve lines by sperm freezing wherever possible as this eliminates the need to maintain large numbers of female donors and stud males to produce fertilised embryos for freezing. All steps in every process will be carefully monitored to minimise numbers. Experimental procedures will be updated as appropriate and new technologies will be introduced as they develop to minimise mouse numbers. Blood and tissue samples will be shared between researchers working on this project. We will use statistical techniques to calculate the number of animals required to detect the effect that we are testing for in each experiment, to ensure that the minimum number of animals are used and that animals are not wasted by using less than the experiment requires. Wherever appropriate, we will randomise the allocation of animals to groups and conduct analyses in a blinded fashion to minimise biases and improve the reproducibility of our results.
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.	Mouse is the species of choice for genetic modifications modelling human disease because of the availability and ease of manipulation of mouse embryonic stem cells. Best practice will be used for all procedures and staff will keep up to date with new methodologies and implement new procedures as they arise. Wherever possible constructs and/or manipulated

	embryonic stem cells will be produced and tested in the bioengineering facility in an *in vitro* system before going on to produce new GM lines. Analgesia will be used wherever appropriate. Mice will be regularly monitored at all times. We will take remedial action as soon as any indication of poor health is observed (e.g. extra palatable food in case of weight loss, or extra bedding for warmth in case of suspected anaemia). If the animal's health does not improve within 48 hours and it is not of scientific interest it will be killed to minimise unnecessary suffering. Animals which are administered substances (e.g. cell labelling agents or gene-inducing agents) will be kept alive for the minimum amount of time required for scientific purposes. Typically they will be killed within one week and often within 12 hours. This will minimise the possibility of animals experiencing unexpected suffering due to the administered substances.

Project	**222. Mechanisms of Hypertension and Renal 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)	High blood pressure (hypertension) and kidney disease are both major causes of poor life quality and of death worldwide. Lifestyle choices play an important role in the development of both high blood pressure and kidney disease. Eating food that is high in sugar and fat and particularly salt is damaging, and diabetic or obese people often have both high blood pressure and kidney disease. Important questions remain about these conditions: how do they develop? how do they interact? what are the genetic factors that determine why some people are affected whereas others are not? This project utilises a portfolio of animal models	

	which have high blood pressure or kidney injury/disease or both in combination. We will use these to understand the biological processes that underpin each disease and unravel the complex interaction between the two.
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 provide new insights into fundamental processes of normal blood pressure regulation and kidney function by showing how these processes fail in disease. This will benefit scientists by stimulating other research projects around the world. Identifying important biological mechanisms may help improve treatment of these disorders which present a major global socioeconomic health challenge. This will benefit patients and doctors and governments .
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use approximately 5000 mice and 1000 rats over the 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?	We examine situations relevant to human disease, focussing on early disease stages and the likely prospective severity of protocols is moderate. Diabetes and hypertension are associated with long-term adverse clinical effects due to uncontrolled hyperglycaemia (diabetes) and barotrauma (hypertension). Progressively, adverse effects, such as polyuria, salt-retention, vascular dysfunction and poor regional blood flow accumulate to increase long-term cardiovascular risk and if untreated significantly increase the likelihood of death. Our use of early physiological events as end-points minimizes the likelihood of adverse events but nevertheless, changes in fluid homeostasis (ie increased urine output and increased thirst) can induce stress and the onset of malignant hypertension is possible, particularly when using new treatments or strains of animals. Similarly, nephrotoxic injury to the kidney, common in humans, can induce renal failure or failure of other organs, although these are not our endpoints and we are focussed on investigating mechanisms that may ultimately contribute to the development of organ failure. All surgical procedures are carried out under general

	anaesthesia, with animals monitored for signs of stress both during and after surgery. For recovery surgery, wound healing is monitored closely. Any animal that does not recover from surgery, or shows signs of distress/adverse effects (eg excessive sustained weight loss, hunched posture, reduced activity and loss of condition) will be humanely killed. Some experiments require single-housing of animals, which may be stressful, but where possible, environmental enrichment is provided. At the end of the experiments, all animals are humanely killed by approved methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project uses animals to investigate the function of the cardiovascular and renal systems in health and in disease. How each part of the system functions is not fully defined and how different parts of the systems communicate with each other is not known. It is highly complex and operates at cell to cell and organ-to organ level and is influenced by our underlying genetic codes as well as factors in our environment It is impossible to reconstruct this complexity in a single cell or non-living model. We do use such alternatives where possible and have a strong history of using cell culture, isolated organ preparations and zebrafish larvae. We also use computer simulations and together these alternative approaches help to inform smart design of our experiments in mice and rats. Overall, we strive to understand how complex physiological processes work normally in order to find out how the processes fail in disease. This approach necessitates the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We often use cell-based systems and computer-based modelling to test whether new concepts hold up in principle. This helps us make informed decisions about the design of experiments in rats and mice and allows us also to use statistical prediction to estimate the lowest number of animals needed to achieve meaningful and robust scientific conclusions. We are very skilled at smart experimental design. For example, we often use experimental procedures that allow us to make careful

	measurements of many different biological processes at once and, if possible, over a longer period of time. This maximizes the information obtained from each animal and provides very important insight into the timing of disease. In combination, these approaches considerably reduce the number of animals needed for the experiments described here.
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 are using the very latest genetic engineering methods to replicate aspects of human cardiovascular and renal disease in rats and mice. These animals have a similar cardiovascular system to humans and comparative studies provide important new information regarding cardiovascular, metabolic and kidney function in health and in disease. We are also equipped to use cutting-edge methods to measure physiology in rodents. We use non-invasive imaging technology that allows us visualise changes to organ function over time. We use minimally invasive procedures to implant devices that then allow us to continuously monitor blood pressure in conscious, unrestrained rodents as they live in their home cage environment. These refinements are possible because we have access to a well-resourced and modern facility, a highly experienced research team and a dedicated group technician to oversee welfare of our animals before and during experimental protocols. Where more refined approaches become available, during the course of this licence, we will aim to incorporate them.

Project	**223. Mechanisms of inflammation, resolution and repair in the airways**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Blood contains cells to kill germs. In order to defend us against germs, they need to leave the bloodstream and travel through the inflamed tissue to reach the germs, engulf them and secrete materials that help kill them. The body does this via a protective mechanism call the inflammatory response. Sometimes this inflammatory response occurs inappropriately in diseases unrelated to infection, which then unfortunately causes self-inflicted harm to the body, for example in asthma, COPD (smoker's disease), or inflammation caused by sudden trauma. Our group has gained understanding on	

	how white blood cells orchestrate this response. We aim to understand the role of different factors affecting the inflammatory response, and therefore effects on lung function and health involved in respiratory diseases like asthma, COPD (smoker's disease), pneumonia, and acute lung injury.
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)?	We anticipate that we will gain a greater understanding of the role platelets and other targets for the treatment of respiratory diseases like asthma, or difficult to treat respiratory infections that lead to pneumonia. This could potentially lead to the development of new drugs for inflammatory diseases like asthma, or to help treat respiratory infections. Asthma currently effects 300 million people world wide, is a major cause of absence from work and is a substantial cost to the NHS. Chronic obstructive pulmonary disease will become the 3rd leading cause of death by 2020 and accounts for 6% of the health budget in Europe. Current treatments only modify the symptoms but not the causes of these diseases. Glucocorticosteroids are not very effective in chronic severe asthma or in COPD. There is therefore a need to find better drugs to treat the underlying causes of these respiratory diseases. Conversely, difficult to treat respiratory infections can lead to pneumonia and are the cause of 40-50% of ICU sepsis patients, of which there is 32% mortality. Infections are becoming more difficult to treat as drug-resistant strains of bacteria become more widespread. Unfortunately, no major new antibiotics have been produced in over 40 years to combat this threat. A potential remedy to solve this is to find new ways of boosting our body's immune response to infection with the creation of immuno-stimulant drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using 17,000 mice of which 4000 have been allocated to breeding over 5 years and 1000 rats in a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at	It is expected that no animal will experience more than moderate severity. Any adverse effects will be monitored carefully. Some animals may undergo surgery and may experience pain, but this will be controlled by providing pain relief

the end?	before, during and after surgery with advice from the NVS. Some animals will be exposed to substances to cause inflammation in the lung or to prevent inflammation in the lung, but we are planning to only use non-lethal doses of substances. In some cases we irradiate mice and this can cause the animals to appear lethargic but animals will be carefully watched and if any animal does not recover after a day they will be humanely killed. At the end of an experimental procedure or if it becomes necessary to alleviate suffering at any time under any protocol then animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are interested in understanding the mechanisms that give rise to the physiological changes to the lung during inflammation. Whilst we can study individual cell types in culture there is currently no test-tube based technique that can replicate the physiological changes in the way the airways constrict in the living animal. Where possible we do undertake experiments using human cells in culture derived from both immortal cell lines and patient samples, or cells derived from blood of human donors. It is increasingly recognised that diseases of the lung such asthma, COPD (smoker's disease) and acute lung injury and infection are complex conditions that cannot be replicated using test-tube experiments alone. Therefore, we have to use experiments on living animals alongside the study of cells obtained from patients and immortal cell lines to fully understand the basis of these conditions and to help us identify improved treatments for these common disorders that still have significant unmet medical need.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are committed to maximising the amount of experimental information we obtain from each individual experimental animal, enabling us to hopefully minimise usage. An example of this is the use of advanced measurements of lung function in conscious animals and the use of advanced imaging techniques whereby animals can be used as their own controls, thereby minimizing the overall number of mice required in

	an experiment. We routinely use single strains of mouse in multiple research areas and use multiple organs collected from individual mice in order to keep animal usage down.
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 are using mice as they have an immune system comparable in complexity to humans and are the most frequently used model for the human immune system. The use of genetically modified mice in our proposed program of work allows the maximum amount of experimental data to be obtained in the most efficient manner. The genetic manipulation of mice enables you to study certain aspects of disease processes/inflammation by the manipulation of specific mechanisms in isolation without affecting the whole animal. We are mindful at all times to minimise welfare costs to the animals we use. All of our protocols are designed to minimise any animal suffering and uneccesary animal usage. All of the models that we use to mimic diseases that kill humans are restricted in our animal studies to only a minimum degree of suffering, whilst still addressing the clinical underlying basis of the disease. In the event that suffering is unavoidable, animals are monitored closely and welfare endpoints[are rigorously applied, so that animals suffering is kept to a minimum. Furthermore when it is possible to take readings/collecting data under terminal anaesthesia, where the animal does not wake up, this is better than completing tasks in a conscious animal and our experimental plans are designed for this.

Project	**224. Mechanisms of interaction between neutrophils and T cells**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	T cells are a subset of white blood cell which generates in the thymus and which circulates the body through the blood and lymph nodes. T cells are critical for clearing infections, but can also induce damage to host tissue, if they are activated in the wrong place or at the wrong time. For example, the subset of T cells known as Th17 are vital for clearing fungal infections, but can also induce multiple sclerosis if they are activated in the central nervous system. T cells interact with lots of other cells in the body; however, we do not know much about	

	how and where they interact with neutrophils, another type of blood cell which is the first responder to inflammation. We know that neutrophils and T cells are together, at the same time and place during inflammation. In the lab, we can see the cells interacting physically. However, very little work has investigated the outcome of this interaction. This project aims to understand the role that neutrophils play in the development of T cell responses during a variety of inflammatory diseases. This will allow us to understand more deeply the pathways leading to damaging T cell activation. It may also lead to identification of new targets for therapy in these diseases.
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 lead to a deeper understanding of how T cells develop, become activated, and proliferate in tissues during inflammatory disease. Identification of points at which neutrophils direct damaging T cell responses would enable targeting of novel therapeutics or treatment strategies for inflammatory disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5000 mice 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?	The majority of the mice will be used in breeding of genetically modified lines. Several inflammatory disease models will be used to assess neutrophil impact on T cell behaviour, including models of infection with fungus and development of Multiple Sclerosis. Effects on these mice in the models of MS include limp tail, paralysis, and weight loss. In fungal models there are unlikely to be any signs of illness but in rare cases we may see a decrease in body temperature, reduced movement and mild weight loss. All models will be of the minimum severity possible and mice will be monitored closely and strict humane endpoints implemented. Following experiments all mice will be culled humanely.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We perform many experiments on human cells isolated from healthy donors; in fact, this is the majority of our work. However, the development of immune responses is an incredibly complex chain of events, which is impossible to study *in vitro* owing to the large number of 'unknown unknowns'. In addition, key experiments include the movement of different types of white blood cell around the body in response to triggers, and the assessment of immune responses in complex and hard to reach tissue such as the brain. This cannot be modelled in culture or removed from human patients. As such, animal experiments are necessary. Throughout the project we will continue to seek alternatives to animal experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Initial experiments are performed on human and mouse cells in culture, so that hypotheses are formed without using animals. Experiments are designed to track animals over a period of time, using repeated measures and a large number of measured outcomes and tissue collection procedures, to cut the number of animals required. Experiments have been designed in collaboration with statistical advice in order to detect significant effects with a lower number of mice. Following initial experiments, statistical analyses will be performed in order to use the minimum number of animals possible to gain significant results.
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 team, both scientific staff and animal technicians, are very experienced in handling and experimenting on animals, which will reduce stress and suffering of the animals. The unit in which the animals are kept is well-resourced and well-equipped. Advice is taken routinely from veterinary staff and all experiments are submitted first to the vets for review. The mouse models used are extensively characterised and replicate human disease and

	immune processes very well. The welfare costs to the animals will be minimised by looking at early disease time points. Published data on these models means hypothesis-forming experiments are much fewer than would be required in other models. Using mice also allows many experimental measures to be assessed, owing to the enormous number of reagents available and the use of transgenic animals. Prior to and during experiments animal health will be maintained using good breeding and handling techniques and housing in ventilated cages. Mice will be monitored closely and assessed using a well-characterised severity score sheet. Support measures such as soft food and analgesia will be provided. And mice will be culled humanely when a humane endpoint is reached, for example a specific level of body weight loss.

Project	**225. Mechanisms of Kidney Injury and Fibrosis**	
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)	"Ischaemia Reperfusion Injury" (IRI) is an inevitable consequence of kidney transplantation that leads to poorer outcomes in the short-term (delay in function or failure of transplant and increased rejection) and long-term (reduced longevity of the transplant and early transplant failure). Understanding IRI and finding treatments for it will improve outcomes in kidney transplant patients.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit	Identifying treatment strategies for kidney IRI will improve outcomes in kidney transplantation – both in the short and long-term. It will also potentially allow use of deceased organs that	

from the project)?	are currently discarded as they are deemed unsuitable for transplantation.
What species and approximate numbers of animals do you expect to use over what period of time?	Adult Lewis Rats. 448 Adult Mice (both wild type and knockout strains). 400
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 animals will undergo an operation to create the Ischaemia Reperfusion Injury to the kidneys, from which one of the adverse effects is postoperative pain. This may be of moderate severity and will be controlled with effective pain relief. The animals will be observed daily for clinical signs of any distress. One of the other risks is that the kidneys are potentially badly damaged, and the animals may go on to develop kidney failure in the long-term. This is unlikely to be a problem as the animals will be humanely killed at 28 days, well before the potential development of chronic kidney failure. This will not be a problem.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Ischaemia Reperfusion injury involves multiple complex biological processes and unknown interactions. To date there is not a suitable non-animal model and therefore the use of animals is the only current method available for the experimental assessment of these interactions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Using established statistical methods (power calculation based on previous literature and results). This minimises the number of animals to get sufficient data.
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 rodent is a reliable subject for surgical work and its use as a model of renal IRI is well known and of widespread use. We are experienced with the surgery involved and the facilities are well established within the university to facilitate both the procedures and husbandry of the animals. We will provide the animals with appropriate pain relief and will humanely kill the animals well before potential development of chronic kidney failure.

Project	**226. Mechanisms of Normal and Leukemic Haematopoiesis**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our goal is to improve the treatment for children and young adults who suffer from a rapidly fatal type of bone marrow cancer called acute leukaemia. Some children can be cured, but the treatment is arduous – lasting up to 3 years – and medical side effects aside, it severely impacts their schooling and physical development and in some cases impairs normal psychological development. Each member of a family with a child blighted by leukaemia is profoundly affected; parents may have to stop working, and siblings may have life opportunities curtailed. Finally, an emerging problem with current treatments is the prevalence of major long-term medical	

	complications in survivors. It is estimated that dealing with these so-called “late effects” now places as big a burden on the NHS as treating the children in the first place.
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)?	Clearly, there is a need to make treatments faster and more focussed so that we can cure all children and minimise the medical, economic and wider societal impacts of leukaemia. We are working towards this by studying the biology of bone marrow cells collected from affected children. We are also looking at ways of improving bone marrow transplantation (an important treatment for patients with leukaemia), and for this we must study normal human blood and bone marrow cells, and cells isolated from human umbilical cord blood. We collaborate with doctors at various hospitals around the country to collect surplus cells that are left over bone marrow tests carried out on patients with leukemia. These tests are painful, and are not undertaken lightly and so the samples are precious. We try to work as much as possible in the tissue culture lab, although some experiments involving mice are inevitable – particularly when we have identified new drugs or treatments which must be tested in mice before clinical studies in children can be contemplated.
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use mice in our experiments and a maximum number of 8500 over the 5 years of the experiment. As most mice are ordered for specific experiments wastage will be kept to an absolute minimum.
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 animal experiments involve mice only. We inject human cells into the animals usually through a vein in the tail. In some cases, it is directly into the thigh bone via the knee. To minimise stress and discomfort to the mouse they are given analgesia. 12-24 hours before the human cells are injected, the mice receive low doses of irradiation. Most patients receiving bone marrow transplants receive equivalent doses of radiation, and experience no immediate ill effects However, over the following weeks their immune systems are suppressed and they are prone to infections. To prevent this from happening to our mice we keep them in very clean conditions. Mice receiving normal human cells experience no ill effects.

	However, those receiving cells from patients with leukaemia can become quite unwell. They are monitored every day by skilled animal technicians, and if there are signs that they are becoming unwell before an experiment is scheduled to finish, they are culled immediately. Whenever this happens the mouse is analysed very carefully, so that we can maximise the information gained from each experiment. Some mice are given chemotherapy or other newly developed drugs. Generally, this is given in the food or water, or by injections either into a vein in the tail or into the abdomen. Occasionally the drugs have to be administered by a feeding tube, and this is carried out by specially trained technicians. Some animals may become unwell after receiving these treatments, and when this happens, they are culled immediately. All animals are eventually humanely culled but, all mice are analysed in detail in our laboratories in order to maximise the data we generate from each experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have explored the possibility of experimental methods that do not use animals, and much of our work is carried out in culture with cells acquired from healthy human volunteers or culled mice. In order to replace animals with in vitro models as much as possible, we have developed a method that allows us to keep normal human and leukemic stem cells alive over a period of weeks. This will help us understand the key features of stem cells. However, one of the main tests of stem cells is that they can live for many months and can produce all types of blood cells, including more stem cells, and at present this can only be tested in animals. Animal studies are also needed to study the impact of chemotherapy, especially to understand why some cells are resistant to treatment and can cause patients to relapse. In addition, when patient leukaemia cells are injected into mice, they are usually able to grow and divide and so the number of cells increases enormously, which does not usually happen under laboratory conditions. These cells can then be harvested and stored long-term, so that these valuable patient
	samples can be used in many more experiments.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The majority of mice used in this project licence will be ordered in for specific experiments rather than breeding. Meaning we can ensure the minimum number of mice will be used for most experiments. We will also use statistical tools to design experiments in order to minimise the numbers of animals used. Where transgenic lines are used efficient breeding strategies are in place. We will replace breeders before their reproductive performance declines and they become un productive and review the breeding continually to make sure we are making the most of the smallest number of animals possible. If we predict that lines or animals are not required for several months we will adjust the numbers of breeders or take advantage of frozen stock when the need arises again.
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.	Stem cells were first identified in mice in the 1950s, and over the years, mice have become the standard model for studying the biology of stem cells and cancer. Indeed, human stem cells were first characterised in mouse models, and the activity of human stem cells is measured in terms of their capacity to transplant in mice. Furthermore, results obtained in mouse models have provided the experimental basis for bringing several new anti-cancer drugs to the clinic. To ensure technical competence, the staff performing the experiments will be fully trained and supervised either directly by myself or senior postdoctoral fellows who have extensive experience in experiments on animals and the techniques we will be applying. To minimise infections in immunocompromised mice extra steps will be taken to minimise cross infection from other colonies, like specific protocols for the cleaning and handling the mice. If mice are in pain or discomfort an analgesic will be given. Transgenic mice exhibiting any unexpected harmful phenotype will be humanely culled. Or in the case of any mouse of special scientific interest, advice will be promptly sought from the Home office inspector or Named Veterinary surgeon and the Named Animal Care and Welfare officer. All the work involving mice will be
	undertaken with the guidelines set out in National Cancer Research Institute (NCRI)

Project	**227. Mechanisms of phenotypic programming across the lifespan**	
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 main aim of this research is to determine the importance of early life conditions in shaping adult brain, physiology and behaviour. By looking at all of these factors we can understand how an animals' developmental experiences can alter the way in which an adult animal copes with challenging environments. Importantly the work will investigate how early life experiences interact with adult experiences to influence these traits. We will also perform experiments that allow us to determine if the effects we see are beneficial or costly to the animal. By studying animals in the wild and those that live in captivity	

	we will be able to see how many different environments can influence development and alter behavioural responses.
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)?	Neuroscientists have become increasingly interested in how the brain changes over the course of the ageing process and into senescence, as this can be highly relevant to several human pathologies. However, there are few studies that have tracked individuals from known developmental backgrounds through adult life to explore the interactions between environmental conditions at different life cycle stages. The developmental origin of health and disease is an important focus for a range of research areas, including those working in basic biomedical and clinical sciences. This work will provide a novel opportunity to gain an understanding of not only how early life can program adult susceptibility to disease pathologies, but also how the environment experienced during adult life can interact with the developmental phenotype to shape the potential for predisposition for a range of disorders, including stress related disorders and cognitive decline. Animal welfare scientists have increasingly adopted behavioural and physiological measures, such as cognitive performance and stress reactivity, for assessing the wellbeing of animals. This project will add to their understanding of how such traits and several others relate to both adult and developmental environmental conditions. This could facilitate direct changes to animal husbandry protocols that would feed into the 3Rs priorities. Finally our work will also look at how developmental conditions and exposure to environmental contamination can impact on the health of bird species so our work has high relevance to conservation organisations and agencies working to understand population changes in our changing world.
What species and approximate numbers of animals do you expect to use over what period of time?	We will study a range of bird species. In many cases our experimental groups will consist of between 15-20 animals per group, and we will typically have 2-8 groups within an experiment. We will use a **maximum** of 1500 captive birds and 1000 wild birds over the 5 year life 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 are interested in how individuals respond to natural variations in environmental conditions during their life and therefore all manipulations will be carried out so to induce changes that mimic those experienced in the natural environment. This will minimise suffering in our study animals and allow us to determine how developmental conditions impact on normal physiology and behaviour. The majority of our procedures are not expected to provide anything other than mild stress or momentary pain during the collection of blood samples or during injection, and indeed it is important that stress is kept to a minimum because this itself could affect the animal's normal behaviour. Where blood samples need to be taken amounts obtained will be such that the health and well-being of the animal is not affected. In the few studies where tissue, such as the brain, will be collected animals will be killed humanely prior to this. In addition we will closely monitor the health of all animals and humanely kill any animal that appears to be suffering during the procedures. The fact that we will manipulate conditions within the natural range experienced for a given species means that we will minimise any potential adverse effects. Animals that experience negative developmental conditions, for example an increase in stress hormone exposure or reduced food availability, may exhibit reduced growth rates, however, our previous work suggests that the at level of manipulation we intend to use, these effects are slight and cause minimal suffering. We have taken several other measures to minimise suffering and the number of animals that will be used in this research. In many cases we will be able to directly manipulate the pre-natal environment, for example by injecting stress hormones into an egg, without causing any distress or suffering to the mother, reducing the overall numbers required to fulfil our objectives.
Application of the 3Rs	
1. Replacement	In order to properly meet our experimental objectives experimental adjustment of

State why you need to use animals and why you cannot use non-animal alternatives	developmental and adulthood conditions is necessary as is the long-term monitoring of many traits into adulthood. Therefore the need to follow living animals through time in this research is essential, since a major aim of the project is to understand how animals respond to environmental conditions and how these can influence a range of important traits throughout life.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Sample sizes of animals used in experiments are always determined carefully via statistical power analyses to ensure the maximal statistical power to detect relevant differences between treatment groups. We also use direct manipulation of egg hormones rather than manipulate the environment of the mother where possible, and thsi reduces the overal number of animals used in experiments.
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 birds as a model in this research as we know a great deal about their developmental trajectories, ageing rates and physiology and behaviour. An important feature of avian species is that they overcome a major constraint of mammalian studies that look at the long-term effects of developmental conditions, namely the direct link between the mother and developing animal during both gestation and lactation. This prolonged link, limits the ability to determine the exact conditions experienced by each animal and/or the effects of subsequent maternal behaviour on later physiology and behaviour. The applicant has been working with avian species for over 20 years and therefore is well placed to ensure the welfare of the animals in her care throughout the project. Avian species represent the best model for the proposed work for 3 reasons: i) the separation of pre- and post-natal development to allow independent manipulation of each stage, ii) the ability to monitor physiological and behavioural traits at each life cycle stage and throughout ageing and iii) characterisation of ‘normal’ and ‘abnormal’ behaviour or physiology. In captivity we have chosen to use species for which we can maximise their welfare as they have been bred in captivity for many generations. In experiments

	we intend to carry out in the wild we have chosen three species that breed in accessible places, such as in nest boxes or at farms or in easily accessible nesting sites. The manipulations we use are designed carefully to mimic naturally changing environments and these have been refined and validated by the applicant over the last decades. This ensures that we not only mimic natural changes in an animals environmental conditions, but it reduces the impact on the animals used in the study.

Project	**228. Mechanisms of regulating immune responses important for controlling infections and cancer progression in the lung**	
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)	Respiratory infections (those that affect the lungs and can affect the breathing) are the leading infectious cause of illness and death in the world. Every winter in the UK, seasonal epidemics of respiratory virus infections cause widespread disease. These infections can cause colds, which are a significant economic burden in terms of time lost from work, but can lead to severe disease and fatality in susceptible groups. These groups include the	

	very young, where respiratory infections are the leading cause of hospitalisation, the frail elderly, and those with underlying long-term health conditions such as asthma or cancer. For many respiratory infections, treatment is only supportive because there are no preventative drugs, they are too expensive or vaccines are not available or effective. Our work aims to understand the body's response to respiratory infections and how this influences the development of cancer in the lung. Our objectives are to understand how the body's responses can protect against infection and how they can sometimes lead to excessive inflammation in the lungs and to disease. We also want to find out how the body's responses to a respiratory infection influences lung cancer growth and spread.
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 project will increase our basic understanding of how the body defends itself against lung infections and how these infections might influence cancer growth. In doing so we will contribute to the field of immunology (study of how the body protects itself from disease caused by infection), respiratory diseases and cancer. In the longer term this basic understanding should lead to new therapies which promote the body's protective responses or prevent unwanted and potentially damaging inflammation. In addition, understanding how cancer growth is influenced by the body's responses to a respiratory infection could result in the development of new treatments or therapies. These may include new vaccines for respiratory infections.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will use approximately 15 000 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	In a typical experiment, we would aim to understand the role of a particular component of the immune (body's defence) system in protecting against infection or causing lung disease. In some experiments we will generate

the end?	different types of cancer in animals in order to study how a respiratory infection influences the growth of tumours. This will typically involve artificially altering the animal's response to infection before infection with a respiratory pathogen. Genetically altered mice may be used. Lung infections can lead to illness in mice and we expect some symptoms including weight loss. However, this is not severe and mice typically regain weight within a few days. There may be circumstances, for example in some genetically altered mice or in tumour-bearing mice, where disease can be worse. We will carefully monitor mice for illness throughout infection. Animals will be humanely killed at the end of the experimental procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Immune responses to infection or cancer are complex and involve interactions between the disease-causing agent, the infected organ and the body's immune system in ways that cannot be reproduced in the laboratory. We need to use a mammalian species due to the similarities in the immune and respiratory systems between these animals and humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always try to get as much information as possible from each animal we use and take many different tissues from each infected animal in order to learn as much as we can about the immune response to infection. Group numbers for each experiment are kept to a minimum. Calculations are used, based on statistics and previous research results, to identify the minimum number of animals needed to provide meaningful and reproducible results Numbers of genetically modified animals bred will also be kept to the minimum numbers required for experiments.
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	We believe mice to be the lowest animal which accurately reflects disease in humans. We have studied lung infections in mice for many years and continuously refine our techniques to minimise distress and suffering of the animals. Doses of the disease agent used are calculated

general measures you will take to minimise welfare costs (harms) to the animals.	so as not to cause severe disease. Mice are continuously monitored for signs of disease throughout infection. In particular, mice are weighed daily, as excessive weight loss is a sign of more severe disease. Limits are set for weight loss and animals are humanely killed if these are exceeded. The progress of tumours in mice used for cancer studies is also carefully monitored along with signs of disease. Particular care will be taken to ensure detection of unexpected tumours. Any animals showing signs of severe disease are humanely killed. Whenever procedures could cause pain or severe discomfort, pain relief is used or animals are anaesthetised. Good, sympathetic animal handling, injection and blood sampling techniques will be used in order to minimise discomfort. When using pups, we scent our gloves with bedding from the cage before handling and limit the time away from the mother. Animals are housed with appropriate bedding and nesting material in individually ventilated cages which provide constant fresh air.

Project	**229. Mechanisms of sensory processing and neuropathy**	
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)	Neuropathy is a major dose-limiting side effect of chemotherapy which affects up to 70% of patients and can persist for months or years following completion of cancer treatment. Chemotherapy induced peripheral neuropathy (CIPN) occurs across a broad range of agents used to treat common cancers and there is currently no effective treatment. This project aims to investigate, using animal models of CIPN, the mechanisms by which chemotherapy causes neuropathy to aid development of novel therapies to counteract or prevent chemotherapy-induced peripheral neuropathy. Through understanding the mechanisms of pain, we can investigate how the application of	

	mechanism-based treatment affects chemotherapy-induced pain-like behaviour in our animal models. Ultimately providing data to aid CIPN treatment, or its prevention, in a clinical setting.
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)?	Through understanding the mechanisms of chemotherapy induced neuropathy we aim to facilitate in the development of novel compounds and/or in the repurposing of existing compounds for treating this debilitating condition in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 800 rats per year. We un-dertake power calculations to ensure the minimum number of animals are used to obtain robust data.
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 generate animal models of chemotherapy induced peripheral neuropathy, peripheral nerve injury and peripheral inflammation in order to study mechanisms of sensory abnormality / hypersensitivity. The likely expected severity of CIPN animals is mild - moderate. Animals will be culled using schedule 1 procedures at the end of the study
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Taking nerve samples from humans is a known cause of chronic pain and testing experimental drugs in humans infeasible. Therefore, we will use clinically relevant rat models that mimic patient symptoms of neuropathy. Non-protected animal alternatives are not yet available for the proposed studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers will be kept to a minimum through use of power calculations to ensure group sizes are adequate to observe a significant effect. Where possible, multiple data sets will be obtained from each animal through extended tissue harvests from each animal.
3. Refinement	Rats will be used as their nervous system pathology closely reflects that of the human. We

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.	are using clinically relevant models to mimic patient reported symptoms and pathology of chemotherapy induced neuropathy. Using these pre-clinical models, we can test potential therapies as a first step towards therapy translation in the clinic. We will ensure minimal welfare costs to rats through routine weight and appearance monitoring. Where appropriate, animals will receive intensive care post-surgery; administration of analgesia and cages will be placed on heated mats.

Project	**230. Mechanisms of synapse function 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 want to understand the mechanisms that control the composition of synapses (specialised connections between nerve cells) in health and disease by (i) monitoring synapses through imaging of brain tissue or living mice, and through isolating synaptic proteins and (ii) by studying synapses in animal models with genetic mutations relevant to human disease and (iii) by modulating synapses using drugs, diet or altered experiences.	

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)?	We have previously reported that over 100 common and rare brain disorders are caused by mutations that disrupt proteins in synapses. Synapse disease, referred to as synaptopathy, can also be caused by brain injury, stroke and neurodegenerative disease such as Alzheimer's. The study of synapses is a novel field in neuroscience and this project will therefore provide useful information that will complement existing research into both brain structure and function as well as brain disease, to help us better understand how the brain works. Understanding synaptophathy and how synapses may be repaired or replenished is important for the development of drugs to alleviate human genetic diseases such as autism and schizophrenia, or to prevent synapse loss in neurodegenerative disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We will study mice, primarily genetically modified mice that are engineered to have fluorescent tags on important synaptic proteins or have mutations relevant to human diseases. We expect to use no more than 3,000 mice each year for 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 majority of animals under this project licence will be subjected to minimal procedures, for example may receive an injected labelling or pharmacological substance that we know from experience results in minimal discomfort to the animals. Some procedures will require surgery and anaesthesia, however pain relief will always be administered to reduce any unnecessary suffering associated with this. A small percentage of animals display post-surgery complications, however, should this be evident animals will be humanely killed immediately. The behavioural tests used are primarily not associated with any adverse effects, but some may require a small amount of food restriction to motivate search for food rewards, short term single housing which is known to be stressful, injection of substances that are moderately nauseating (but cause no long term adverse effects) or small foot shocks that are designed to cause only minimal, transient discomfort (aversive stimulus). All animals throughout

	these experiments will be monitored carefully and if there are any signs that the animals are under distress or unwell they will be humanely killed immediately. Some animals will have their sleep/wake cycle altered (disturbed during normal hours of sleep) which the likely adverse effects of are moderate weight loss but may be as severe as death if prolonged. To avoid this, animals will only be deprived of sleep for short amounts of time initially which may be incrementally increased but will be checked daily and video monitored constantly. As for all procedures, we expect no more than transient discomfort to the animal but careful monitor regimes will ensure that any animal that goes above that will be humanely killed. At the end of all experiments, animals will be humanely killed and brain tissue will be extraction and used for imaging or extraction of synapse proteins. We expect minimal adverse effects as all of our protocols have a moderate expected severity and the majority of animals will only experience procedures of mild severity. Experiments that are of moderate severity, e.g. live imaging that requires anaesthesia, are important to translate our findings in mice to clinically relevant imaging approaches in humans. Throughout these and similar experiments animals will be regularly monitored to minimise any unexpected adverse effects and unnecessary suffering. The genetically altered mice which are developed to model human diseases primarily affect neurological behaviour and are not associated with adverse effects that impact negatively on animal welfare. In rare cases where adverse effects develop, animals will be studied prior to this time point. The animals will be humanely culled at the end of the experiments and the brain tissue will be used for imaging or extraction of synapse proteins.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are unable to use non-protected animal alternatives such as invertebrates because we are studying the role of genes unique to vertebrates in whole-brain systems. The brain is a very complicated structure with a

	number of different cell types necessary for its maturation and functional development, including synapses and their proteins. There is substantial evidence showing that synapses keep changing throughout life, for example in response to learning and memory, and in disease. It is impossible to understand how synapse proteins are regulated and re-distribute throughout life and in response to disease, or from altered experiences in anything other than a living organism. Mice provide a valuable animal model because there is high conservation between the genes of humans and mice. That means that we can study human disease-relevant gene changes in mice to monitor the effect on synapses. Further, studying mice means that we can understand how changes in synapse proteins ultimately affect function, using behavioural studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise animal numbers we try to reduce the variation of our experimental data, this is done by (i) using standard protocols and conditions assuring that outcomes can be compared between normal and disease-relevant mice as well as between different lines of disease-relevant mice, (ii) using mice that are all of the same age and genetic background, (iii) design studies that generate both structural (e.g. imaging) and functional (e.g. behaviour) data in the same group of mice (iv) studying synapse proteins over to whole mouse brain rather than very small areas We also, based on over thirty years of experience studying genetically altered mice, reduce the number of animals required by designing appropriate breeding and maintenance strategies.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are a vertebrate model with high conservation of human genes, which can also be manipulated to create genetically altered models. One such example is genetic tagging of fluorescent markers to proteins of interest. In our case, that means that we can monitor a number of different synapse proteins in the brain of the same mouse, as they light up with different colours when imaging the brain tissue. This is a very refined model for which we have developed highly sophisticated methods of data analysis which generates enormous amounts of information on synapse proteins from single animals. Importantly, these fluorescent tags are not associated with any adverse effects. Secondly, mouse genes can be manipulated so that they resemble human disease-relevant changes, and these models can be validated using behavioural studies. The most refined approach is achieved by crossing both of these types of mice to visualise several synapse proteins in disease-relevant mouse models. Together with functional information acquired from behavioural studies this experimental design allows for a vast amount of data to be acquired from single animals.

Project	**231. Mechanisms to explain the influence of early-life iron and zinc supplementation at the ImmunoMetabolic-Microbiota interface**	
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)	Iron and zinc deficiencies are a major health concern world-wide, especially in malnourished children. However, supplementing foods with these nutrients is often linked to poor gut health, especially in terms of increasing susceptibility to infections, but little is known about why this should be the case. The overall aim of this project is to help understand the interaction between the immune and metabolic	

	systems of the animal and the bacteria living in its gut - following supplementation with iron and/or zinc. Specifically: 1. To measure immune and metabolic development in response to iron and/or zinc supplementation in a piglet model of human infants. 2. To assess how that immune and metabolic development is affected by the developing gut bacteria in piglets fed both supplemented and unsupplemented diets.
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)?	Pigs and humans are very similar and so the results we obtain from these studies will be very relevant to humans. Far more so than if this program of work was carried out using mice or rats. The major benefit will be seeing if iron/zinc supplementation in formula feed is good or bad for gut development. This will be in both normal and deficient animals. It will also go on to see which types of iron/zinc supplementation might be best for gut health For example, iron/zinc added to formula milk, or injected straight into the body. This second option means bypassing the gut. This is important because iron supplementation can change the population of bacteria living in the gut, which can be bad for health. Furthering our understanding of why intervention with iron and/or zinc can lead to poor gut health will eventually aid the development of novel strategies to combat iron and zinc deficiencies which do not carry such risks. Most other animals are very reliant on their mothers for nurturing and milk when they are born, so their feed cannot be controlled. Pigs are born running around and so they can easily be taken away from their mothers and given especially formulated feed. This means the experiments are very tightly controlled and the results are very reliable. For these reasons, results from this study will be much better in furthering our understanding of what is going on at the biological level that if they were carried out in other species. We have only recently become aware of the considerable interactions which occur between the immune and metabolic systems, and that these systems interact closely with the bacteria living in all our guts. However, this is a new area which we do not know much about. We have also

	become aware that these systems are implicated in several conditions affecting humans, including diabetes, metabolic syndrome and inflammatory bowel disease. It is important that we understand exactly what is going on at the biological level in order to address these conditions. This study will go some way to doing this.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the next 5 years, we would expect to use about 100 young piglets (0-8 weeks of age).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We expect to see relatively few adverse effects caused by the proposed experimental procedures. Those that we do see are expected to be mild and short-lived. Piglets will be very young when we transport them to the research site and may have navels that have not completely healed. Infection is a possibility although previous experience tells us that this will be very unlikely. Piglets on the unsupplemented control diets may exhibit signs of anaemia. Again, previous experience suggests this is very unlikely, but if we do see it, iron supplementation will be given at once. Distress or short-term pain may happen when blood, saliva or rectal swab samples are taken. We expect these effects to be mild and very short-lived. At the end of the study, the piglets will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	So far, it has not proved possible to model the interaction between gut bacteria and the complex immune, metabolic, nervous and hormone systems of humans without using experimental animals. Less advanced, non-protected, animals do not show the same complex interactions and do not provide reliable models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Professional statisticians will be consulted to ensure that experiments in this project are designed using only the number of animals needed for the research questions to be answered. Litter-matching will also be deployed wherever possible. This is where members of the same litter are split between treatment groups to reduce genetic variability between these groups (effectively ‘twin’ studies).

	Coupled with housing animals in individual units, this strategy means far fewer animals are required than if the treatments were applied to group-housed piglets.
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.	Pigs have been chosen for this project because they are one of the best non-primate models for humans. As a consequence, findings will be very relevant to human healthcare. Pigs are especially valuable in early nutrition studies since they can be safely removed from their mothers and therefore infant diet can be very tightly controlled. Animals will be cared for using standard husbandry practices in order to reduce harms and, in addition, their environment will be enriched to permit normal behaviours.

Project	**232. Mechanisms to medicines in neurodegenerative diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, are a group of fatal conditions that cause loss of memory and mental ability due to death of brain of cells (dementia). Currently, no treatments exist for these conditions and they pose an ever-increasing burden on families, society and healthcare systems worldwide. We recently discovered a key process that causes brain cell death in mice with these diseases, which we have targeted with drugs, curing disease and preventing death of brain cells in mice. The same process is affected in human neurodegenerative diseases. We have recently discovered a safe, licensed drug which is ready for clinical trials in dementia patients. We now aim to increase our knowledge and understanding of	

	the way that this drug works and identify more pathways and compounds which protect brain cells and drive the discovery of new, safe, treatments for dementia and neurogenerative disease.
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 impact of possible new treatments for neurodegeneration on the lives of individual patients and carers, on healthcare services and on the global economy in the context of an ageing population, is potentially enormous. Finding drugs to target the processes underlying brain cell death will benefit learning and memory, as well as protecting brain cells from dying. By testing drugs that are already safe to use in humans we hope to be able to transfer these to clinical trials more quickly. In the long term, even modest delays in disease onset or progression could have significant impact worldwide.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, both wild type and genetically modified. To date we have used ~16,000 mice in 5 years. However, where possible we replace mice with cellular models (see 3Rs section). We would nonetheless expect to use up to ~25,000 mice over 5 years for developing new models of dementia and testing of potential treatments.
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 mice will be models of neurodegeneration i.e. dementia models such as Alzheimer's and prion, and frontotemporal dementia models. Some of the animals will never show any visible signs of distress however some will show signs of disease such as movement and behavioural changes. Those that do will be carefully monitored by daily observation and examination of weight to limit both the length of the experiment and any suffering caused. Where needed, these animals will be given pain relief and easier access to food and water. In most cases, mice will not exceed a level of moderate severity. Mice will be humanely killed as soon as relevant clinical signs of disease are seen or other suffering. When new compounds are used, adverse effects may result, these will be rigorously monitored and appropriate action taken. Occasionally mice will undergo surgery, to deliver substances to specific brain regions. During these 30 min-1 hour surgeries, very small holes will be drilled into the skull and an ultrafine needle used to deliver these substances. Mice recover quickly and we be given pain relief and post-operative care. At the end of each study, animals will be humanely killed and tissue taken for analysis and
	further studies.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Neurodegenerative diseases are fatal disorders for which there is no cure. To understand what causes them and how to treat them, studying them in experimental models is essential. Mice provide an excellent model for many aspects of human disease. They are ideal for studying prion diseases as they reproduce all key features of the disease: long incubation times, loss of normal brain function and cell death. They also model brain cell loss and changes in movement and mental ability of other neurodegenerative disorders such as Alzheimer's disease. By using mice with prion disease, we have discovered new reasons for brain cell loss and new treatment targets for dementia. We now need to understand the role of this in neurodegeneration more broadly, and the effects of modifying it for new treatments for dementia. The complexity of the brain, and the need to use systems that can accurately model neurodegenerative diseases, means that there is no substitute for animal experimentation. Insufficient information exists to generate accurate computer models that can predict the complex responses of brain tissues. Whilst many studies can and will be done in cells in culture, intact brain with its full complement of brain cells is the only system in which mechanisms can be fully tested and therapies be accurately evaluated. Mice share many similarities in brain structure with higher mammals, including humans and many of the mechanisms, processes and pathways are identical to those in humans. Further, the availability of genetically modified animals with particular genes knockouts or overexpression make them useful tools for testing the importance of particular process in neurodegeneration. Thus, these species are the most appropriate for testing basic aims, which can be relevant to human health. However, where possible we will use cell culture for testing new drugs for the treatment of neurodegenerative disease. However, the clinical validity of these and their relevance to human disease ultimately requires validation in mouse models.
2. Reduction Explain how you will assure	We will use the minimum number of mice needed in all experiments for reproducible results and statistical validity in line with the ARRIVE

the use of minimum numbers of animals	guidelines www.nc3rs.org.uk/ARRIVE. We will use published protocols to guide statistical validity in all our experiments. We will also use the PREPARE guidelines (https://norecopa.no/prepare) and the NC3RS Experimental design assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda) to help plan and design our future experiments. We are committed to keeping the numbers of mice to a minimum by maximising the use of each experimental animal (see below), e.g. taking tissues from different halves or parts of the brain for biochemistry and histology after behavioural assessment and neurophysiology, therefore maximising readouts from any one animal. For genetically altered animals, where suitable lines already exist, animals will be obtained from the relevant supplier. Otherwise, we will have the required lines made by reputable companies. We will ensure high standards of animal care, welfare and utilize the most appropriate breeding methods. Colony sizes are monitored and adjusted within a formal forecasting system to meet the requirements of the research programme. Breeding colonies are always kept to their minimum size so as not to over-produce and to avoid wastage. Where it is possible to avoid using animals by growing primary cell cultures from their brains, we will use this approach. A single new-born pup can provide enough cells for whole experiments that we will use wherever this is a feasible alternative.
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.	Non-mammalian species (such as flies and worms) do not share sufficient commonalities in their central nervous system to make them appropriate models for human disease. Rats and mice share many similarities in brain structure and processes with higher vertebrates. Furthermore, the availability of transgenic animals with particular genes knocked out or overexpressed make them useful tools for testing the importance of particular genes in neurodegeneration model systems. Thus, these species are the most appropriate for testing basic hypotheses, which can be relevant to human health before moving into higher vertebrate species. For genetically altered animals not infected with prions, the most invasive procedure is likely to be intracerebral injection with modified viruses or other substances. This

	is carried out under general anaesthesia and the animals are given pain relief for the craniotomy scar. Most animals will receive one set of bilateral injections and not more than two, on separate occasions, not less than one week apart. Administration of other substances will be given with due care and pain relief if appropriate as well as staged dosing, in general not more than daily dosing. To study cooling as a method of neuroprotection we will use a technique that mimics the biochemical changes that occur during hibernation in small mammals and in which the mice return to normal temperature within a few hours with no lasting signs of stress.

Project	**233. Mechanisms Underlying Human Genetic Disorders of Insulin Action and Adipose Tissue Function**	
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)	Rising levels of obesity all over the world have caused a major increase in premature disability and death due to complications such as heart attacks, strokes, liver damage and cancers. One reason for this is that as some people become obese they become resistant to insulin, the hormone in the body that keeps blood sugar levels under control. This “insulin resistance” is closely associated with disease, but why some people are very prone to it, and how it causes disease, is not well understood. It is now believed that how fat tissue responds to high levels of energy intake is	

	critical. One way to try to understand common disease is to study rare diseases where the same problems are caused by changes in only one gene. It is important to understand these diseases to improve treatment for rare people affected, but such study can also give important information about how fat tissue works and is related to disease. In this project a series of very rare human diseases affecting fat tissue, each caused by changes in a single gene, will be reproduced in mice. These mice will then be used to study how the gene change controls fat tissue function and how they determine when disease appears. The mice will also be used to understand how the change is influenced by environmental factors, and how these conditions can best be treated.
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 sole purpose of this project is to increase understanding of human disease – both rare diseases caused by single gene changes, and common obesity-linked diseases. Using mouse studies for conditions where affected people are very rare, often very young, and where they live far apart, is critically important. It allows the consequences of rare disease across the whole lifetime to be studied much more quickly than would be the case in people, it allows study of tissues than cannot be studied in people, and it allows testing of a range of possible treatments or factors that might make the disease worse. This helps to design the best clinical studies in humans, and helps to give the best advice to affected people. It also commonly teaches us lessons about the normal regulation of fat tissue and metabolism, and helps to understand how this goes wrong in common obesity-linked disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years, approximately 2500 mice will 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?	The mice to be studied will be genetically altered to mimic human disease. Across their lifetimes the effects on their metabolism, body weight, body make up (e.g. how much fat tissue they have), and organs will be studied. The large majority of experiments will mimic studies that are undertaken in people, including changes in diet or

	temperature, assessment of ageing, and the effect of different body temperatures. A variety of forms of imaging will be used, and a variety of types of blood test, sometimes after injecting test substances to assess how the body responds, will be used. The responses of mice with different gene changes to possible treatments, or possible factors that worsen metabolism will be assessed. The possible adverse effects of each study are well known from previous experiments, and from experience in people, and mice will be carefully monitored for each of these. They include abnormally high or low blood sugar levels, obesity or changes in body fat distribution, stress from being moved to different environments, or some features of the human conditions being mimicked. At the end of studies, and to ensure the maximum information is gained from every mouse and each experiment, animals will be humanely sacrificed so their key tissues can be examined and stored, with findings made available to other doctors and scientists.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our studies all start with people with rare genetic disease, and all possible information is extracted from their medical records, and from additional research studies which affected people may consent to. We also extensively use cells grown in the lab. Despite all these measures, there are critical questions for affected people that we cannot answer without studying animals. This is often partly because only very small numbers of people with the disease are known, and they often live far apart, are often very young, and usually have a high medical burden already. Studying mice allows a. much faster study of longer-term outlook in the rare disease across the lifespan b. study of organs (e.g. liver, heart and brain) that cannot be obtained in almost all cases in humans, and c. evaluation of potential new treatments to look for signs of benefit. This is of crucial importance as small numbers of patients makes clinical trials very challenging. Finally, genetic alterations in mice allow assessment of which organs contribute to observed disease, a critical question which can never be answered with confidence in humans when all organs are usually
	affected.

2. Reduction Explain how you will assure the use of minimum numbers of animals	A key aspect of reduction involves studying only carefully selected mice that faithfully mimic human genetic diseases. This research focuses on highly targeted changes that have been studied extensively in humans and proven to causes disease. Careful attention will be paid to experimental plans, ensuring the most economical types and sequence of tests that minimise animal use and maximise information gathered from each animal. For example, painless X-ray and other imaging techniques will be used as much as possible. Careful study design that allows statistically robust conclusions to be drawn is another critical element of our approach, and we will involve experts in the planning phase to maximise our changes of convincingly answering our questions, and thus not having to repeat experiments unnecessarily. We shall follow other aspects of best practice in animal studies to minimise animal use, for example using “pilot” studies to ensure our assumptions are correct before proceeding to larger scale studies. Available information on any related models, and deep understanding of corresponding human diseases will be taken into account. All samples obtained will be stored and made available for sharing where possible, and results of the work will be published in scientific journals, which will help reduce animal studies in the longer term. Importantly, we shall also publish negative studies, increasing understanding of mice as a model of human disease, and accelerating understanding of optimal approaches to their study.
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.	None of the procedures used in this project are considered to cause severe distress, and the large majority will produce negligible or only mild distress. We shall extensively utilize the equipment and support of the highly-trained staff of the animal facility, who provide husbandry and technical assistance to researchers. Close contact will also be maintained with colleagues across the country, allowing sharing of refinement to techniques and optimal use of noninvasive approaches. Cage environments will be “enriched” to provide opportunities for mice to exhibit normal

	behaviors such as, nesting, hiding, gnawing, foraging and exercising to reduce stress. Suffering will be minimised by having tests performed by experienced staff familiar with the well established techniques to be used. When pharmacological/chemical stressors are used, pilot studies will be undertaken to determine the most refined dose. Where necessary, painkillers and/or anaesthesia will ensure that pain, discomfort and distress is minimal. This is not only important for animal welfare, but will also minimise the potential impact of stress on the experimental results.

Project	**234. Mechanistic studies to support respiratory drug discovery**	
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 support the discovery and investigation of new ways to treat serious human lung diseases such as COPD and asthma. We will use mice and rats in experimental studies to increase our understanding of novel mechanisms which either lead to disease or could be used to aid resolution or repair. As the cell types in the airway are very similar to those found in the skin, a small number of preliminary studies which could help us understand the potential role of these mechanisms in eczema and skin wound healing will also be carried out.	

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)?	• New understandings and insights into the mechanisms responsible for inflammation and tissue repair in lungs • New treatments for patients with diseases of the lung such as asthma and chronic obstructive pulmonary disease (COPD), which could bring life changing improvements to their quality of life. • There is potential for this research to also benefit patients with skin diseases such as eczema. • Benefits to society from the development of new treatments, reducing healthcare burden
What species and approximate numbers of animals do you expect to use over what period of time?	• Up to 4074 mice over 5 years • Up to 500 rats over 5 years • Most of our studies will be in mice as this species is the most thoroughly characterised, but some studies will need to be performed in rats due to differences in biology or differences in the response to treatment. • Most of these animals will be adults, although in some cases we may use aged animals (more than 12 months old) to help us understand the effects of aging on our mechanisms of interest.
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?	All animals will kept in a modern hygienic animal facility, with free access to clean water, food, bedding and nesting materials and other life enriching materials such as shelters. They will be checked at least twice per day and a vet is available to the animal care staff if they have any concerns. The staff handling the animals are highly trained which reduces the likelihood of procedures such as injection, blood sampling or restraint causing injury. Most of the studies we will conduct will involve inducing some sort of inflammation or challenge, so that we can study the biological consequences including inflammation and healing, and we would expect the animals to experience some discomfort and subsequent changes in their behaviour. We will use common techniques such as injections into or under the skin, or into a vein, muscle or the abdomen in order administer treatments or other substances. In some studies we may dose

	directly into the stomach via the mouth, or into the lungs using a special tool under anaesthesia. These ways of dosing animals may cause a very brief discomfort, for example injection into the skin would likely result in a brief sensation. We will always use the smallest needles we can for any injection and avoid sensitive areas. Pain relief medication will not be used routinely (except for surgery) as we need to understand the biological processes at play and these drugs can affect our ability to interpret experimental studies. None of the studies on this Licence will be in the Severe category. Some studies may be conducted at Moderate severity. For example, in some experiments we may inject a substance into the abdomen to activate inflammatory cells so that we can then kill the animal, collect the cells and use them for experiments outside the body. This is likely to cause abdominal discomfort but not acute pain. In other studies we will induce skin inflammation that is similar to human eczema and that can result in animals scratching the affected skin, or we may make a small wound in the skin (a biopsy sample) so we can look at how the skin tissue repairs. We will also perform studies that fall under the Mild category, for example we may inject normal healthy animals with a substance that will deplete specific immune cells. This is not expected to have any obvious effect on the animals' welfare. Blood samples may be taken to monitor the cells in blood. Where genetically altered animals are used (for example animals which have been given a copy of a human gene to replace a mouse gene, or have had a particular mouse gene removed ('knocked-out')) they will not be expected to have any outward signs of illness or reduced welfare, as we are targeting immunological mechanisms and we know from experience that these rarely have impact in unchallenged animals. We will in some cases need to understand how the immune and repair systems change with age, and old (greater than 12 months old) animals will be used. These animals will receive additional checks on their welfare and health and will be removed from the study if necessary. All animals in these experiments will be humanely killed at the end of the procedure to enable collection and scientific analysis of tissues, usually with a lethal dose of anaesthetic.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Much research is done using human tissues and cells in vitro prior to any in vivo studies, but there are limitations with these systems as the conditions under which they are used are often very different to the real conditions in a living animal. There are many researchers seeking alternatives to animal testing (see for example http://altweb.jhsph.edu/ and https://norecopa.no/). These approaches are improving and can replace specific well understood types of study. However for exploratory studies where the biology is not yet understood these models are not always adequate. For example, single cell types grown in isolation or even in mixed cell cultures do not behave in the same ways as cells in a complex living animal where they can move between places in the body to receive different signals at different times. So although there are more complex non-animal systems becoming available, understanding immune cell, nerve cell and structural cell responses in live animals is still very important in discovering new mechanisms and understanding new potential treatments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For many studies we will want to test a specific question and will need to understand if the result we have is likely to be a real or simply a result of chance, so mathematical methods (statistics) are used to analyse this. If we use too few animals the findings may not be valid, but we also do not want to use too many, so for our studies a statistical assessment is made of existing data to ensure that we optimise the number of animals used. This involves looking at how much variability there is in our data and predicting how many animals we would need to use to have a good level of certainty that the conclusion we have reached is correct. We have access to professional statisticians to guide us and advise on the most appropriate methods.
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	Mice and rats are mammals with well-studied immune and physiologcial systems which are closely related (but not identical to) the equivalent human systems. Because of this there are many established techniques and tools

the general measures you will take to minimise welfare costs (harms) to the animals.	we can use such as genetically engineered mice and mouse- and rat-specific cell labelling technologies. The models we will use are the simplest and involve the least amount of suffering while still addressing our need to understand the mechanisms behind many human inflammatory diseases. Most of the studies will be looking at inflammation, or tissue responses to inflammation, in a simple system such as in the abdomen or in an 'airpouch' which is a pocket of air introduced under the skin on an animals' back which we can use as a space to draw in immune cells. In some studies we may block or deplete some specific types of immune cells to see how that affects the inflammation so that we can understand the potential for a new treatment to work in human disease that involves that cell type. Other studies may look at responses in the skin or airways to help us undertand how particular cells respond to injury or stress, and how the tissues then repair to restore their normal function. All animals are closely monitored to ensure that they receive high quality care during these procedures. Although we expect many of our procedures to induce discomfort which would in a clinical setting be treated with pain killers (analgesics), we cannot use these in all situations as the purpose of the experiment is to induce the inflammation and study it, and these medicines will directly interfere with our ability to do this. We will use analgesia when performing surgical procedures including skin biopsy.

Project	**235. Meniscal Repair**	
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 helps to develop new treatments to repair the meniscus in damaged knees. The clinical need is for a reliable treatment of meniscal damage that can induce repair or regenerate a stable meniscus and prevent the progression to degenerative and painful joint disease.	
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 new treatments will help patients by relieving pain, restoring function and protecting undamaged joint structures. The new treatments will require less invasive surgery, permit shorter hospital stays, allow faster recovery and permit easier repair. Early intervention will also prevent/reduce	
	progression on to total joint replacement.	

What species and approximate numbers of animals do you expect to use over what period of time?	The estimated numbers are likely to be no more than 200 sheep over the course of the 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?	Whilst much of the early research work can be carried out in a laboratory or using a computer, once the prototypes/devices have been developed they have to be tested in an animal in order to find out how they behave in a real joint and how the surrounding bone, cartilage and meniscal tissues will respond to the treatments. Each study is peer-reviewed and approved by a group of experts and a layperson. This is to check that the study is absolutely necessary, to minimise the number of animals used and to further refine the protocol where possible. Every attempt is made to minimise the pain and trauma associated with using the treatments because pain-free joint repair is one of the key objectives of the project. The expected level of severity is moderate. The animals are humanely terminated at the end-points of each study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cell culture and other non-animal types of testing cannot fully replicate the loading, physiological and anatomical conditions required to demonstrate safety and efficacy of novel meniscal repair prototypes/devices, therefore animal studies are necessary in the development of new meniscal therapies. There will be extensive non-animal testing to select and help improve the prototypes but animal studies will be necessary at some point in the development of these devices to show safety and efficacy in comparison to appropriate controls and/or currently approved treatments as required by regulatory authorities. Sheep will be used under this licence to evaluate meniscal repair devices / therapies due to their joint size, which facilitates surgical procedures of this type, weight bearing and the acceptability of this model to regulatory authorities.

2. Reduction Explain how you will assure the use of minimum numbers of animals	There will be extensive non-animal testing to select and help improve the prototypes but animal studies will be necessary at some point in the development of these devices to show safety and efficacy. Consultation with a biostatistician and other experts at the planning stage will help to optimise study design, minimise the number of animals required, and meet the study objectives.
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.	Choice of Species After a thorough review, sheep have been chosen because of their relatively large joint size, weight-bearing nature and their similarities in anatomy and cell/tissue (histology) structures to humans. We have built up an extensive amount of expertise in using sheep for the evaluation of prototypes /devices on other project licences for cartilage and meniscal repair. Minimising suffering Animal suffering is minimised by: (a) consultation with people with expertise in orthopaedic surgery and animal welfare; (b) thorough laboratory testing and refinement of the techniques and equipment before any surgeries take place; (c) starting with pilot studies using small numbers of animals to monitor animal behaviour when a novel type of surgery or prototype is being tried for the first time. This is to ensure that it does not cause suffering before continuing to a larger study; (d) standard veterinary procedures are used to administer anaesthetics and pain-relief before, during and after surgery. (e) each animal is carefully and closely monitored throughout the study and health checked beforehand. After surgery, pain-relief is given until there is no further need.

Project	**236. Metabolic alterations of pregnancy**	
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)	Pregnancy is associated with a series of metabolic changes in the mother that are necessary to support the nutritional needs of the developing baby. These can have consequences for the health of the pregnant woman and her baby during pregnancy and in later life. In normal pregnancy, these changes include raised cholesterol levels as well as increased insulin resistance, a condition that usually leads to diabetes, and high blood levels of bile acids (chemicals made by the liver as a way to remove cholesterol from the body). In high-risk women, these changes cause metabolic disease of pregnancy. Metabolic	

	disease of pregnancy can cause increased rates of sickness and death of the pregnant woman and her baby. They also have implications for the subsequent health of the children of affected pregnancies. Moreover, metabolic changes in pregnancy may have important health consequences for women who do not have diseases of pregnancy *e.g.* women who have had a large number of pregnancies have an increased risk of developing heart disease in later life, and this is thought to be due to continuous exposure to raised levels of cholesterol. This work aims to elucidate the factors that drive gestational metabolic changes and how these factors can lead to metabolic disease of pregnancy. The impact on the embryo and children will be also determined. Additional experiments will enable evaluation of therapies that can be applied to prevent metabolic disease in offspring from affected pregnancies.
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 proposed research will impact the health of a wide spectrum of individuals. The results will be of relevance to women with metabolic pregnancy disorders, e.g. gestational diabetes, cholestasis and obesity. Children of affected pregnancies who are more susceptible to obesity and metabolic syndrome may benefit from this work. There will also be economic benefits to the NHS if this research identifies effective treatments to reduce metabolic disease of pregnancy and susceptibility of children and young adults to obesity and metabolic syndrome. This work will inform affected women of ways they can improve the subsequent health of their children. Pharmaceutical companies that invest in strategies to prevent obesity, diabetes and fatty liver will benefit from our proposed research. The work will also have an impact in the field of the developmental origins of health and disease, as we have developed new experiments to investigate factors of pregnancy that cause, and could treat, subsequent susceptibility of the children of affected pregnancies to metabolic syndrome.
What species and approximate numbers of animals do you expect	Over a period of 5 years, we estimate that a maximum of 3000 mice will be used.
to use over what period of time?	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The proposed research plan involves mating of animals and characterisation of the metabolic profile of the offspring through collection of organs after killing the animals in a humane way. Metabolic disease will be induced by administration of modified diets which are not expected to cause any harm or suffering to the animals. In the cases of more invasive methods, such as surgical procedures e.g. to remove reproductive organs or supply a compound or imaging, general anaesthetics will be used in combination with analgesics, painkillers and appropriate post-operative care to keep pain and suffering to a minimum. Surgery will be carried out using the same kind of aseptic techniques that are used to avoid infection in human operating theatres. Routine tests such as to assess glucose and insulin function are not expected to cause any pain and animals will be treated in a humane way in every occasion. No animal is expected to experience more than moderate severity and the majority will experience no more than mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not ethically or practically possible to obtain samples from the tissues of interest for this research from pregnant women and their children, therefore it is necessary to use mouse models. Due to the complexity of trying to understand signaling in pregnancy and disease, this cannot be fully replicated using cell culture or computer modelling methods.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will employ non-animal experimental tools as alternatives to the use of live animals wherever possible. For studies of metabolic alterations, we have an active human research programme to collect certain samples from pregnant women and the fetus where possible, such as blood, urine, faeces, placenta and amniotic fluid.Animal data will be correlated with population studies where possible. The proposed experimental designs and methods of analysis are always discussed with statisticians so that we can

	maximise the information obtained from the minimum resource. Also, more than one researchers share the same animals to address their questions. In this way, we aim to minimise the numbers of animals used for our 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.	Mice have similar mechanisms for regulating glucose, lipid and bile acid metabolism as humans, which will be studied in these experiments. Moreover, use of animals is a useful method to determine causes of disease as genetic and lifestyle influence, often referred to in population studies, can be eliminated. This will allow better evaluation of data and more solid conclusions to be drawn. Also, based on studies performed by the applicant and others, there is already a considerable amount of background information on the hormonal and metabolic parameters of mice that will facilitate experimental planning and validation of the results. Adverse effects are not expected from the protocols that will be used for these experiments, but in the unlikely event these do occur they will be humanely killed.

Project	**237. Metabolism, Pharmacokinetics and Biomarkers**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the work carried out under this Project Licence is to support the discovery of medicines for unmet medical needs. Specifically, experiments will be conducted to determine and understand the metabolism of novel therapeutic agents in laboratory animals and their concentrations within tissues of interest. This information is necessary to select optimal drug candidates for further evaluation and will be used to design *in vivo* studies to establish how well the agents work and their safety as potential medicines.	
What are the potential benefits	This Project will benefit humanity in general by	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	helping to accelerate the advancement of therapies to the clinic for unmet medical needs, particularly where work is being undertaken on the behalf of clients that lack the necessary facilities to perform in vivo testing. It is also anticipated that Biomarkers (natural substances) may be discovered that are relevant to disease progression in humans. This information will reduce the development time of new therapies and treatments that have the potential to reduce human suffering.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (non-genetically altered) 23250 Rat (non-genetically altered) 13250 The exact number of animals used will be dependent upon external factors such as the number of clients and the success of those clients in designing suitable drugs.
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 an attempt to reduce animal suffering, protocols have been designed to have the lowest severity limits possible. To improve animal welfare, the following considerations will be met; where possible animals will be group housed with extensive use of environmental enrichment, surgical techniques will only be used where there are no alternatives and if used, appropriate analgesics will be given and all protocols will be assigned humane endpoints. Most of the animals used on this Project will experience a single injection or dose followed by a series of blood samples, typically over a 24 hour period via a temporary cannula or directly via venepuncture of the tail vein or alternative accessible vein. If tissues are required, then animals will be terminally anaesthetised or schedule 1 killed and tissues and/or blood will be taken. Minor, transitory discomfort is anticipated upon injection/dosing. Adverse effects due to the test substance are anticipated to be rare. Any animals exhibiting continued distress (after the transitory discomfort of dosing), will be killed by a Schedule 1 method. A small percentage of animals used may receive test substances by more than one route but this figure is anticipated to be very low. A small percentage of animals used may be surgically cannulated but this figure is anticipated to be very low, depending on client requirements. All surgical interventions will be performed under general anaesthesia. Systemic antibiotics may be given prior to, during or after surgery on veterinary advice. Pre and post-

	operative analgesics will be used as advised by a veterinary surgeon. Animals will be allowed to recover from surgery for 48h prior to use. Once cannulated, the animals will typically receive 1 dose of test substance which may involve the same degree of discomfort as previously described and blood samples will usually be collected over a 24 hour period, ending with Schedule 1 killing. The remaining animals will have terminal procedures performed under general anaesthetic. Protocols may end in a Home Office approved, Schedule 1 method of killing. Alternatively protocols may end in a non-Schedule 1 method such as deep anaesthesia,(AC) for blood sampling via cardiac puncture/CSF sampling/ body fluid withdrawal, perfusion or tissue donation followed by confirmation of death.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In silico and *in vitro* approaches are becoming increasingly powerful tools in the design of drug molecules with optimal properties and are being used extensively throughout Industry and Academia. These approaches can help in understanding various isolated aspects of a molecule's suitability as a medicine and these methods are used to screen out compounds that clearly do not have the desired properties. However, because the suitability of a molecule as a medicine depends on many factors, not just those that can be studied in isolated *in vitro* experiments, *in vivo* studies need to be conducted to determine the actual properties of a new molecule in the body. Although not identical, small mammals (e.g. mice and rats), have a similar enough physiology to Man to be able to use these to characterise a potential drug's ADME (Absorption, Distribution, Metabolism, Excretion) properties. Species selected for this Project represent the lowest form of vertebrates in which these types of studies can be conducted and are species that will be used for later pharmacological and safety evaluation of drug candidates. *In vivo* studies are required because the multiple processes involved in determining how well a drug is absorbed, where it goes to, how it is metabolised and how it is excreted are difficult to replicate in

	the *in vitro* situation. The use of *in vitro* screens prior to in life studies will minimise the numbers of animals used. Also, compounds going into further development will have appropriate pharmacokinetic profiles in those species that are likely to be used in safety assessment studies. This will minimise the use of animals in the later stages of drug development projects by identifying unsuitable compounds at an early stage. Other efforts to reduce animal use include combining PK and efficacy studies and the use of PK samples to investigate markers of disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The PK for a particular compound in a given animal species is fairly reproducible and given that drug or metabolite concentrations are measured rather than efficacy or a safety effect recorded, the number of animals used for a PK study is relatively low (typically 3 per dose). Experience has shown that plasma exposure data obtained following intravenous dosing in rats is very reproducible and so where appropriate and particularly when in vitro screens are not predictive of metabolic stability, i.v. (n=3) studies will be performed at an early stage to investigate in vivo metabolic stability. Data obtained from these experiments could negate the need for further work on a compound or a particular structural series of compounds and hence reduce potential animal usage. Reduction has been a focus of attention throughout the protocols of this licence; approaches regularly used include the use of software to simulate multiple dosing PK, rather than repeat dosing animals. Cassette dosing strategies where applicable can provide multiple compound evaluations in one animal and increased assay sensitivity has led to serial sampling in mice where full PK profiles are generated in single animals rather than the multi animal approach utilising 3 animals per time point.
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	Species to be used in this project will be determined by the pharmacology of the compound in question. For pharmacological evaluation it is necessary to use a species whose relevant receptors/biochemical processes give a good model for those in Man. The species used for PK modelling such as mouse

costs (harms) to the animals.	and rat will be appropriate for the treatment area or where specific features of disease are being investigated. In order to improve general animal welfare and the scientific integrity of experiments, where possible the following considerations will be met : Animals will be group housed where possible. Group sizes will not exceed ASPA stocking densities. All animals will receive environmental enrichment including a selection of nesting material and refuges/hiding places. All animals will undergo acclimatisation before use.

Project	**238. Mice with transgenic immunoglobulin loci**	
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)	REDACTED are currently getting sick from the same conditions that we do, and that we can successfully treat in people with antibody medicines. The problem is that these medicines are species-specific REDACTED. This is because antibody drugs are proteins and so the REDACTED immune system would recognise the human drug as foreign and get rid of it, stopping it from working. We are developing a way to make sure these diseases can also be treated in REDACTED. We will use the same cutting-edge science used to make human therapeutics, but are building a version that can do it for REDACTED. We will	

	mainly conduct work that will benefit REDACTED, but could potentially begin work to benefit other species, for example horse. It is unlikely that we would conduct mouse work related to more than three REDACTED species over the five years of the license. The best way to make antibody medicines is using mice that express the antibody genes of the species you want to treat (which to date has always been human). Our main objective is to generate mice that can express the antibody genes of REDACTED animal species REDACTED. Once we have done this, we will immunise the mice (a process that is similar to a human having a vaccination) with the proteins or other substances that we want to make antibodies against. We will then test these antibodies to see if they can be used as medicines. Most of this work will not involve mice, but once we've narrowed down some antibodies that have good properties based on our experiments, we will then want to test them in a mouse model of the REDACTED disease. This will allow us to see if they work like they are supposed to, and successful ones will be taken into clinical trials in REDACTED (work that will not be carried out here or in any way related to this license, but which will be carried out by experienced professionals at licensed establishments). In summary: Aim 1 – Make mice that express REDACTED antibodies Aim 2 – Immunise these mice to make antibodies Aim 3 – Test these antibodies in mouse models
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 range of veterinary medicines available is much smaller and less advanced than for human medicine, and so REDACTED are often being treated with drugs that we no longer really use in people, simply because there isn't a better alternative. For example, we treat lymphoma, a type of blood cancer that's very common in REDACTED, with the same chemotherapy we've been using in people for a very long time. The

	thing is, the REDACTED don't understand why they are suddenly experiencing all of the side effects of chemotherapy and it can be very distressing for them REDACTED. As a result, they tend to be given a lower relative dose than people are, to reduce the side effects, but this also means it doesn't work as well. Lymphoma is just one of many diseases for which we have highly effective human antibody medicines that mean that people can have treatments that work, without the awful side effects of previous therapies. It is our hope that the antibodies produced in, and later tested on, mice that come under this license can be used to treat REDACTED diseases in the future and to deliver the same benefit that we have seen in people. Given how little innovation there is in veterinary medicine (compared to human medicine), this new class of drugs could have an even bigger impact on REDACTED health than it did on human health. And because we have thirty years of experience in making human therapeutics to work from, we have a very good idea of what will and will not work, and so we can make sure that we can deliver the maximum benefit with the fewest mice used. Part of the lack of innovation in veterinary medicine is because less fundamental research has been done in these areas. Therefore, as part of our project, we will need to conduct work ourselves, as well as with external collaborators, to learn more about how these diseases work and how best to treat them. This work will be published and made available to the rest of the field, so that we can all benefit from the greater level of understanding of REDACTED disease and its treatment. In the long term, it is our aim to move from just learning from human antibody medicines and to use these mice to help us learn what are good drug targets. We can then use this information to help improve drug development for people by giving more accurate information on what goes wrong and how we can treat it. So this project will go from REDACTED being benefited from what we know from human medicine, to being able to help the other way around, overall making the most of the advantages of each area. In summary: Short-term benefit – New veterinary medicines based on what we know from human health Mid-term benefit – Increased understanding of REDACTED disease Long-term benefit – Potentially this work could be
	used to gain insights that benefit human health.

What species and approximate numbers of animals do you expect to use over what period of time?	Members of our team have carried out, over the last ten years, almost exactly the same process to make mice that have human antibody genes. We therefore know exactly how many mice were needed for that project. Whilst the species REDACTED is different, the science is the same. We are therefore able to confidently predict, based on these past numbers and statistical predictions based on normal inheritance patterns, how many mice this project needs. This project will use mice, and we estimate that the project will need 85,250 mice in total over the five years of the project. All experimental work will be carried out on 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?	We are going to edit parts of the mouse genome in some embryonic stem cells, and then put these cells into a 3.5 day old mouse embryo. All of the work up to this point is not regulated as it doesn't work on sentient animals, only cells from them. We then take that embryo into a female mouse that will give birth to mice that contain our edited DNA. This is a female that has been mated to a male that has had a vasectomy (which is an equivalent process for mice and people), undergoes a simple procedure under anaesthetic, and then is pregnant in a normal fashion. As such, it is a very mild, and largely natural process. The changes we make to the DNA are not expected to have any effect on the mice, and so they will be healthy and normal. This project involves changing the DNA at multiple sites in the genome, and so we will need to take mice that have each of these changes and breed them together so that we end up with a mouse line that has all of the relevant REDACTED in the same mouse. These mice can then be used to make antibodies. To do this we will inject them with what we want the antibody to be effective against, such as a cancer protein, and this should only cause temporary discomfort for the animal – it's just like you or I getting a vaccination. These mice will then be anaesthetised and humanely killed – at which point we will be able to collect their blood and spleens, and the antibodies in them. When it comes to testing the antibodies in mouse models, what they experience will depend on what the disease is. We only want to use very well-

	established models, and so our work will be routine for this area. For example, we may use a well known mouse cancer model, and try to treat the cancer in the mouse with our antibodies. This will carry the accepted thresholds for the mice with the cancer or other disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune system is very complicated, and you need all of the parts of it working together in a healthy animal in order to make good antibodies. Over the past thirty years, hundreds of millions of pounds have been spent on trying to make a system that does not need live animal models to make antibodies for human medicine but none of them works well enough. Hence we are using live animals to make these veterinary medicines. Simply put, there is no non-protected animal alternative that works well enough.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The main area of reduction is by maximising the amount of work that is carried out in cells so that we don't need to use mice. This is both in the stem cells before we make the mice, and in the experiments, we will run to test the antibodies, before using them in mouse models of disease. The stem cells we use will be extensively pre-screened to make sure we only use the ones most likely to work. We will also try to do as many of the genetic modifications in cells, rather than mice, as possible in order to reduce the amount of breeding steps (and therefore animals needed). In the stage where we make antibodies, we will want to knock out the target protein in these mice. We will check available databases to see if someone else has done this before, and if so what happened, as we will only undertake this work if the mouse produced is healthy enough to be used. In other words, we won't just make the model and see what happens, we will reduce numbers by not making bad models in the first place. Then when it comes to the antibody testing, we will carry out a wide selection of non-regulated experiments in order to be sure that we only go

	into mouse models if we are really confident the antibody could work. We are also going to use the most up to date immunisation methods in order to use the fewest number of mice for this step. Finally, given that we only wish to use established disease models, and therefore shouldn't need to run pilot trials – reducing mouse numbers, there will be a lot of data available about how the model works, and so we can carefully calculate the statistically significant number of mice we need to see if the antibody works. The team's experience should also help, as it means we know what mouse experiments are actually required, and overall ways to be as efficient as possible, reducing mouse 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.	**Choice of the mouse** The mouse has been the first choice means of producing therapeutic antibodies for thirty years. Using it for this project means we can benefit from that knowledge and experience, increase the probability of success, and not require extensive work (including the use of many experimental animals) characterising an alternative mammalian model system. The mouse is also a very well characterised model for the diseases we wish to treat, and so we can be confident that the data will be relevant and that we are using the right model and number of animals. **Minimising suffering** For this project to work, the REDACTED genes we are putting in have to work in the same way as the mouse ones we are taking out. These mice will therefore be healthy and not different from normal. The other changes we might need to make for the project will also only be useful if the mice are healthy. **Group housing** Animals will be kept in socially compatible groups. **Enrichment** Mice will be kept in suitable environment as specified by the Code of Practice for housing and care of animals bred, supplied or used for

	scientific purposes. We use a sophisticated animal tracking system to ensure welfare data on all animals can be readily accessed/analysed.

Project	**239. Microglial targets in brain pathology and ageing**	
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)	Nerve cells (neurons) and their connections (synapses) can be 'eaten' by another type of brain cell, called microglia. The aims of this project are: (1) to find out whether the loss of nerve cells and their connections during ageing and brain diseases, such as Alzheimer's disease, is due to microglia eating these synapses and neurons, and (2) to find drugs or potential treatments that prevent loss of brain nerve cells and their connections by blocking microglia from eating them.	
What are the potential benefits likely to derive from this project (how	Alzheimer's disease is now the most common cause of death in the UK, and there are no	

science could be advanced or humans or animals could benefit from the project)?	treatments that affect progression of the disease, so that if we found a treatment, it would be beneficial. Reduced ability to learn and remember is very common with age, and there is no known treatment, so if we found a treatment, it would be beneficial. By blocking the microglial cells from eating nerve cells and their connections during brain ageing or Alzheimer's disease, we may be able to prevent brain ageing and Alzheimer's disease. We have found that this is true in: brain cell culture and some simple mouse models of Alzheimer's disease and ageing. In this project we aim to: i) test whether blocking the eating of neurons is beneficial in animal models of brain disease, and ii) test how best to block this eating in order to protect the brain from ageing and disease. The animal models of brain disease used in this project will be more realistic than those previously used because , for example, we will be using the human disease-causing proteins expressed in the mice.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats 1100 Mice 2440 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 mice and rats will be killed within a few days of birth, causing transient pain. Brains from these animals will be used to isolate brain cells, which we will use to investigate how to prevent microglia eating nerve cells. Some experiments will involve injecting into mouse brains substances that cause loss of nerve cells, so that we can investigate how this happens and how we can prevent it. The mice will be anaesthetised when we do this, but when regaining consciousness or as a result of the substances injected they may feel mild or moderate signs such as humans have during a cold or flu, including clinical signs such as fever, disrupted sleep, reduced activity and loss of appetite. A mouse model of Alzheimer's disease will cause impaired memory and wobbly gait, but we will use a welfare-monitoring system to make sure that mice do not develop marked disability/disease before being humanely killed. All animals will be humanely killed at the end of the experiments,

	and tissues from the animals will subsequently be analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have already done extensive experiments that do not use live animals. However, it is now essential to determine whether the eating of nerve cells by microglia occurs in realistic animal models of human disease, and how we can prevent this to protect the brain. We have previously used some animal disease models in which blocking the eating of nerve cells by microglia was strongly protective, but these models have limited application to human disease, and only have relevance to some diseases but not others. We now need to use more realistic animal models of human disease in order to test whether we can protect the brain, otherwise we will not be able to progress towards developing treatments for human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The lowest numbers of animals will be used consistent with obtaining a reasonable estimate of experimental variability to test for meaningful results between treatment groups. Previous results indicate that about 8 animals per treatment group is the minimum required to achieve statistical significance. We will only test drugs in animals, when we know they work with isolated brain cells. All the experiments will have appropriate controls, and will be designed to reduce variation and bias, and increase the reproducibility of the results obtained.
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 only be using rats and mice for this research (the lowest vertebrates for which there are established models of brain disease relevant to human disease). We will be using the minimum numbers of animals compatible with obtaining statistically significant results. And we will be using disease models with the minimum suffering compatible with relevance to human brain disease. When using models of Alzheimer's disease, we will use a welfare-monitoring system to make sure that mice do not develop marked disability/disease,

	and that any suffering is minimised by pain relief. Animals that have been transported (which can be stressful) will be acclimatised (destressed) for at least three days before being used for experiments. The housing environment of the mice and rats will be enriched with bedding, play, nesting and gnawing materials.

Project	**240. REDACTED behaviour of 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)		Basic research
		Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To increase knowledge and understanding of the REDACTED behaviour REDACTED of REDACTED fish so as to provide evidence and advice REDACTED	
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)?	Basic information on e.g. fish REDACTED, behaviour, REDACTED is lacking for many REDACTED species REDACTED. REDACTED REDACTED In addition, the integration of fish behaviour with environmental data will contribute to improving the 'ecosystem approach to REDACTED management', REDACTED. REDACTED	
What species and approximate	Adult, maturing (sub-adult) and juvenile fish. Up	

numbers of animals do you expect to use over what period of time?	to 1600 animals would be used over the 5-year period of the work.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The REDACTED procedures we propose to undertake are assessed as 'Mild' or 'Moderate' severity, while the biological sampling protocols are assessed as 'Mild'. Where appropriate, analgesia will be applied to the REDACTED site to reduce likely pain. The most likely adverse effect in the medium-term is deterioration of REDACTED wounds, which may lead to scarring and/ or infection. The risk of deterioration will be minimised by using established and researched REDACTED attachment or implantation techniques. The risk of infection will be minimised by using aseptic techniques during the procedures. Most fish will be used in REDACTED studies REDACTED
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of the project is to increase knowledge and understanding of the REDACTED behaviour of REDACTED fish REDACTED. The knowledge being pursued does not exist and is typically species-specific. It is therefore not possible to find a non-protected animal alternative.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental methods and numbers of animals used are based on previous experience and research. As part of the REDACTED Animal Welfare and Ethical Review Process, each programme of study is considered by staff from our in-house statistical team and their sign-off is required before any study is undertaken. REDACTED. Opportunities to reduce the number of animals used will be assessed throughout 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	The aim of the work is to improve knowledge of the REDACTED behaviour of REDACTED fish REDACTED in relation to their environment. A range of species REDACTED needs to be studied. The methods we have proposed are based both on direct experience and development of fish REDACTED techniques

welfare costs (harms) to the animals.	developed over 20 years, as well as on peer-review research that has been shown to provide robust evidence on fish REDACTED, behaviour, population structure and life-history. The identified procedures have therefore been refined over many years of practice within a culture of continuous improvement. When fish undergo a procedure with recovery, appropriate anaesthesia and analgesia will be used to minimise welfare costs. Individuals will be monitored for a suitable time following procedure(s) to assess any adverse effects. All fish that are caught for the purposes of the proposed project will be assessed for their fitness REDACTED and those not fit will be treated in accordance with Home Office guidelines. Advice on refinement of techniques will be sought and taken from the Named Veterinary Surgeon and the Named Animal Care and Welfare Officer throughout the project as appropriate.

Project	**241. Minimally Invasive Cloaca Repair Operation (MICRO): Development of a Novel Anisotropic Tissue Expander for Non-Keratinised Epithelial Tissue**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 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)	Cloaca is one of a group of severe, nonhereditary anorectal malformations affecting female babies. It is characterised by confluence of the urethra, vagina and rectum into a single common channel. The current standard repair involves many surgeries, is highly invasive and often leads to tissue necrosis after surgical damage. This project licence aims to use a minimally invasive technique in a pre-clinical	

	(porcine) model to establish efficacy of using an intra-luminally implanted hydrogel to expand the vaginal epithelium of a young piglet to allow for – in humans – easier surgical reconstruction. The project has two clear aims and objectives: 1) to characterise the extent of new tissue formed after 10-14 days vaginal tissue expansion in young piglets and 2) to repeat the experiment but in older post-pubescent pigs, to establish efficacy or not at older ages when tissue has differentiated to a greater extent. Tissue obtained post-mortem, not possible in human neonates, will be characterised in terms of new cell formation (as oppose to stretching existing cells), tissue viability (e.g. blood and nerve supply, is not necrotic), epithelial microbiome, is non-mutagenic and is hormone sensitive. We also wish to check that newly formed tissue does not simply contract back after removal of the intraluminal device.
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)?	We will develop a functional hydrogel that is ready for first-in-man implantation after all the necessary experimental trials. An alternative method for surgical repair of cloaca malformations will be established, this will be less invasive, better tolerated by patients and should provide patients with functional innervated tissue. All data collected will be made available to other clinicians, surgeons and scientists through publication in peer-reviewed journals and presentations at scientific conferences and meetings. We will characterise the effect of tissue expansion on the vaginal microbiome – this has not been done in any other animal model or clinical study to date. In collaboration with clinical colleagues we aim to inform (and change) clinical practice in regard to surgical soft-tissue repair operations. We will build upon this work to develop new hydrogels for other applications, to move the work into clinical trials in patients which will have a significant impact on those patients' lives.
What species and approximate numbers of animals do you expect to use over what period of time?	We do not expect to use more than six pigs per experiment. Pilot studies have used n=3 pigs per time point and variation in study end-points is minimal between pigs. Full project proposal may require approximately double these

	numbers of pigs for each study, plus further pigs kept for a longer period of time. Pilots studies 1+2 = 24 pigs. Approximate number of pigs over lifetime of licence, 3yrs = 42-48 pigs.
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?	Pigs will undergo surgery that requires the insertion of a small, solid cylindrical hydrogel (around 40mm long and 8mm wide), using endoscopic guidance, into the vagina. A natural stricture present at the interface of urethra and vagina (hymen) prevents backflow of urine into the reproductive tract. The cervix forms another natural stricture. With the device inserted and a purse-string suture in the hymen, there will no backflow of urine as per normal and the expander will be secure within the vaginal cavity. The pigs will receive appropriate sedation and/or anaesthetic in order to insert and remove devices. Post expansion, animals will be killed humanely and their tissue taken for analysis after death.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This is a surgical research project in which the medical device to be developed is to be used in humans. Therefore pre-clinical testing must take place using relevant animal models. Testing must be conducted in vivo to allow for possible interactions with live body systems. The use of less sentient animals has been considered thoroughly. The majority are inappropriate as they lack functional urogenital tissue (flies, worms, fish, mice, rats, sheep). The project will characterise the effect of tissue expansion on anatomy, histology, physiology and microbiology of the urogenital sinus and vagina of pigs. Such end-points cannot be achieved using in vitro models. It is important we characterise new blood vessels formation to support the growth of new cells. We must also characterise whether new tissue has become hypoxic or necrotic as these outcomes are clearly unacceptable clinically. An objective of this project is the analysis of histology and physiology of expanded tissue.

	For this to take place, samples must be obtained from a live animal in order to be able to investigate the immunological response to the implants as well the properties of the neotissue that has grown.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have conducted extensive characterisation of the suitability of the pig for the current project using schedule 1 cull of piglets, including animals used for other projects. This has informed our decisions on the number of animals to be used in a pilot study design. We have considered anatomy and histology of the urogenital sinus and vagina, transcriptomics and meta-genomics to come to this decision. For a pilot in vivo study, we consider n=3 piglets per group to be satisfactory for confidence in the general applicability of our results to a wider population of pigs.
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 human and porcine female genital tract compares well as adults. Our extensive pre-clinical characterisation has confirmed these observations. The anatomical and morphological construction and proportion of mucosal layers together with oestrus cycle alterations are similar between humans and pigs. The natural urogenital anatomy of the pig includes a urogenital sinus, which is similar to cloacal malformation presentation. Rodents are an inappropriate model for the project as the murine vagina has a keratinised squamous epithelium during estrus, whereas the porcine and human epithelium do not keratinise at any point in their reproductive cycle. In mammals, the majority do not develop a functional urogenital sinus of a size that is reminiscent and similar to human (excludes rodents, rabbits, guinea-pig, sheep, and horse). In the rabbit, the vagina connects externally via a long urogenital sinus, but histologically it is dissimilar to human, with a single cell, ciliated epithelium. We have considerably refined our surgical approach as a result of our schedule 1 characterisation to date. Equally, due to the nature of an anisotropic expander (i.e. only

	increasing in length rather than length and girth) we expect far less pressure on the ureter and thus no impedance of urine flow.

Project	**242. Modelling and treating primary ciliopathies**	
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)	Cilia are antenna-like structures that most cells possess. Some cilia, called motile cilia, can actively move, whilst others, called primary cilia are used as communication centres for the cell. Primary cilia in the kidney are important for the orientation of the cell and for sensing the external environment of the cell. If the cilia within the kidney are abnormal this invariably leads to kidney disease, most often cystic kidney disease. Because cilia are found in most parts of the body, diseases affecting the cilia can affect many organs. These diseases are known as ciliopathies and affect up to 1 in 2000 people worldwide. These conditions typically lead to kidney failure which may be within the first 30 years of life, but may also	

	cause problems such as visual loss, liver fibrosis, skeletal abnormalities and brain disorders. The outcomes from these conditions are variable, but they are a serious and life shortening set of disorders. The project investigates how underlying genetic defects of the primary cilia lead to genetic and developmental diseases affecting the kidney and other organs and how this can be affected by other inherited and environmental factors. To do this we will use zebrafish and zebrafish embryos which have had genetic changes induced by using genetic editing and engineering techniques to introduce changes within the genes of interest. This is done by using specially designed RNA molecules to edit the DNA of the genes of interest.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By finding out which genes cause these ciliopathies and the way in which they work, we can understand the biological mechanisms and pathways involved. This will help in finding ways to treat, prevent and diagnose these conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use approximately 42500 zebrafish over a five year period. The zebrafish is a tropical minnow that has a simple kidney that develops in a similar way to the human one. The use of animals can be divided into two parts: (i) Most studies are performed on very young embryos (less than five days old) which are not protected animals and are therefore not counted. (ii) Much fewer studies are performed on adult fish but adult fish are also required to breed the embryos used in (i). Adult zebrafish lay hundreds of eggs each week. To ensure healthy adult stocks we select some embryos from several pairs to grow and then further select healthy male and female fish to produce the next generation. Selection takes place over the first few weeks of life. As all embryos greater than 5 days of age have to be counted as they are protected animals, we record use of about 40,000 embryos to produce and maintain several thousand adult fish over a 5 year period.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the fish we will use have genetic changes induced that we can study. The adults are mostly completely healthy, or have minimal features of any genetic disturbance, but by interbreeding these adults, young embryos with genetic abnormities can be produced and studied. The genetic changes in the adult fish are expected to cause no symptoms of disease or discomfort and are categorized as sub-threshold effects. Where fin clipping is required, in order to identify fish carrying a specific genetic change, the procedure may cause some short term discomfort, categorized as a mild effect. We will observe genetically modified adult fish to ensure they do not become unwell as they become older. If fish become unwell they will be humanly killed. Unused embryos beyond five days old will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	These inherited problems of the cilia involve many different cell types and how they interact to form organs such as the kidney. There are currently only very limited cell culture or theoretical modelling techniques that enable study of these processes such that these interactions cannot be fully studied without using animals and these zebrafish are the simplest animals that we can use. By using zebrafish, and in the main their embryos at very early stages (which are less than 5 days old and are not protected), we can avoid using other animals such as mice. Furthermore, we have pioneered the culture of kidney cells from patients' urine and use these cells to complement our animal studies, thereby reducing the numbers of animals needed for our research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Most of the experiments we want to do can be done using embryos at very early stages, (less than 5 days old and are unprotected), as their kidneys are forming. By carefully planning our experiments and using the latest methods, for example the most modern microscopes and computer based analyses, we can reduce the

	numbers of animals used in each study compared to historical methods by >50%. The appropriate statistical analysis has been performed to identify the optimum numbers of animals to be used in order to obtain meaningful results. We will store sperm from genetically modified male animals, so that we can maintain the specific zebrafish lines without having to keep breeding animals.
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.	Zebrafish have kidneys that are similar to human ones and develop in a similar way. Most of the features of these kidney ciliopathy diseases will be present in the early embryos. We will carefully observe adult genetically modified fish in case they are affected by their genetic changes. Where possible we will kill adult fish after they have produced offspring and before they exhibit any possible ill health. A small fraction of adult fish will be used to maintain stocks. No fish exhibiting ill health will be maintained, they will be humanely killed.

Project	**243. Modelling and treating scarring and fibrosis 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)	Wound healing provides mammals with a very efficient mechanism to repair damaged tissues following injury. Tissue damage that can arise from trauma, infection or diseases such as autoimmune disorders initially causes inflammation. Inflammation activates the resident connective tissue cells to make scar tissue and heal the injury. Once healing has taken place the scarring process stops. These processes need to be very carefully controlled as chronic or prolonged inflammation can lead to excessive wound healing promoting pathological scarring. Persistent and extreme scarring results in tissue fibrosis where the normal tissue/organ architecture is gradually replaced with scar tissue. Ultimately the affected tissues and	

	organs fail. The processes governing wound healing and why these continue and fail to terminate causing fibrosis are not fully understood. The objectives of this project are to uncover the role of fibroblasts in fibrosis and determine why and how inflammation drives scarring and fibrosis and discover new ways to modulate inflammation and scarring to prevent fibrosis.
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)?	Excessive scarring and fibrosis are major health concerns for animals and humans. Fibrosis is often associated with many rare as well as common diseases and can affect almost any body tissue and organ. Once fibrosis starts, it is a progressive process that has proved very difficult to halt. This project aims to better understand the molecular pathways processes in the inflammatory response and subsequent scarring that promote connective tissue remodelling and fibrosis. We wish to reveal the important cell types that make scar tissue and the key molecules and pathways involved. By investigating these pathological mechanism(s) of fibrosis and testing the impact of novel therapeutic approaches to attenuate scarring and fibrosis, we aim to identify and develop effective treatment for patients with fibrotic diseases, which are currently very difficult to treat.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rabbits provide a unique system to understand connective tissue repair and formation and explore key biological pathways especially as there are close similarities between human and mouse biology and mice have been characterised genetically and unique genetically modified strains are available. Between 100-300 mice will be used in each of the major protocols for this project license although numbers will be minimised to that required for robust and reliable interpretation and analysis. Up to 300 mice per year will be used in breeding of genetically altered for these experimental protocols, therefore we expect to use a total of ~1500 animals over 5 years. Up to 150 rabbits will be used to assess the benefit of therapeutic substances in skin scarring and fibrosis.
In the context of what you propose to do to the animals, what are the expected adverse	Mice and rabbits will be used to provide samples for analysis of connective tissue in growth and development, including novel genetically modified

effects and the likely/expected level of severity? What will happen to the animals at the end?	animals. Experiments to assess response to tissue injury (e.g. skin biopsy), lung injury or therapeutic substances will be examined. In mice and rabbits skin irritation can occur in response to shaving and treatment with bleomycin to induce dermal fibrosis. In mice bleomycin treatment in the lungs to induce pulmonary fibrosis can result in some weight loss, this will be carefully monitored. This is classified as moderate severity. At the end of experiments animals will be humanely killed by a schedule 1 procedure and the levels of inflammation, scarring and fibrosis of tissues will be examined by biochemical and immunostaining/histological analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have developed and will continue to make extensive use of in vitro models which allow us to study the effects on of inflammatory cytokines or growth factors, on biological functions such as cell proliferation and differentiation. We believe that two areas of study in vitro which we are employing have the potential to reveal new avenues for replacement. These are the use of complex co-culture systems where we can model in vitro more closely how certain cell types interact within whole tissues and secondly the use of single cell studies. Here single cells from whole tissues are separated and isolated and their individual characteristics examined. By investigating the abundance of the various cell types within the whole tissue and their features it is possible to determine their relationship, how they interact and what happens as they change in disease. Findings from these in vitro models have helped and will continue to assist us in identify mechanisms that may be important in the disease mechanisms. Nevertheless, the in vitro models only replicate some aspects of the disease. The much greater complexity of the healing process in vivo requires the use of an intact animal to validate in vitro findings and test novel therapies. Therefore animals, and specifically mice, provide the only way of studying connective tissue repair, scarring and fibrosis in vivo that permits detailed exploration of key pathways and mechanisms and cell types involved. The use of rabbits is essential as the biological therapeutic antibody being used

	in the study has limited species cross-reactivity restricted to human and rabbit proteins only. The models used within the project protocols are moderate, induce inflammation and repair where suffering is kept to a minimum. The models are designed to allow detailed investigation of healing and fibrosis and provide important insights that can be developed further into new treatments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The scientific objectives of the project are very clear and the aims have been defined in order to gather robust, informative and definitive data. We have carefully designed the experimental approaches and harnessed statistical expertise to ensure that we have reduced the number of animal involved to the minimum required to maintain statistical power. The protocols have been refined, are reliable and designed to reduce severity where ever possible. By using mice and rabbits for development of parallel cell and tissue based experiments and through careful experimental design to minimise variability the number of animals used will be reduced to the minimum essential to provide reliable experimental results. Good cataloguing of animal samples and storage will maximise the use and future value 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) to the animals.	Studies will be carried out mainly in mice and a small number of studies using rabbits. Mice provide a unique system because strains that reflect human diseases or have key targeted alterations in relevant biological pathways exists and can be used to test specific scientific questions. All procedures will be undertaken within a well-managed and regulated animal facility by suitably optimally-trained staff and there will be close and careful monitoring so that welfare cost are minimised. Rabbits provide the only animal species with which to explore the anti-scarring activity of the therapeutic antibody under study due to restricted cross-reactivity of the antibody which is only reactive towards human and rabbit protein and tissues. Work from our previous studies and the development of new genetically modified animals will enable us to further refine our understanding of the pathological mechanism(s) underlying scarring and fibrosis and make major advances towards treatment.

Project	**244. Modelling cardiovascular diseases, tissue repair and heart 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
		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 main aim of this project is to learn more about the cellular response to tissue damage in the skin and heart, with a particular focus on white blood cells since there is substantial evidence that this immune response has a profound influence on the outcome of both the healing process and contributes to complete tissue regeneration in organisms such as zebrafish. We want to know precisely how immune cells interact with other cells in their environment and how they influence scar deposition and scar resolution. We also want to study how cells in the heart communicate with	

	one another after injury, test new genes that might be involved in tissue repair and regeneration in the skin and heart and to create new, much needed, models of cardiac arrhythmias.
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)?	We envisage that our programme of work for the next 5 years will reveal much about the mechanisms underpinning normal tissue repair in the skin and heart and also reveal more about regeneration, particularly the resolution of scarring. We hope to uncover the key genes and signalling pathways, particularly those associated with the inflammatory response to injury, which could be modulated in order to promote scar-free healing and tissue regeneration in the clinic in the future. We also hope to generate zebrafish models of human cardiac arrhythmias which can result in sudden cardiac death, allowing more studies into what goes wrong in the cells of the patients and allowing us to screen existing drug libraries to potentially identify new therapeutics to prevent or manage arrhythmias.
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish – 14,000 (10,000 for breeding of existing lines, 4,000 for injury experiments and to generate new lines - 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 our fish numbers relate purely to breeding via natural spawning to maintain each of our different lines and to provide larval fish for experiments. We do not expect any adverse effects from this. Some fish will undergo surgical procedures under general anaesthesia to generate tissue injuries to their heart, skin or fins so that the repair process can be studied. These injuries mimic the damage patients experience during a heart attack, surgery or trauma but are generally tolerated well by the fish. Occasionally, some fish do not recover from the anaesthetic applied during the procedure (approximately 5%). The majority that do recover from anaesthesia typically swim slowly for approximately 10-15 minutes before recovering completely and show no adverse effects. If any fish ever show any adverse effects (e.g. abnormal swimming behaviour) that indicate pain they will be humanly killed immediately. All fish undergoing procedures will be killed humanly at the end of the study in
	order to obtain tissue for analysis.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, cell culture studies in a dish can not as yet replicate the complex interactions that occur in a whole organism. Many aspects of tissue repair and regeneration cannot be accurately studied without understanding the complex coordination and communication of lots of different cell types. However, cell culture studies will be used to address certain aspects of our work e.g. when we want to determine “messages” being sent between just two different cell types or when we want to understand what goes wrong in single heart muscle cells when they have mutations that cause cardiac arrhythmias, and we will use these whenever possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all of our tissue repair and regeneration studies we use the minimum number of animals possible to provide rigorous, statistically significant data, based on previous work and power calculations performed with specific software packages. We consult regularly with REDACTED statisticians about appropriate animal numbers for our studies and we consult with colleagues REDACTED doing similar experiments. Results will be monitored as experiments are undertaken to determine whether subsequent experiments could use fewer animals if possible. Additionally, we extract multiple different tissues (e.g. skin, fins and heart) from every fish after termination so we can acquire as much data as possible from each fish, this allows us to use less fish as we can answer different questions (how many immune cells do they have in their skin versus heart, for example) from each individual.
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	We use zebrafish as our model system for two main reasons, firstly because it is relatively straight forward to modify their genome so that we can make certain cells fluorescent or change the function of certain genes. This allows us to track these cells and determine their function in a vertebrate model, especially because zebrafish

to minimise welfare costs (harms) to the animals.	are transparent allowing us to perform live cell imaging. Secondly, because zebrafish can regenerate lost cells in lots of different tissues including the skin and heart, we can attempt to work out how they can replace these lost cells, something that will be important if we want our cells to be more regenerative in the future. The majority of the animals we require are only used for creating and maintaining lines where specific cells are labelled with a fluorescent marker. In comparison, a relatively small number are required for tissue injury experiments where we can study the role of specific immune cells in the healing process. To minimise harm to the animals, they are monitored daily (more often when undergoing procedures) and where there is any concern, advice is immediately sought from the Named Veterinary Surgeon and Named Animal Care and Welfare Officer, before appropriate action is taken.

Project	**245. Modelling the Prostate Cancer Genome**	
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)	Prostate cancer is a significant cause of death and morbidity in men. Over recent years, we have made major advances in understanding the changes that occur in the DNA of prostate tumours. These chang-es turn out to be complex and are likely to change the behaviour of tumour cells in many ways, determining whether the cancer is stable or aggressive, and influencing the response of the can-cer to treatment. We now need to perform precise genetic experiments in which the DNA changes we see in humans are reproduced in a stepwise and systematic manner so that we can understand which gene mutations are important in controlling the disease. We will focus on genes with roles	

	in regulating genome stability. We will use both in vitro experiments with cell cultures, and transgenic mice to address these questions.
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)?	Currently, there is limited understanding of why some prostate cancers remain stable for years, but others rapidly progress to a lethal disease that is incurable. and. difficult to treat. This project will produce new information about the genes involved in driving aggressive prostate cancer – i.e. which genes do this and how? By answering these questions we expect to identify new opportunities to monitor and treat prostate cancers. The long term goal is to use the data produced in this project to develop new treatments and tests that provide better management of prostate cancer, but the immediate aim of this project is to generate new information.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year period of this project we may use up to 2450 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?	Some mice will be modified genetically to pre-dispose them to the development of prostate cancer. In some cases we will also use a chemical to induce the genetic modification and subsequent tumour growth. In some mice prostate-derived tumours will be implanted onto the back of the mouse for ease of monitoring. Tumour growth is not associated with pain during the period in which we conduct our observations. Tumour growth will be monitored regularly by either use of callipers, or by imaging method, following dye injection, for internal tumours. For some procedures that involve surgery, we will administer pain killers and monitor closely. The mice will also have blood samples taken either from the tail vein or by sampling from a heart chamber under anaesthesia. Occasionally mice may be administered organ preservative whilst under non-recovery anaesthesia to allow us to undertake microscope investigations on slices of selected organs. At the end of a procedure
	mice will be humanely killed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For the vast majority of experiments we don't use mice, but instead cells grown in the laboratory, however, their use enables us to answer many questions regarding specific scientific problems. As this project concerns the genetic changes that occur in human prostate tumours, we need a model of prostate cancer that is possible to genetically engineer. Other non-protected animal alternatives (e.g. insects) lack the prostate organ. Whilst we can engineer cell lines to mimic some of the genetic changes found in humans, this doesn't allow us to understand how a tumour arises 'naturally' from within prostate tissues that are initially normal. We cannot at this stage model in vitro the changes in hormone signalling that occur during mammalian development, or the interactions the tumour has with its neighbouring cells and the immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use the smallest number of mice necessary to answer a specific scientific question. This will be backed up by many years of experience in choosing the right number of mice and by seeking advice on statistical methods to ensure the experiment will be conducted properly. We will design very simple experiments in which a group of control mice is compared to a test group. For example, a group of normal mice will be compared to a genetically altered group, or a placebo / standard of care group will be compared to a treated group of mice. We will aim to always include 5 – 10 mice per group and compare groups of similar sizes. This design will allow us to detect an effect size of 30% or greater. More subtle effects would require larger groups, but we would be reluctant to use large numbers of mice to prove a subtle effect. Therefore we think that this simple design will suit our work and allow us to conclusively measure important changes in biology. We will take steps to maximise the amount of useful information we generate

	from each mouse in order to keep the number of mice used to a minimum. We will carefully fix and archive our mouse tissues so they are available for use in the future.
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 order to study prostate cancer we need to use a mammalian species, because mice possess urogenital organs that are similar to the human system, together with the hormonal regulation (e.g. testosterone) of these tissues. Mice represent the least sentient mammalian species in which we can conduct genetic experiments, allowing us to model the genetic changes found in human cancers. Although genetics can be employed in less sentient species, (e.g. fish), these do not have a prostate, and are not suitable for our work. Wherever possible we use experiments that affect only the cell type that is under investigation, rather than the whole animal (i.e. we will alter genes only in the prostate). This ensures that possible suffering is avoided or kept to a minimum. After surgical procedures we will monitor mice for signs of pain and administer effective pain relief for as long as it is required.

Project	**246. Modulating and Resolving 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
	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)	Disordered inflammation is responsible for most of the diseases afflicting western society, including hardening of the arteries (causing heart attacks, stroke and peripheral vascular disease), lung diseases (including chronic bronchitis and asthma), crippling rheumatoid and osteoarthritis, and the commonest form of liver and kidney disease necessitating transplantation. Despite the huge burden of illness, loss of work and death, there is yet no effective drug therapy for most of these conditions. Our major early discovery was that certain damaging white blood cells need to undergo a form of ‘silent suicide’ and to be quietly removed by local	

	scavenger cells in order for inflammation to resolve effectively. The 'breakthrough' came at the beginning of our previous REDACTED programme grant when we showed that agents called cyclin-dependent kinase inhibitors (CDK inhibitors) not only drove the cell suicide process in the test tube in the presence of survival factors, but greatly accelerated the rate of resolution in mouse lung models of human inflammatory/scarring diseases. We have now clearly identified the molecules REDACTED for close attention in our new research programme, and shown that CDK inhibitors not only drive cell suicide but also cause the 'scavenger' cells to release anti-inflammatory agents, thus adding to their potential therapeutic benefit. Furthermore, we have shown that CDK inhibitors cause resolution of inflammation not only in the lung but also in other mouse and zebrafish models of the inflammation. Thus our research aims to extend our studies to models of blood vessel disease, in the likelihood that our work will have widespread relevance for the development of new treatments for inflammatory/scarring diseases of key relevant organs.
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 initial work on CDK inhibitor drugs in lung inflammation has paved the way for a new therapeutic approach to treating inflammatory disease with our continued research REDACTED leading to better targeted drug approaches with better toxicity profiles. The extension of our findings into potential new treatments for blood vessel disease, liver and kidney fibrosis, as well as ventilator-induced lung injury has added enormously to the impact of our on-going research in chronic inflammatory lung disease. Importantly, the use of our evolving disease REDACTED have proved and continue to prove to be invaluable in shaping and defining our more selective and specifically targeted mouse models in other important target tissues ie vascular, renal, hepatic, etc. These in turn will help to shape and develop next generation drugs and importantly better drug targets to help in the treatment of patients.
What species and approximate numbers of animals do you	Total maximum numbers over the 5 year project will

expect to use over what period of time?	be: 7600 mice and 9800 zebrafish.
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?	Regulation of acute and chronic inflammation will be assessed in our well-established mouse and fish models; at times making use of genetic modification to help identify key mechanisms contributing to the onset and resolution of inflammatory processes. A range of adverse effects, such as weight loss, short periods of breathlessness and lethargy can be associated with these models. After the indicated periods of experimentation, animals will be culled using approved methods and their tissues will be extensively studied so that the maximum amount of information can be gained from each animal. The impact of these techniques will be managed by good experimental technique and post-procedure care. Appropriate use of anaesthetic and analgesics (pain killers) is important in reducing the impact on animals. Potential adverse effects of procedures that do have a high impact on animals are well understood and will be monitored and treated accordingly to reduce any potential suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of the experiments in our programme will continue to use human cells (from volunteers and patients) in order to, as far as possible, assess the relevance to human disease. These studies will not only guide the number and experimental design of the animal work but will replace the mouse usage where possible. It is not feasible to produce an adequate *in vitro* or *in silico* model of the immune system and the body's response to infection, thus in this regard there is no substitute to live animal studies to determine systemic responses to infection and novel treatments. Utilising zebrafish embryos (before day 5 post fertilisation) will allow us to perform *in vivo* experiments on the pharmacological manipulation of the resolution of inflammation thereby replacing mice and adult zebrafish in certain circumstances.
2. Reduction Explain how you will assure the use of minimum numbers	Non-invasive *in vivo* imaging techniques are used to reduce the number of animals required. Furthermore, in vivo analyses are extended by the use of

of animals	complementary techniques (histology/immunohistochemistry, molecular biology, cell isolation and culture, tissue culture, and functional analysis) for analysing tissue samples post mortem. Similarly, relevant *ex vivo* models (e.g. cell culture) are used to model processes that occur in vivo. The advances in transcriptomics and bioinformatics have greatly increased the information obtained from experiments and allow powerful, detailed analyses of signalling pathways, improving target identification and assessment of new interventions.
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 team, both scientific staff and animal technicians, are very experienced in handling and experimenting on animals, which will reduce stress and suffering of the animals. The unit in which the animals are kept is large, well-resourced and well-equipped. Advice is taken routinely from veterinary staff and all experiments are submitted first to one of the vets for review. The welfare of animals used in this work is extremely important. They are kept in state-of-the art facilities using best husbandry practices, with regular checks by suitably qualified staff and recourse to veterinary advice. Animals are provided with environmental enrichment (nests, nesting materials, tubes, chew sticks), as appropriate. All procedures are performed by well-trained staff using good aseptic technique and appropriate anaesthesia and pain relief, to minimise distress and discomfort. Animals are individually monitored to assess the actual severity of the procedures they experience; they are killed humanely at the point of the experiment that allows the most meaningful analysis of outcomes. Any adverse effects of procedures that exceed expected limits will be referred to the named veterinary surgeon and, if necessary, affected animals will be euthanized. In our animal models we will continue to optimise existing protocols in order to ensure quality of data provided in keeping with guidelines from appropriate academic bodies (e.g., American Thoracic Society Experimental Acute Lung Injury guidelines AJRCMB 2011)

Project	**247. Molecular and neural mechanisms of social behaviours**	
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)	In this project we aim to tackle the problem of how the brain is capable of coordinating social behaviours. In particular, we are interested in three aspects of this problem: 1) how the brain processes the sensory information relevant to another individual (i.e. how does a mouse know when it is looking at another mouse?), 2) how the brain then uses that information to produce a behaviour (e.g. parenting), and 3) how an animal's social behaviours develop over time (*i.e.,* when a male mouse reaches a particular age it will start to attempt mating with female mice, when previously it did not show this interest).	

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)?	Social behaviors are fundamental to the survival of species, and represent a fascinating problem because despite their complexity animals know how to do them without ever having done or seen them before. They are hard-wired in the brain. What is additionally of great interest, is the fact that this hard-wiring can be altered throughout an animal's lifetime. One of the benefits of this project is that we will advance our scientific knowledge of these captivating phenomena, using cutting-edge techniques and a rigorous scientific approach. A second benefit of this project is the translatability of the findings to humans. The brain structures involved in social behaviours are largely conserved in mammals, including humans. Tackling our aims will provide an understanding of how these processes occur, and hence will also indicate the ways in which these processes can go wrong. This is important because deficits in social interactions are a recognised symptom of some psychiatric diseases in humans, and so new medical targets may be gained through the findings of this project. For example, a reduction of motivation for social interaction is a symptom of depression in humans. Furthermore, autism is a disease where the perception of social cues is impaired.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use 2,000 mice for this 5-year project. We use mice because they have a large repertoire of social behaviours, and because they rely on olfaction as a key sensory modality for their social behaviours which can be easily controlled. This control allows us to investigate which stimuli are important. In addition, science has discovered a lot of knowledge about the genetics of the mice we will use, and this allows us to do more powerful experiments when determining how the brain works.
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	In order to investigate how the brain works we need to perform surgeries on mice to attach devices that enable us to record its activity, as well as to alter how the brain is acting to find out what activity is necessary for particular behaviours. These surgeries will be the primary

the end?	source of adverse effects to the mice. Surgeries will introduce a short-term pain to mice as they recover, and this will be managed by the administration of analgesics, as is the case for humans after they have gone through surgery. Other procedures will introduce only a minor or no distress to mice since most of our experiments will monitor the natural behaviours of mice, and they will be monitored in familiar environments such as their homecage. At the end of our experiments, and also in rare cases where mice show signs of ill health that cannot be treated, they will be humanely killed without causing pain.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have considered using different animal models for the studies of social behaviours, but mice are by far the best model system to learn the fundamental principles of social behaviours in mammals. The studies of behaviours in mammals unfortunately cannot be replaced by the study of invertebrates, and we are far from a point where we have the knowledge to generate sophisticated computational models that will allow investigation of social behaviours. However, whenever appropriate, we will use in vitro methods and this can partially replace in vivo physiology experiments examining neural activity and gene expression.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have achieved a significant reduction of mice used in our research in the last three years through two main improvements in our workflow. One strategy is to use wild type mice, whenever possible, to avoid using transgenic mice, which require larger numbers to maintain. The second way of reduction is to obtain combined molecular, physiology and behavioural data from single animals, which allows us to obtain rich datasets which allow powerful data analysis and require less mice overall.
3. Refinement Explain the choice of species and why the animal model(s) you will	We have made a number of refinements over the years. For example, we have made surgical refinements such as reducing the time for surgery and recovery by using inhalation

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.	analgesics such as isoflurane whenever possible to do so. For the current study we intend to use inhalant analgesics such as isoflurane for >90% of all procedures that require anaestheic. We have also made refinements for husbandry of mice in non-stressful conditions, especially for pregnant females. In the experimental designs, we worked with biostatisticians to design experimental cohorts and comparison groups prior to mouse breeding. We are also devising methods to monitor natural behaviours of mice in their home cages. We are committed to refining methods to reduce mouse stress, pain and suffering and to maintain their well-being, for example, inclusion of housing materials and toys as enrichment in their home cages.

Project	**248. Molecular imaging of the tumour microenvironment**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our goal is to improve cancer imaging by developing more sensitive and lower cost imaging technology. A growing tumour is composed of more than just cancer cells. For example, the tumour may stimulate the growth of new blood vessels to improve delivery of the oxygen and nutrients that it needs to grow. It may also recruit cells that deposit a structural scaffold upon which it can grow, and try to avoid detection by the immune systems by many different mechanisms. In this project, we aim to understand how all of these supporting factors, referred to as the tumour 'microenvironment' aid cancer development. Our first objective is to create new imaging methods	

	that could be used by doctors in the clinic and report on different facets of the tumour microenvironment. Unfortunately, not all of the molecules or cells in the body can be seen by imaging methods directly. Sometimes we need to send in a contrast agent that acts as a molecular 'beacon' that can be seen by our imaging methods. Our second objective is therefore to design new contrast agents that report on important facets of the microenvironment, for example, the complex range of immune cells, which are not normally seen by imaging. Our third and final objective is to apply these new imaging methods to address unanswered questions that remain about the role of this physical and cellular microenvironment in cancer and how it is important in the evolution of the disease.
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)?	Cancer patients could benefit from our approaches as they will improve not only our ability to detect the earliest signs of cancer, but also to understand whether a particular tumour is likely to be more aggressive. Science could also be advanced because being able to image these processes in mice and humans will help us to understand how cancer develops, assisting in the creation of new methods to tackle the disease, such as novel drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, 550 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 likely level of severity in these studies is moderate. Adverse effects arising from imaging procedures are uncommon (less than 5%) and are mainly associated with anaesthesia-related effects. In some specialist procedures, depilatory cream may be used remove hair from the mice and this can occasionally lead to skin inflammation. We will use 4 main types of tumour model: • Tumour cells or tissues implanted under the skin of the mouse; adverse effects may include: complications from surgical procedures or hormone supplementation (if used); and those associated with ageing (if used). • Liver tumours developed using carcinogenic chemical substances; adverse effects include: liver fibrosis and impairment of liver function. • Skin tumours developed using

	carcinogenic chemical substances; adverse effects include: mild skin irritation, impairment of liver function associated with metastasis and those associated with ageing (strain dependent). • Colorectal tumours developed using carcinogenic chemical studies; adverse effects include: mild peritoneal inflammation, diarrhoea and reduced food intake. These models will allow us to develop new imaging tools and test them in the context of tumour evolution and response to anti-cancer drugs. At the end of the studies, each mouse will be killed and its tumour will be removed for subsequent analysis, which will help us to validate and understand the new imaging methods that we are developing.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research alternatives, which do not involve the use of animal models, are used in the initial testing phase of all our new imaging methods. However, testing of novel molecular imaging technologies for the monitoring of tumours in living animals is also necessary. This is due to the inherent stress that is placed upon cancer cells that grow outside of the body, and the lack of models that can mimic the combined actions of supporting cells such as blood vessels, immune cells etc.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Full statistical analysis will be used to guide our studies. We will use a power analysis to calculate the minimum number of animals that will be needed to evaluate our new imaging methods.
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 anaesthetic/analgesic during imaging. Our ongoing efforts in preclinical imaging standardisation initiatives will ensure that we minimise animal suffering while carrying out our work. This can involve performing multiple different imaging scans within a single anaesthetic period, to avoid repeated exposure. We have chosen models of solid tumours that best enable us to achieve the scientific benefits while producing the minimum pain, suffering, distress or lasting harm: the majority of our studies will be performed in tumours that are transplanted under the skin of the mouse and therefore do not impede

	normal function. Environmental enrichment will be provided to improve animal welfare and promote the expression of species-appropriate behaviour and mental activities. Animals will be housed on an appropriate light/dark cycle with control of room temperature and humidity. Animals will be allowed 7 days to adapt to their new environment upon arrival to minimise stress and will be handled prior to the imaging studies to ensure no unnecessary duress is caused by the procedures. No protocols are defined as severe and all moderate protocols will be continuously reviewed to ensure that any new advances that afford opportunity to reduce the severity limit still further are duly incorporated.

Project	**249. Molecular mechanisms of metastasis of melanoma 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)	We seek to understand the mechanisms by which pancreatic cancer and melanoma develop and spread throughout the body. We study how genetic changes can make cancer more aggressive in the hope that we can discover how to prevent cancer spread and better treat patients that may have a more aggressive cancer. There are currently very few treatments available to patients whose cancer has spread beyond the initial (primary) tumour, so it is our hope that this research will lead to knowledge that speeds up the search for such treatments.	

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)?	We hope to advance knowledge about how cancer spreads so that better diagnosis and treatments can be found that prevent cancer spread. Our work focusses mainly on pancreatic cancer and melanoma, but could be relevant to other types of cancer as well.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 5,000 mice per year over 5 years for this project. Around 80% of these will not undergo any scientific procedures, but will be used only for breeding and maintaining colonies.
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 bred to develop pancreatic cancer or melanoma or in some cases will receive a surgical or injected transplant of tumour cells from mouse or human cancer. Approximately 80% of mice will not show any adverse effects beyond simple ear notching for identification and genetic testing. Many of these animals will be used by us and others as "normal controls" for experiments, where tissues may be harvested after they are humanely killed. Some proportion of the animals, around 20%, will develop tumours and will be monitored carefully for clinical symptoms. Symptoms include weight loss, swelling of the abdomen or development of a visible skin tumour. Carefully trained staff will monitor mice with tumours and if the tumour interferes with normal behaviour or reach a certain allowable size, the mice will be humanely killed and the tissues will be analysed. In some cases, we will treat animals with chemical compounds (such as experimental chemotherapy drugs) and measure the effects on tumour growth and spread. This may involve adding substances in the food or drink or injecting the substances. All animals receiving treatments will be monitored closely and any animals that display signs of being unwell, such as ruffling of the coat, reluctance to eat or move, weight loss of 20% or more, will be humanely killed. Any animal undergoing a potentially painful procedure, such as surgery, will be given anaesthetic and anaelgesic in consulation with a named veterinary surgeon. At the end of the study, all animals will be humanely killed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cancer metastasis is a very complex process, involving many different systems- such as the blood, organs (e.g. pancreas, liver), lymph nodes and the immune system. It is not yet possible to fully model the metastatic process in the laboratory without using live animals. Where possible, we do studies on cells rather than animals and most of the work done in our laboratory uses cells. We are constantly developing better models, such as cells growing in 3-dimensional cultures and co-cultured with other cells to replace animals where possible. However, mice are currently the most accepted model for cancer metastasis, as they can closely recapitulate the human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We plan our experiments with the help of statistical experts who help in experimental design and calculation of the number of animals needed for each experiment to make sure that we use the right number for the experiments to be meaningful, but not an excess. When we do a new type of experiment, we generally perofrm a small pilot study, with just a few animals, to make sure that the design is good, before proceeding with a full study. We share animals and tissues with other groups and we save tissue samples in archives, so that often many studies can be done with the same tissues from groups of animals. We frequently interact with other groups to optimise our experimental design, breeding strategies and types of models used. We use transplant models, which require fewer animals than breeding studies, for some of our experiments.
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.	Since our goal is to try to recapitulate human disease as closely as possible, we use genetically modified mice that have been developed to mimic human cancer progression and spread. These models are widely accepted as resembling human cancers and being useful for pre-clinical trialling of new ideas, base don modulation of genes or treatment with compunds such as experimental anti-cancer drugs. We will always try to use the most

	appropriate model with the least harmful side effects possible- this includes limiting the effects such as gene expression or deletion to the specific organ of interest by using mice engineered to show tissue-specific and/or inducible gene modulation. All animals will be under the care of the local named veterinary surgeon as well as trained staff who monitor them for normal behaviour and any animals exhibiting moderate adverse effects will be humanely killed.

Project	**250. Molecular mechanisms underlying neurovascular dysfunction**	
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 aim to investigate a type of RNA molecules, which do not translate into a protein and are therefore called non-coding RNAs. We want to determine the role of non-coding RNAs in the dysfunction observed in the blood vessels of the brain, also known as the blood-brain barrier (BBB) not only in disorders related to the central nervous system (CNS) such as multiple sclerosis but also in normal ageing. These RNA molecules can be classified as small (microRNAs) or large (long non-coding RNAs) and have been implicated as potential targets for developing drugs in many diseases such as cancer,	

	cardiovascular diseases, and inflammatory diseases. CNS pathologies and healthy CNS ageing (i.e. in the absence of disease) share a common feature of BBB dysfunction, inflammation. However the underlying molecular mechanisms remain elusive. Hence, delineating the role of non-coding RNAs in BBB dysfunction would help to identify potential therapies for CNS pathologies and age-associated brain disorders. There is also lack of information about ageing and/or inflammatory processes in females and we will focus our studies on gaining this information and determining differences between males and females. The project involves first identification of non-coding RNAs deregulated at the BBB in ageing and inflammation models and, second, modulation of non-coding RNA levels to determine their effects on BBB dysfunction.
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 information obtained should have a high value and it is hoped that this will allow direct-translation of the laboratory findings to the clinic and provide new knowledge regarding BBB dysfunction and repair, which will in the longer term, contribute towards the development of additional treatments to improve the lives of people with CNS pathologies and allow ageing population to enjoy quality of life.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5500 mice will be used over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals may be exposed to inflammatory substances using standard routes and best practice techniques or allowed to age naturally. For some experiments, substances such as tamoxifen will be administered using standard routes to animals in order to manipulate their genome prior to inflammation or ageing. No harmful effects are expected as best practice protocols will be followed. However, there is a low risk that genetically altered mice may develop unexpected effects such as consistent lethargy or sudden acute weight loss as a consequence of altered microRNA levels and these animals will be killed humanely before

	symptoms reach the moderate severity limit. Some other adverse effects include the following: a) There is a very low risk of injury to females caused by rough treatment by stud males during breeding. b) There is a low risk of mild bleeding during ear notching or tail tipping used to identify genetic background. c) There is a low chance that animals will be stressed by handling and a low risk of peritonitis or infection from injections. d) There is a small chance of vaginal injury in females during lavage. In all instances, mice will be closely monitored for signs of illness or distress and killed humanely before the symptoms exceed the moderate severity limit (e.g. 10-20% loss of body weight, intermittently hunched posture, intermittently laboured breathing, subdued response to stimuli). Finally, in order to assess the function of brain blood vessels, substances will be administered either using standard routes or, if not, under terminal anaesthesia. At the end of the experiments, animals will be humanely terminated or transferred for continued use in another protocol under this or another project licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst some elements of inflammation, neurodegeneration, ageing and repair can be modelled in cell culture, the complexity of the brain cannot all be currently modelled using cell-culture or computer-based models. The use of live animals is needed for investigation of mechanisms of dysfunction of blood brain barrier and to identify potential therapeutic targets in situations relevant to human disease. It is not ethical to conduct experiments on humans in ageing, multiple sclerosis or other CNS pathologies, therefore, there is no feasible alternative that would entirely replace the use of a living animal that would allow the objectives to be met. Nevertheless, in parts of the project we will conduct experiments both in cultured cells and in brain tissues (of both animal and human origin) prior to animal studies in order to identify potentially relevant non-coding RNAs and their function in ageing and inflammation.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use in vitro cultured cells and human brain tissue prior to or in parallel with animal studies. Written protocols will be provided for each experiment including methods of analysis, to minimize experimental variation and reduce the number of animals needed to generate reproducible data. The number of animals needed per experiment has been calculated in consultation with statisticians to use the minimum number of animals needed to generate statistically meaningful data.
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 chief mouse model to replicate MS will involve a systemic inflammatory challenge for a short period of time that has proved reliable and far superior to other models that cause long-term clinical disability. To investigate dysfunction of the BBB, we will use the C57BL/6 mouse strain in the first instance as it is the source for specific non-coding RNA deletions in comparison to other strains, which will considerably reduce the number of animals used in order to determine the role played by non-coding RNAs in BBB dysfunction. We aim to continue to refine this model to detect effects of knock-down/over-expression non-coding RNAs in specific cells of brain blood vessels and enhance the utility and reproducibility of the model. A reproducible neuroinflammatory model will allow us to reduce the number of animals used and for a shorter time reducing the occurence of adverse effects on the same animal. Technical-support staff and researchers are trained so that variability between users is minimised further improving reproducibility. In addition, their highly skilled training further reduces the probability of adverse effects while applying the protocols described in this project. The ageing model to be used has been selected because normal ageing leads to less harmful effects compared to other ageing models such as accelerated models of ageing.

Project	**251. Molecular neurobiology of circadian clocks and sleep**	
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)	Our lives are built around daily cycles, the most obvious being sleep and wakefulness, but underlying that rhythm are pronounced changes in our physiology and metabolism that we may be aware of as changes in appetite, mood and ability to concentrate. These daily cycles are synchronised to and adapt us to the day/night cycle, but they are generated internally by a biological clock mechanism. In almost every cell and tissue there is a time-keeping mechanism that oscillates with a period of approximately one day (circadian). We know that this circadian body clock system is important for health, since when it goes wrong or gets out of synch with the day/night cycle (as in shift work), people are	

	more likely to suffer from conditions such as diabetes, cardiovascular disease and various forms of cancer. Under normal conditions, our vast array of internal clocks is co-ordinated by a small cluster of about 10,000 cells in the hypothalamus of the brain, the suprachiasmatic nucleus (SCN). Overall, the purpose of our work is to understand how this body-wide timing system works and contributes to health. Our specifc aims in this project are, first, to understand how the circadian clock genes and the proteins they encode work together inside cells to generate a daily circadian time-signal. Second, we wish to understand how the cells of the SCN work together to generate such a precise and powerful timing signal, and how it is synchronised by the retina. Finally, we wish to identify the neural pathways and mechanisms that allow the SCN to broadcast this signal and thereby control daily rhythms such as sleep
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 discovery science and so the potential benefits from our work will be new knowledge. Circadian clocks are pervasive and so decoding how they are controlled by the SCN will provide a mechanistic basis to determine how they go wrong, and potentially how to avoid or mitigate that, in various diseases (e.g. dementia) and lifestyles (shift-work). Additional benefits will be technical developments of value to other neuroscientists and molecular biologists
What species and approximate numbers of animals do you expect to use over what period of time?	60,000 mice, principally genetically altered, 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 cellular mechanism of the clock is created by a small number (<12) of genes. Our work will use mice carrying variants of these genes in order to understand how they, and the proteins they encode, behave within cells and thereby control daily rhythms. We shall measure this in tissues collected for microscopic analysis and also in living mice carrying such variants. We shall also use mice with benign genetic modifications that allow us to select which brain cells we analyse – a procedure that brings significant experimental refinement. Because our work will use various mixtures of such

	genes, the majority of mice to be counted in the project will simply be used for breeding to create these genetic mixtures, and so their experience will be categroised as “mild” with no adverse effects anticipated. The first steps in our analyses will also involve mild procedures, including monitoring the pattern of daily wheel-running behaviour as a “read-out” of the clock, and testing memory by exploiting the natural curiosity of mice. Some mice with interesting clock-patterns (<10% of overall total) will then go on to more intensive measurements, for example recording sleep with electrodes attached to the skull, or monitoring brain cell activity via implanted fibre-optic cables and microscopic lenses. Inevitably these require surgical approaches and so are classed as “moderate”. Surgery is conducted to the highest standards and adverse events (infection, wound-opening) are very rare and readily dealt with. To monitor daily rhythms of gene expression across the brain or in tissues such as the liver, mice will be placed in an apparatus that can detect bioluminescence emitted by active genes. For this to work, the mice will carry sub-cutaneous pumps to deliver light-emitting reagent. At the end the mice will be killed humanely by a regulated procedure, and where useful tissues will be collected for further analysis. A small number may be supplied to other Researchers.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	My research seeks to understand the regulation and function of circadian rhythms and sleep. These are products of an intact nervous system. It is the case that a large part of my work uses ex vivo brain tissues and so the requirement for animals is in suitable breeding programmes to generate intact tissues. Beyond this, the use of conscious adult mice is unavoidable if we are to study the integrated circuitry and the dependent behavioural states and outputs: cell lines or computer simulations cannot yet come close to reproducing the complexity of the brain. Importantly, the starting point for our analysis of molecular mechanisms is our broad view of the existing literature of biophysical and molecular biolgical studies of

	clock genes and proteins, and much of this is derived from in vitro and cell-based studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We significantly reduce our reliance on animal models by performing preliminary studies in cell lines, and as far as possible, in vitro and ex vivo techniques will always be employed to minimise the use of intact animals as well as inform that use. Where animals are necessary, tight control over breeding programmes means that we produce very few animals surplus to our experimental needs (less than 10%). Robust experimental design taking advantage of within-animal, repeated-measures cross-over designs, predicated on power analyses will enables us to generate statistically valid results from the minimum requirement of experimental stock. Importantly, by using inbred strains alongside top quality facilities and kit we shall ensure consistency of results, and minimise the variations between individuals, thus allowing us to keep the experimental cohorts relatively small.
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 least sentient mammals that can be used for satisfactory tests of the circadian organisation of behaviour, physiology and sleep. They are the species of choice because the characteristics of circadian rhythms and sleep and the neural circuits that control them are highly conserved with humans, offering direct translational relevance. Genes, transcriptional networks and expression patterns are shared between mouse and human, and a wealth of data on mouse molecular neuroanatomy is available to inform interrogation of relevant circuits and molecules. Third, the simple behavioural tests of memory and emotional state, are experimentally robust and mice can perform them very easily. We shall exploit the unsurpassed genetic refinement offered by mice. They have readily modifiable genetic systems, including lines carrying genomically encoded recombinase drivers and conditional alleles, which are widely available from central repositories including. A large number of genetic tools have been developed and validated specifically for mice:

	AAV-, Rabies- and LV-based viral vectors; optical reporters; genetic manipulators such as CRISPR, siRNA, opto- and chemo-genetics etc. Finally, because of the local and targeted nature of the modifications and AAV transduction, the resulting phenotypes usually result in highly specific changes in behaviour (usually disruption in the sleep-wake cycle, and/ or circadian behaviour, or responses to drugs) and rarely show any morbidity. The genetic and electronic/ imaging technology now available ensures very refined experimental procedures and datasets. In terms of welfare costs, the mutations to be used are benign, the surgical procedures and associated kit are well established and so the needs for after-care monitoring and analgesia are well understood and the behaviours we shall study are spontaneous and within the normal repertoire of mice. It is critical to our scientific success that the mice behave normally because that is our measured dependent variable. It is therefore paramount in our design of studies and use of animals that we minimise all aspects of stress and discomfort.

Project	**252. Monoclonal antibodies against difficult proteins**	
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)	There are many diseases for which we have an understanding of the underlying problem, and where we know how we could intervene to improve the patient's condition, but we don't have a medicine whose specific mechanism of action can be used to address that underlying problem. In many cases the disease-causing proteins – the 'targets' - sit in the membranes of our cells. This kind of protein is extremely difficult to work with and to discover new drugs against. This project will address this problem, by allowing us to use a technology we have developed that lets us discover a class of drug called 'monoclonal antibodies'. A critical part of the technology is injecting mice, and then	

	harnessing part of their immune response as raw materials for discovering the monoclonal antibodies.
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 aims to discover treatments for severe diseases. We currently have programs is two different areas: • Pancreatic cancer currently has a 5-year survival rate of 3.3% with approximately 10,000 new cases diagnosed each year in England. The survival rate for this disease has not improved in the last 40 years and there is therefore an urgent need for effective new treatments. • Fibrotic disease is the end-stage of a number of chronic conditions and affects many organs. For example, idiopathic lung fibrosis is thought to cause up to 1% of deaths in the developed world. Existing treatments are poorly efficacious and leading candidate drugs have recently failed in clinical testing. Over the course of the project we expect to expand this list of diseases and we are currently considering a range of diseases including vascular disease, chronic pain and organ deformities. As well as making good therapeutic drugs, monoclonal antibodies are also useful for detecting chemical markers of health and disease called 'biomarkers'. A part of the personalised health revolution is the miniaturisation of biomarker detection devices such that they can become consume devices. High quality monoclonal antibodies will be essential tools to facilitate this process.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a five year period we expect to use 7250 mice and 500 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?	At the end of all experiments, the animals will be humanely killed, in many cases under anaesthetic. During the immunisation protocols, the animals will be injected with preparations designed to stimulate an immune response, similar to the administration of a vaccine to a human patient or a pet. The adverse events are expected to be similar to those expected after human vaccination, as a result of the immune response: some local soreness and some systemic effects which cause temporary discomfort. The animals will be carefully

	monitored and if the symptoms do not resolve by 72 hours after the injection the animals will be painlessly killed as a humane end-point. We will also perform non-invasive imaging though the use of luminescence technology that allows us to determine the physical location of the immunising proteins within the host antibody. This information will allow us to rationally optimise our immunisation protocols. To perform this imaging, animals are injected with a biologically invert dye-like molecule called luciferin. A luminescence camera can then detect luciferin in the body of the live animal, which will be anaesthetised in order that it stays still during image capture. Some of the mice we will be working with will be genetically modified (GM). In most cases, these lines of GM mice will experience no adverse effects as a result of their genetic modification. Those mice that do experience moderate suffering will be handled under a separate protocol to those with mild or no adverse effects, and mitigation strategies will also be put in place to minimise the suffering experienced by these animals. Again, animals judged to be at risk of exceeding the moderate suffering banding (judged by both the severity of the phenotype, but also whether it is continuously experienced by the GM mouse) will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are some methods that do not involve live animal injection for discovering monoclonal antibodies, however these have not been successful for working with target proteins that sit in cell membranes. We have shown that our technology is superior to all alternative methods in benchmarked studies, specifically when working with membrane proteins.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When working with any new target protein we always perform pilot studies to ensure that the immunisation method we are using is optimised for the specific target protein. This means that when we start a large-scale immunisation campaign, we are confident that our immunisation method will work well. We are

	also trying to develop our technology which already works with mice to work with rats. Because rats are much larger, we can use fewer of them to discover the same number of lead monoclonal antibody medicines. Repeated non-invasive *in vivo* imaging on the same animal decreases animal numbers itself, but also increases the statistical power to draw inferences from experiments.
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 mice and rats for a number of reasons. Most of the stages in monoclonal antibody medicine discovery that come downstream of the animal immunisation step are best optimised for mouse antibodies, which means that there is a higher likelihood of successfully developing a new treatment. There is also a variety of genetically altered mice that potentially deliver an increased rate of antibody discovery, reducing the number of individual animals required. Because mice are very small and yield only a very small amount of material, this necessitates using a larger number of animals. To reduce numbers we want to work with a larger species. Rats are preferable to rabbits or guinea pigs because they are more similar to mice in their physiology and immune systems. This should make it much more efficient to translate our technology which works in mice across to rats than it would be to translate across to guinea pigs or rabbits. Non-invasive *in vivo* imaging represents a very refined method for understanding the evolution of the antibody response. When injecting substances, we use refined routes of injections and where appropriate to reduce distress, we use anaesthetics. We will work with the world-class team of vets and husbandry specialists at to monitor animal welfare. All animals are monitored daily for signs of suffering after injection.

Project	**253. Mouse Models of Human 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 overall aim of this project is to develop new treatments for human cancers based upon an improved understanding of how cancer develops and grows. There are three key objectives: 1. To investigate how mutations in the DNA of cancers regulate how cancer is initiated, and how it progresses and spreads. 2. To develop models of human cancer in mice that accurately reflect human cancer. 3. To use these models to assess the	

	effectiveness of anti-cancer treatments. To evaluate the role of the immune system in the biology of our chosen cancer models, and how the immune system may impact upon activity of anti-cancer treatments.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this work are the development of new anti-cancer treatments for use in patients. The ability to assess how well new treatments or combinations of treatments work in mouse models that are an accurate reflection of human cancer is an essential step on the road to clinical trials in patients. In addition, this work will help us to understand the complex interactions between cancer and the immune systems.
What species and approximate numbers of animals do you expect to use over what period of time?	The only animals that will be used will be mice. In 5 years, we estimate that we will use between 12,000 and 14,000.
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?	Level of severity: Mild-Moderate Tumours will be induced in animals, but the size of tumour needed for this research is unlikely to affect the health of the animals. Adverse effects due to drug administration will be monitored in accordance with NCRI guidelines. Animals will be humanely killed at the end of the experiment and also if there are indications during routine monitoring that the animal may be subject to undue pain or distress, in order to minimise suffering. These indications will be based on recognised symptoms, which, in turn, will depend upon the site and type of tumour that has been induced. However, general indications will include weight loss exceeding 20% of initial body weight and/or other signs of illness, such as hunched posture, rough fur, poor appetite, inability to groom and immobility.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal	In order to minimise animal usage, we are already utilising highly sophisticated non-animal models, including growing primary patient tumour cells as three-dimensional

alternatives	masses. However, such techniques fail to model some critical features of novel anti-cancer agents, in particular the interactions with the immune system. These aspects can only be assessed in realistic models of cancer in whole animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will reduce the numbers in three ways 4. Using sophisticated laboratory models as above 5. Statistical calculations to ensure that the minimum number of animals are used to address the scientific question 6. Using powerful imaging techniques similar to those used in patients, to assess tumour growth in small groups of animals over time.
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 refinement is to use sophisticated models that accurately reflect the biology of the human cancer, for example by ensuring the correct mutations within the tumour and also that the cancer grows in a manner that is equivalent to human disease. We will only use mice as they are mammals with similarities to humans, and there are thousands of reagents available, making them the most relevant species for the proposed research. At all times, mice will be cared for by experienced staff under the guidance of the Project Licence holder and REDACTED staff. All experiments will have clear severity limits (none more than moderate), and any animal reaching or exceeding those limits will be killed by Schedule 1 methods. In addition, all animals will be housed in groups where possible, with appropriate environmental enrichment and fed according to current institutional best practice

Project	**254. Multisensory integration: Olfaction and Metabolism**	
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)	This project has 4 objectives that aim to determine how changes in the level of hunger alter brain activity responsible for processing odours and how these changes result in altered perception, feeding behaviour and metabolism. 1) We will use advanced brain imaging methods to observe how the neural circuitry responsible for processing odours is altered when the metabolic state is changed from hungry to satiated. Depending on the neuronal population of interest we will use mice expressing genetically encoded indicators of neural activity restricted to a single cell type or we will use alternate methods to express indicators in the	

	neurons of interest. Prior to an experiment an animal will be fasted overnight to induce hunger, it will then be anaesthetised and a small window, to allow imaging of the brain, will be placed in the skull. The activity of the labelled neurons will be recorded in response to odours before and after manipulation of satiety by inflating the stomach or injection of for example glucose. At the end of the experiment the animal will be humanely killed under anaesthesia. The imaging technique to be used enables the activity of hundreds of neurons to be recorded simultaneously, a refinement which will reduce the number of animals required. 2) We will then use the same experimental paradigm described in 1 together with pharmacological tools to determine the molecules and receptors responsible for sensing satiety and which cause the resulting changes in brain activity. 3) To link the observed changes in neural activity to altered olfactory perception, feeding behaviour and metabolism we will use genetic strategies to selectively block the molecules we identified in aim 2 in the specific neural population identified in 1 & 2. This will be achieved with intracranial injections of viruses designed to selectively knock down the receptor of interest and will be performed under stable and balanced anaesthesia. Animals are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital, they are unlikely to experience any adverse effects. Once the receptor of interest has been knocked down we will determine whether this manipulation affects olfactory sensitivity using simple behavioural tests and will also determine whether metabolism is altered. This will be determined by giving the mice a high-fat diet and monitoring food intake, weight gain and energy expenditure. The results from this work will describe how changes in the activity of a brain circuit cause altered perception and feeding behaviour and may identify potentially druggable targets to reduce food consumption. 4) To determine the brain circuit changes that occur during learning of a food odour and

	whether food odours are more susceptible to modulation by satiety, we will use the same imaging strategies as described in 1 but will perform the experiments in awake animals to allow comparison of neural activity before and after learning. Similar to the experiments in aims 1& 2 this requires implantation of a device to stabilize the head and placing a window over the brain area to be imaged. This will all be performed under stable and balanced anaesthesia and with post-operative pain relief mice recover from this surgery quickly and are unlikely to experience any adverse effects. Once the mice have recovered they will be trained to remain with their head fixed on a treadmill where, under their own volition, they are free to groom, run/walk, remain stationary or drink from a water spout. Mice quickly become accustomed to this protocol and during these periods neural activity will be imaged in response to delivery of odours. ,This will allow us to reveal how the activity of the brain changes as a mouse learns to associate an odour with food reward e.g. sugar.
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)?	Identifying molecular targets that modulate the neural circuitry in the olfactory bulb responsible for the control of feeding and metabolic health could lead to drug therapies to improve metabolic health. Additionally the insights gained from studying how neural activity is altered during learning will have broad benefit to the neuroscience community as this is still an outstanding question.
What species and approximate numbers of animals do you expect to use over what period of time?	Genetically altered mice ~ 610 used in experiments over a 5 year period. Around 2000 will be bred to achieve the correct genotypes
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There are negligible adverse effects expected. The aspect of the project with moderate severity involves recover from general anaesthesia after surgery which is classed as moderate severity. The animals will be euthanised at the end of experimental protocols
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To study multi-sensory integration there are no alternatives but to use animal models. Furthermore, the ability to measure neural activity in defined components of the neural circuitry is only afforded in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our aim is to reduce the number of animal experiments whenever possible. All experimental parameters are monitored and recorded to ensure the reproducibility of the experiments. The methods we use record from 100s of neurons simultaneously which will reduce the number of animals used.
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 studies aim to characterise the properties of the neural circuitry of the olfactory bulb and how it responds to altered metabolic state with the hope of a better understanding of olfaction and metabolism in humans. The organisation of the mammalian olfactory bulb is distinctly different to invertebrates or even other vertebrates. The observation that olfaction and metabolic health are linked has been made in both humans and rodents. The genetic amenability of mice also means that many genetic tools are available to study circuit function. This project relies on such lines of mice, which express an optical reporter of neural activity in defined cell types.

255. Mechanisms of movement generation and inhibition

Key Words

motor cortex, mirror neurons, movement inhibition, subthalamic nucleus, hyperdirect pathway

Expected duration of the project (yrs)

5

Purpose of the project (as in section 5C(3)3

- Basic research
- Translational and applied research

Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)

The observation of the actions of others is central to our social lives. Action observation rarely triggers movements in ourselves, despite the presence of significant activity within the brain motor network. Moreover, these signals clearly survive in paralysed patients and therefore can be harvested to help them to control external devices by means of brain-machine interfaces.

We propose that studying the mirror neuron system, which is active both during action/observation and action-execution, could offer novel insights into both the command signals that do characterise motor execution, and the mechanisms for suppression of unwanted movements, a key feature of human behaviour. We will search for the signals within motor system which are specifically associated with movement generation and movement inhibition. We will look for different brain sources of movement inhibition that occurs during action-observation.

Suppression is exaggerated in Parkinson patients leading to the pathological slowing of movement. Therefore, I will investigate cortical neurons which are directly connected to the subthalamic nucleus (STN), a brain structure belonging to the basal ganglia, and heavily implicated in Parkinson’s disease. Stimulation of this structure, known as deep brain stimulation (DBS), profoundlyameliorates the symptoms of Parkinson’s disease but it is still not known how exactly this therapy works.

Research in NHPs has been fundamental to the development of DBS for clinical treatment of movement disorders.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

My research will advance understanding of the brain activity evoked by action-observation as useful signals for brain-machine interfaces, and the mechanisms underlying the therapeutic effects of DBS for motor symptoms in PD.

What species and approximate numbers of animals do you expect to use over what period of time?

6 NHPs over 5 year period

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

All animals are pair-housed and are provided with an enriched environment and have large home cages, exercise pens and forage areas. They interact regularly during the day with investigators.The procedure involves a number of stages for preparing NHPs for long term recording in the awake state. This includes a number of separate and well-spaced surgeries under general anaesthesia. These are carried out under full aseptic conditions and involve a full regime of pre and post-operative analgesia. All anaesthetic procedures are carried out by our NVS and all surgeries are performed under his supervision. Training and recording sessions involve head and body restraint while recordings are taken from multiple microelectrodes advanced into the cortex. Neuronal activity is recorded while the NHPperforms its trained task. Recordings are usually taken from pairs of cortical sites. During the course of these studies, which typically last for 2-3 years, both cerebral hemispheres are investigated. The expected level of severity is severe. At the end of this procedure, the NHPs are humanly killed by an overdose of anaesthesia.

Replacement

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

To understand the human mirror neuron system and its role in suppression of unwanted movements, we need some invasive work that will allow us to interpret correctly and to calibrate the results of human non-invasive studies. It is not yet possible to sample activity of single neurons in the healthy human brain, and recordings from small populations of such neurons in a non-human primate model are essential for our understanding and interpretation of non-invasive methods such as fMRI and TMS.

Reduction

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

We use advanced experimental techniques which allow us to record more data simultaneously from different brain areas in a shorter time from a single subject. This directly leads to smaller number of animals being needed before enough data has been collected to allow thorough statistical testing of the scientific hypotheses.

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 an NHP model is essential for this project. Mirror neurons were first discovered in NHPs. The macaque’s motor system, including its mirror neuron component, closely

resembles that of the human. Rodent models could not be used for this research due to substantial anatomical differences and their inability to perform the complex tasks required to achieve the aims of the project.

Optimisation of animal welfare is achieved by a variety of approaches, which include:

- Positive Reinforcement Training (PRT) of animals to co-operate with the procedure so as to reduce stress
- Giving animals a small dose of oral sedative for the first few days after new procedures are first introduced
- Use of appropriate pre- and post-operative analgesic and antibiotic regimes
- Use of additional NC3Rs approved refinements to improve the outcome of the experiment and improve animal welfare.