

# **Animals (Scientific Procedures) Act 1986**

Non-technical summaries for project  
licences granted during 2018

Volume 1 (granted between 1<sup>st</sup>  
January and 30<sup>th</sup> June 2018)

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## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 1. Understanding formation and diseases affecting the limb musculoskeletal system</b>
<b>Key Words</b>	limb formation, limb birth defects, bone fracture repair
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We aim to understand how two key tissues of the musculoskeletal system-bone and muscle-are formed normally and how these tissues our maintained and repaired throughout life.

Diseases that affect proper functioning of the musculoskeletal (MSK) system are common and diverse. MSK disease can arise from birth defects, sports injuries, trauma, disease, normal wear-and-tear and age-related diseases. There are approximately 200 different musculoskeletal conditions that together account for 1 in 5 visits to the GP in the UK. This represents an annual cost of £5 billion to the NHS and results in 30.6 million working days lost a year, adding a further cost to the UK of £7.4 billion annually REDACTED

By understanding more about the underlying biology of how limb tissues, specifically muscle and bone, are formed and how they repair and can degenerate in old age or through disease we aim to address some of the challenges of MSK disease and contribute to health benefits that will enhance quality of life, health and productivity.

**What 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 tissues of the limb are formed normally we can better understand and diagnose birth defects caused by these processes being disrupted. This research will help contribute to the development of therapies to engineer components of the musculoskeletal system, muscle and bone. It will also aid in the development of improved diagnostic and treatment regimes for individuals with limb birth defects.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We use mouse as an animal model. We estimate to use approximately 3000 animals 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 levels of severity? What will happen to the animals at the end?**

The expected level of severity is moderate. The bulk of our work involves breeding and maintenance of mouse lines that have been genetically altered with the specific intended purpose of carrying out research into how limbs form and how diseases affecting the limbs can occur. Much of our work involves the breeding and maintenance of a reporter line in which a cell type involved in bone fracture repair is labelled fluorescent green. This allow us to enrich for this rare cell type and facilitates us in carrying out further research in vitro on the function and capabilities of these cells. In other work we carry out crosses of genetically altered mice to generate embryo material that is harvested after humanely killing the female mice and embryos. This approach allows us to model human disease and study how gene disruption can cause birth defects. In order to generate, store or replenish stocks of live animals it is necessary to carry out some surgical techniques on animals. These methods can help use produce frozen stocks to secure lines that we have produced and to enable these resources to be shared. Overall, these methods help reduce the total number of experimental animals used

## Application of the 3Rs

### Replacement

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

### Replacement

in parallel studies we are using human tissue samples from controls and patients with congenital limb defects. This work is limited to tissue culture experiments. Work with human cells lacks many of the powerful genetic tools available with the mouse model and we are only able to work with the human cells in culture and by analysis of (fixed) tissue biopsies. Nevertheless, they serve as an excellent complement to our work using (whole) animal models with our animal models providing valuable insight into design and interpretation of our experiments using humans tissues.

Tissue culture studies of muscle and bone can replicated some aspects of their normal behaviours but cannot replicate the entire tissue and the environment it is normally exposed to-the role of innervation, vascularisation and interaction with other tissue/body physiology for example. We therefore require use of the whole animal for some experiments.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We aim to use the minimum number of animals in order to generate statistically significant and/or robust data.

When possible we maintain lines and design breeding regimes that maximise the yield of mutant embryos or pups obtained in crosses. This reduces the breeding schedule, genotyping and numbers of animals used. Where possible, animals are genotyped based on a visible scoring of a consistent observable phenotype that has been established to be diagnostic of a particular line. We routinely use cryopreservation techniques to store our genetically altered lines and thereby minimise breeding schedules. This enables us to only maintain breeding pairs when animals are required for experimentation and reduces the numbers of animals used.

When appropriate pilot studies will be carried out to enable refinement of experimental design. Commonly we will carry out pilot experiments *in vitro* and *in vivo* using chick embryo model (at stages prior to the stages covered by the ASPA) that will reduce the number of experiments we plan to carry out using the mouse model. This approach allows us to replace the use of the mouse in some instances and also to refine our experiments prior to the use of the mouse model system.

We produce cell lines from the animal model that extends and expands the number of experiments that can be carried out from each animal produced. Established cell lines can replace some of our *in vivo* work. Some of the source material will be obtained from animals prior to regulated stages and typically prior to mid-point of gestation

## Refinement

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

### Refinement

The mouse is an excellent model system to use. The relatively close evolutionary relationship between mouse and humans make it an excellent model for studying the causes of human disease. Powerful genetic resources are available in the mouse, which enable us to perform precise experiments that can be accurately interpreted.

When appropriate pilot studies will be carried out to enable refinement of experimental design.

For some experiments, parallel studies will be carried out using human cells/tissues from control and patient samples. The results from experiments using either cells from human or mouse source will be used to refine experimental design.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 2. Central nervous system development, plasticity and repair</b>
<b>Key Words</b>	central nervous system, neural plasticity, learning and memory, glial cells, interneurons
<b>Expected duration of the project</b>	5 year(s) 0 months

## Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The purpose of this Project is to understand the development of different classes of brain cells, how they interact to form electrically active circuits and how those circuits drive normal behaviour. This information is needed before we can fully understand what goes wrong in neurodegenerative and psychiatric disorders, or what underlies age-related cognitive decline.

**Background**

There are two broad classes of neural cells in the brain – neurons (the electrically excitable cells) and glial cells (which support and modulate neurons in various ways).

Neurons communicate with one using "neurotransmitter" molecules that pass from one neuron to another at specialized contact sites called "synapses". They can reach out to one another, sometimes over long distances, via long, slender extensions of their surface called "axons".

There are two types of glial cell, oligodendrocytes and astrocytes. Oligodendrocytes ("multiply-branched cells") wrap lipid-rich membrane spirally around axons, forming fatty insulating sheaths called "myelin". Myelin allows electrical impulses to travel along axons much more rapidly and with less energy expenditure than along unmyelinated axons. Astrocytes ("star-like") envelop neuronal synapses and regulate neurotransmission in various ways. A major part of our research is

focussed on glial cells – how and where they are formed during embryonic development and what their functions are in the mature brain.

We also study "interneurons", an diverse set of small neurons that interact with and regulate the larger "projection" neurons that form long-range connections across the brain. Interneurons can excite or inhibit firing of projection neurons and hence are crucial modulators of circuit activity. We study interneuron diversification during development in order to illuminate their adult properties and how they influence circuit activity and behavioural outputs.

Our studies are mostly performed in mice. Our general approach is to perturb molecular or cellular pathways in transgenic or mutant mice in order to determine how those pathways normally influence the normal development of glial cells or interneurons, including the numerical balance of the different cell types, and the behavioural and cognitive abilities of the mice.

## **Aims**

1. To understand the role of glial cells in learning and memory. It used to be thought that learning was solely a property of neurons, involving strengthening or weakening of synapses. We now understand that glial cells can also detect and respond to activity of neural circuits in "real time" altering circuit properties according to need or experience. 1. To understand the role of glial cells in learning and memory. It used to be thought that learning was solely a property of neurons, involving strengthening or weakening of synapses. We now understand that glial cells can also detect and respond to activity of neural circuits in "real time" altering circuit properties according to need or experience. Thus, glial cells are more responsive to their environment and susceptible to modification (i.e. they are more "plastic") than previously imagined; we aim to understand 1) the nature of this plasticity, 2) its effects on circuit properties and behavioural outputs and 3) the cell-cell interactions, signalling pathways and molecules that drive glial plasticity.

2. To understand the molecular regulation of interneuron development, maturation and their interactions with other cells. We also wish to understand how subtle perturbations of normal interneuron development can influence mouse cognition and behaviour, potentially leading to ASD-like symptoms (e.g. altered social interactions).

### **What 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 primarily directed towards increasing basic knowledge of how the brain's cells develop and interact with one another to create functional neural circuits. This is pre-requisite to an understanding what goes wrong during neurodegenerative diseases like multiple sclerosis or Alzheimer's disease, or in neuropsychiatric disorders like schizophrenia or ASD.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

For most of our studies we will use wild type, transgenic and mutant mice. During mouse breeding we inevitably generate large numbers of offspring, many of which will not be useful experimentally (e.g. because they do not carry the desired combination of transgenes or mutations) and will be culled humanely soon after birth. The total number of mice generated by breeding will be ~4,000 per annum. For studying early stages of development we will use chick embryos, which can be manipulated easily in ovo (e.g. by electroporation of DNA into the embryonic neural tube). (~100 chick embryos 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 levels of severity? What will happen to the animals at the end?**

Adverse effects include unexpected events such as infection following surgery (very rare), or harmful phenotypes resulting from crossing mutant mice that individually are not harmful but interact genetically to cause an adverse phenotype. Adverse neurological phenotypes can include tremor, ataxia, circling behaviour or general discomfort manifest by lack of grooming, weight loss or inactivity/lethargy. Any such animal will be humanely killed immediately or, in the case of scientifically informative and important phenotypes that would be classified "moderate", the REDACT Management and Named Veterinary Surgeon will be consulted and the Home Office Inspector informed. Any animal that develops adverse effects classified "severe" (e.g. seizures) will immediately be humanely killed. All other animals will be humanely killed at the end of the experiment. Some experiments, such as focal demyelination, are predicted to cause adverse effects; in such cases the animals will be closely monitored and humanely killed if they are obviously distressed.

## Application of the 3Rs

### Replacement

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

### Replacement

We are trying to understand the cellular correlates of learning and memory, which can only be observed in intact, behaving animals. We are especially focussed on the role of oligodendrocytes and astrocytes, which do not exist in invertebrate animals such as *Drosophila*.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

We will design our mouse breeding strategies carefully to minimize the number of generations necessary to reach the desired endpoint (i.e. desired combination of transgenes/ mutations), and to cut down the number of unwanted offspring. Where appropriate, we will use otherwise unwanted offspring for negative control experiments.

## **Refinement**

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

## **Refinement**

We use mice because this is the only mammalian species that can be easily manipulated genetically, and for which brain anatomy, physiology and function has been well documented

Our mice are group-housed where appropriate to avoid stress. Environmental enrichment is used whenever possible (e.g. nesting box included along with adequate nesting material). Our behavioural tests (e.g. running wheels, T-maze or radial maze) can arguably be considered environmentally enriching in their own right. Atmospheric conditions and health status in the animal facility are closely monitored and kept within strict limits. Biological Services staff and our own researchers are experienced in animal handling and welfare and trained to spot signs of discomfort. Any mice that are in unnecessary distress or discomfort will immediately be killed humanely.

Where possible and appropriate, we will administer drugs (e.g. EdU) via the drinking water rather than by injection or other invasive routes.

For studies of early neuro-developmental processes, which are largely conserved among vertebrate species, we will use chicken embryos rather than mice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 3. Causes and consequences of genome instability</b>
<b>Key Words</b>	Chromosome, Mutation, Cell Cycle, Stress
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The human body contains many hundreds of different cell types, each of which could theoretically become cancerous. In reality, certain cell types form tumours much more frequently than others, and in many cases the reasons for this are poorly understood. This project will investigate two possible explanations for the different rate of tumour formation in different cell types. First, we will investigate whether the speed at which cells divide can influence the amount of damage to DNA, which is a common cause of cancer. Second, we will investigate whether different types of cell respond differently to DNA damage, for example by dying, remaining alive but no longer dividing, or continuing to divide.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Research on cell division has traditionally been conducted in yeast and human cancer cells grown outside of the body, and so we know relatively little about how basic cellular processes such as DNA replication and mitosis occur in living tissues. In particular, how cell division processes differ between cell types is not known. Because cancer arises due to errors in cell division and different cell types give rise to tumours at drastically different rates, it is essential to study cell division in its natural context in order to gain new insights into the causes of cancer. This project will study the speed of cell division in different cell types of normal and tumour-prone mice, the amount of DNA damage that arises when cell division goes wrong, and how different types of cells cope with damaged DNA. We expect that this will give insight into why certain cell types become cancerous more frequently than others, which is one of the biggest unanswered questions in cancer biology. Although not a primary aim of this project, it is anticipated that the knowledge gained could provide new molecular targets for cancer therapy and prevention in high risk individuals.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 6000 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 levels of severity? What will happen to the animals at the end?**

This study will use genetically altered mice that develop tumours. All of the animals will be housed in a modern animal care facility and will be monitored daily for signs of illness due to tumour formation or other causes. We are interested in the early stages of tumour formation and thus the expected adverse effects due to tumours will be kept to a minimum, mostly at the mild level of severity (although occasionally moderate). At the end of the studies the mice will be humanely killed and dissected to analyse phenotypes in premalignant cells and tumour formation. The mice will be treated with labelling agents that allow cell division to be monitored in living tissues, and chemotherapeutic drugs that either cause DNA damage, or affect the cells' ability to detect damaged DNA. These compounds are widely used in the laboratory and the clinic, and are expected to cause no more than mild distress to experimental animals.

## Application of the 3Rs

### Replacement

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

### Replacement

The aim of this project is to better understand how cell division happens in mammalian tissues to gain insight into cancer initiation. This cannot be achieved without at least some studies using protected animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Where possible, mice with existing genetic abnormalities will be imported rather than generating new ones. We will perform pilot studies using both *ex vivo* cell cultures and *in vivo* modelling; where the results are consistent, *ex vivo* models will be used preferentially.

Use of previously published genetic alterations will allow effect sizes to be estimated for power calculations that will ensure only a minimal number of animals are used in order to achieve statistical power.

Where possible, we will extract cell types of interest from mice after they have been humanely killed, to minimise the number of regulated procedures such as drug treatments to which the animals are subjected.

### **Refinement**

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

### **Refinement**

Mice represent appropriate experimental subjects as they are of small size, have relatively short lifespans 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 as set out here.

Our emphasis will be focussed on sound experimental design to test our hypotheses, based on experience from our own previous work, reports available in the literature, appropriate use of small scale pilot studies to refine protocols to be used, use of organ-specific or tissue-specific gene alterations, and appropriate use of statistical tests of significance, such as the Kaplan-Meier. Harm will be minimized by careful daily observation of the mice for signs of illness and use of well designed protocols based on previous experience.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	Project 4. Hereditary diseases of the eye
<b>Key Words</b>	Eye, Disease, Retina, Development, Therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Genetic eye disease causes a significant loss in quality of life and affects approximately two million people in the UK. Despite an increased understanding of the genetics that underlies these diseases, much less is known about the function of the causal genes in normal and pathological conditions. It is the aim of this project to develop mouse models of human eye diseases in order to better understand visual function and how it is disrupted in individuals suffering from inherited eye disease. We aim to use the knowledge gained from these models to design and test new therapeutic 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)?**

This project will firstly benefit patients suffering from eye disease by providing them with a better understanding of their condition and potentially leading to new therapeutic strategies to combat their symptoms. Additionally, ocular disorders represent a large financial burden to the NHS, so new treatments and better diagnoses based on the research output of this project could alleviate financial spending. Lastly, this project will advance science by increasing our understanding of eye biology, which can be applied to other vertebrate organisms including humans.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will utilise mice as they have been shown to be an excellent model for studying eye function and the lowest suitable species that can be used. We plan on using 16000 mice in total over the five year duration of this project. This includes both wild type and genetically altered mice and includes mouse models currently under, and generation of future mouse models based on newly identified eye-disease causing genes from the clinic.

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

The majority of mice used in this study will be aged and subjected to visual inspection and behavioural analysis (no anaesthesia) and tissue collected for analysis after humane euthanasia (expected severity = mild). It is well documented that loss of vision in mice does not affect their ability to feed, drink, groom or reproduce. Other eye monitoring procedures and drug administration will require general anaesthesia (expected severity = moderate). Careful monitoring, use of appropriate pain medication and anaesthetics together with humane end points will be used to minimize animal suffering.

## Application of the 3Rs

### Replacement

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

### Replacement

The eye is an extremely complex organ with many different cell types that contribute to eye development and function. At present there are no cell culture models that can recapitulate a fully functioning eye. For this reason, animal models are still necessary to study eye function. Mice have proven to be excellent models for studying visual function, so as such, the techniques used to study the eye in mice have been extensively refined. After phenotyping of mouse models, we do have available different eye-derived cell lines

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Number of animals required for the project will be kept to a minimum through the implementation of good husbandry practise. Furthermore, the number of animals required to confirm statistical significance of the retinal degeneration phenotype will be determined using a power calculation before the experiments begin. A statistician will be consulted to ensure that our calculations are correct.

Mouse models will only be generated for genes where no existing mouse models have been generated or the disease-causing potential is already well understood.

### Refinement

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

### **Refinement**

The species of choice for these studies is mice. This is because they are mammals and as such have a retina highly similar to humans and have been proven to display retinal degeneration similar to that observed in humans. The NVS will be consulted prior to all experimentation to ensure that the most appropriate methods to minimise animal suffering. All mice will be closely monitored for signs of distress, and analgesia or euthanasia used as appropriate to minimise animal suffering.

To ensure that any resulting phenotype in our mice are truly representative of human disease, and thus a valid model, we will utilise the latest genome editing technology to knock in exact patient-identified mutations into the mouse genome. This approach ensures that the models we generate are the most appropriate and representative of the human pathology.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 5. Understanding the role of inflammation in dementia</b>
<b>Key Words</b>	Alzheimer's disease, Dementia, inflammation, co-morbidity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Alzheimer's disease (AD) is the most common cause of dementia in the elderly with over 850,000 people in the UK being affected, and this is expected to double within 20 years. Therefore, AD represents a significant medical problem, especially as there is currently no cure and no way to slow progression of the disease. There is therefore an urgent need to understand in more detail the underlying mechanisms that contribute to AD, so that new treatments can be developed.

Here we aim to understand how inflammation contributes to dementia such as that seen in Alzheimer's disease. The inflammatory response is how our immune system reacts to a stress or danger. Danger signals that are released in the brain of someone with Alzheimer's disease (AD) alert the immune system and inflammation then occurs. It is thought that this inflammation is bad for the brain and contributes to the problems seen in AD such as memory loss. However, we do not fully know how this inflammation happens and we aim here to find out. We also want to try to find ways to stop this inflammation from happening or from stopping it doing damage.

**What 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 benefit of this programme of work is related to new knowledge about how inflammation affects dementia and AD. In defining this contribution we hope to be able to identify and test new ways to help these conditions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice (5000) and rats (1500) 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 levels of severity? What will happen to the animals at the end?**

Mice (and rats) will be used as there are several available mouse models of AD and some new rat models. These animals appear normal and healthy but develop problems in their memory over time. Disease progression will be monitored over several months by mainly studying pathology and behaviour. Pathology will be tested post-mortem, but we will also scan the brain, which will allow the progression of AD to be studied in the same animal over a long period of time, which will reduce animal numbers. We will also test the memory using a series of well-described tasks that are not harmful to the animals. Much of our analysis will be carried out post-mortem on tissue from animals that undergo no treatment and therefore no adverse effects are expected in these animals. Where there is treatment or an intervention (induction of inflammation) some mild changes may be observed e.g. infection may cause a fever and sickness-like behaviour in the animals, but these effects are transient. One of the behavioural tests (Morris Water-Maze) involves placing the mice in a pool of water, which may induce mild stress. Animal welfare will be continually monitored and any issues will be discussed with the named veterinary officer. No serious adverse effects are expected and we are very well aware of minor adverse effects that may be seen, and have taken great efforts to reduce them.

## Application of the 3Rs

### Replacement

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

### Replacement

Mice and rats will be used in this project. Studying the mechanisms involved in dementia and AD is extremely complex and involves understanding the interactions between different systems in the body (e.g. nervous, immune, and vascular). It is very difficult to mimic such complex interactions *ex vivo*, and whole animal *in vivo* experimentation is therefore vital in order to obtain a greater understanding. In addition to the pathological changes in the brains of AD patients, the disease is characterised by problems in learning and memory, and as such this behaviour is very difficult to model *in vitro*.

The proposed studies could not be undertaken in lower species because they do not show such similarities to humans (e.g. do not have some of the immune molecules), and *in vitro* experiments do not allow the study of interactions between different body systems (i.e. immune and vascular), which are critical for this project. Thus, the questions and hypotheses to be addressed cannot be fully studied *in vitro* alone and require *in vivo* studies.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Several factors lead to a reduction of animal numbers, including reducing variation and good experimental design involving the use of appropriate statistics. In particular statistical tests will be used to ensure that we use the minimum number of animals possible to reliably interpret our data.

## **Refinement**

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

### **Refinement**

There are several models AD and currently the most relevant model available to study AD are mice and rats. They are also the lowest vertebrate species that share common pathways to humans with respect to inflammation. We will use well-established methods to cause inflammation without causing severe or long lasting harm to the animals. Sometimes we will test the behaviour of the animals but the tests we will use do not cause any distress or lasting harm and usually rely on natural behaviour of the animals (exploration, social interaction) However, all animals will be constantly monitored to ensure that they suffer minimum distress

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 6. Targeting of B cells in rheumatoid arthritis</b>
<b>Key Words</b>	B cells, inflammation, rheumatoid arthritis, autoimmunity, antibodies
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We have identified a population of B cells in the joints of patients with rheumatoid arthritis, which contributes to inflammation and disability. They express a surface protein which is unique to this type of cells. We have produced human recombinant antibodies specific for this protein and have extensively tested and selected them for their ability to deplete these B cells in vitro. As the next step towards development of a new drug we now need to test whether these antibodies deplete B cells expressing this marker protein 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)?**

Many patients with rheumatoid arthritis benefit from a drug that removes a type of immune cells known as B cells. However, this therapy has disadvantages because some of these cells are needed for the response to new infections and for vaccinations to work. We are developing a more specific therapy that targets only a small subset of pathogenic B cells to overcome these disadvantages for better treatment of patients with rheumatoid arthritis and other related diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We estimate that we will need ca. 250 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 levels of severity? What will happen to the animals at the end?**

In one series of experiments the mice will be injected with cells. It is possible that these cells will grow excessively. If they affect welfare of the mice, we will humanely

kill the mice according to the prescribed protocols. The mice will be injected with our drug and we will monitor the signs of inflammation. For all our work we will seek the advice of the named veterinary surgeon to ensure we give appropriate levels of pain relief. At the end of the experiments the mice will be humanely killed according to the prescribed protocol. In all experiments the severity we anticipate is moderate.

## Application of the 3Rs

### Replacement

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

#### Replacement

Prior to this work, we have extensively tested our drug on in vitro models. We initially used cell lines and used several experimental models to test whether the drug could deplete the population of cells we are targeting. We have found clear evidence that the drug works in three of these in vitro models. We then moved on to testing the drug on cells isolated from the inflamed joints of patients with rheumatoid arthritis. These experiments also showed a clear effect of our drug.

While these experiments provide evidence that the drug is effective in vitro, they do neither accurately replicate the complex conditions within the body. To determine how the drug functions in a way that is meaningful, it is essential to use an in vivo model. This is an important step that is mandatory for all new drugs as part of the regulatory process.

However during the course of this licence we will continue to systematically review the literature for any developments that could replace the use of mice in our work.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Initially, small pilot experiments will be used to determine how large the groups of mice have to be to achieve a reliable result. This preliminary work will allow us to limit the number of animals required in each experiment to a minimum.

For the experiments with transferred cells we will use a novel scanning technology that means that we can measure the number of transferred cells in the same animal multiple times. This will allow us to greatly reduce the number of mice needed.

Experimental designs will be supported by the NC3R's research design tool, and we intend to publish in journals that support the ARRIVE guidelines.

### Refinement

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

## **Refinement**

Choice of species:

Mice are an established, well-understood and reliable model for human disease. Consequently there is a wide range of established techniques, and that make them more suitable than other animal models.

Non-mammalian animals are limited in their use because they either do not possess the cells we need to target or are too far removed from the human immune system to provide relevant results.

Methods

Most of our experiments do not last more than a few days, and therefore have only a limited impact on the animals.

The methods outlined in this licence are all established. As a group we have many years of experience with these adverse effects are rare. However, we do anticipate some discomfort on injection and acknowledge that recovery from anaesthesia, whilst not painful, is unsettling for mice.

Continuous monitoring and refinement

We shall work closely with the vet and the REDACT to discuss appropriate monitoring of the mice. Pilot experiments will allow us to refine our procedures, a process that will continue throughout this licence.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	Project 7. p53 pathways in cancer and metabolic diseases
<b>Key Words</b>	cancer, metabolic diseases, GA mice, therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Cancer, a major health issue worldwide, is caused by a series of changes in genes (genetic mutations). Understanding how these mutations change the normal cell to a cancer cell, and how such changes alter the way in which cancer cells interact with the rest of the body, is vital if we are to prevent and treat cancer. Only in the context of the complete living animal can we fully understand how cancers develop, invade and spread to other organs (metastasise). Using genetically altered mouse models with the same genetic mutations as in the human disease ('patient-like' animal models) we can investigate the biological consequences of these mutations in cancer progression, and identify how cancer cells co-opt normal cells to drive invasion and metastasis. These models can also be used to understand how preventable factors, such as obesity, promote cancer development. The genetic changes that occur in cancer can be very variable and complex, but a few alterations are consistently found in many types of cancer and appear to be fundamentally critical for tumour development. One such alteration is mutation within the p53 gene, which changes the function of the p53 protein. Alterations in p53 lead to many changes that contribute to cancer development. We are specifically interested in how p53 controls metabolic pathways within the cancer cell and how p53 affects the interaction of the cancer cell with surrounding cells, such as fat cells and immune cells. Defining metabolic functions and adaptation of tumour cells will enable us to design new cancer drugs, and understanding the effect on the immune response may help us identify patients who will respond to the new generation of immunomodulatory cancer therapies. We are also looking at how p53 works in other responses such as aging, tissue regeneration or metabolic disease. This information will help us identify less toxic cancer therapies and may allow us to repurpose drugs that are presently used for the treatment of metabolic diseases such as diabetes for cancer therapy.

These questions can be addressed using well established protocols that will allow us to explore the interplay between p53, inflammation, immune responses and

metabolism. Our ultimate aim is to develop new therapeutic strategies that can be taken forward into human applications

**What 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 deepen our understanding of the underlying causes of cancer and metabolic diseases in general and of specific types of cancer which currently have a poor prognosis such as pancreatic and liver cancer. Knowledge of the genetic causes will dramatically improve our ability to diagnose, treat and prevent cancer, which affects almost half of the human population. We will also use mouse models to identify and test new therapies which will benefit cancer patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project uses mice (including genetically engineered models). We expect to use up to 7,000 mice per year over 5 years. It should be noted that 70% of these will not undergo scientific procedures, but will be used solely for breeding and maintenance of colonies.

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

Animals will be bred to achieve test subjects which may be predisposed to cancer and/ or obesity/diabetes. Approximately 70% of the mice will not show any adverse effects relating to their breeding and not undergo any procedures except for ear notching for identification and genetic testing. These will be humanely killed when they are no longer required for breeding. A proportion of animals (no more than 25%) will develop diseases because of their genetic makeup or because tumour cells have been implanted and allowed to grow. This may require administration of an agent to switch on/off particular genes which only causes momentary discomfort but reduces off-target effects in other tissues. Animals will be monitored closely by highly trained staff for well-established clinical signs such as weight loss, swelling of the abdomen, and development of visible or palpable tumours. Some of these animals (15-20%) will be given anti-cancer treatments, changes in their diet or cancer causing agents (for example chemicals/irradiation). All animals on treatment will be closely monitored and may be blood sampled to follow changes in biomarkers which should cause only mild handling stress and momentary discomfort, or may be imaged. Any animal that displays signs of illness such as immobility or ruffling of the coat will be humanely killed. At the end of the study all animals will be humanely killed and tissues collected at post-mortem to gather as much information from the study as possible.

## Application of the 3Rs

## **Replacement**

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

### **Replacement**

Although many aspects of cancer research can be conducted using cells, it is impossible to fully model the complexities of a tumour, which is an interaction of many different cell types (tumour cells, immune cells, blood vessels). Furthermore, the ability to monitor how cancer cells invade and spread to other organs requires an animal model. Finally we know that cancer cells respond differently in the lab to anti-cancer therapies as they do in the context of the living organism and so testing the efficiency of such therapies requires a complete animal system. For metabolic diseases, changes of whole body metabolism or organ functions due to different diets such as high sugar high fat diet cannot be recapitulated using cell system.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We perform preliminary experiments using only a few animals, before scaling up to the appropriate numbers for a full study. Numbers are calculated based on our experience using the same models, published literature and advice of our in-house statistical experts. We also share animals between experimental groups where possible e.g. when we need normal animals for controls, we can often obtain these from our breeding colonies where they would normally not be needed in a study. We constantly optimise our breeding strategies to minimise the number of animals needed to achieve the desired genotypes for our studies and we use tumour transplant models where appropriate, which do not require breeding of genetically altered animals and thus use fewer animals in total per study.

To reduce numbers of experiments we also perform studies using cell lines or 3D models so that only our strongest hypotheses are tested in the mouse.

## **Refinement**

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

### **Refinement**

We use mouse models with the same genetic changes that are known to cause human cancer – so accurately replicating the human disease. These genetic changes are specifically altered in the tissue of interest so that unrelated effects in other tissues do not occur. All animals are monitored regularly for signs of normal behaviour and are humanely killed if they exhibit moderate adverse symptoms. All staff are expertly trained in these clinical signs. Regular monitoring of mouse welfare allows us to complete studies at the earliest endpoint in which we observe a significant result to prevent unnecessary suffering resulting from high tumour burden.

We always refer to previous studies for adverse effects of anti-cancer or anti-diabetic therapies and when a group is given a treatment for the first time, we initiate the study with a small number of animals (n=3-6) which is closely monitored before extending to a larger number.

Animals are housed in a dedicated facility proactive with environmental enrichment and receive anaesthesia and analgesia as appropriate.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 8. Respiratory tract regeneration and tumorigenesis</b>
<b>Key Words</b>	Respiratory, Regeneration, Cancer development, Repair
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this licence is to establish the cells and the molecules that are responsible for maintaining the respiratory tract (lungs, airways, ear, nose and throat) in healthy individuals and the processes and factors that drive respiratory disease and 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)?**

We will increase our understanding of respiratory tract biology in health and disease and in the development of cancer. This will enable us to identify and develop new therapeutic agents that could potentially be used to treat human respiratory diseases and/or to halt or reverse the progression of cancers. In addition, increasing our knowledge of the cells and processes that maintain the respiratory tract will enable us to bioengineer segments of the respiratory tract for tissue engineering applications, which could eventually be used to treat patients with irreparable damage and poor quality of life.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

9,500 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 levels of severity? What will happen to the animals at the end?**

The majority of animals will be used in models of lung disease, tumour development or airway tissue engineering. These animals may develop tumours but these are generally well-tolerated. Some animals may experience reduced mobility, reduced

lung function and some short periods of respiratory distress, but are not expected to show prolonged signs of breathing difficulties. In addition, some animals used in this project will experience weight loss but this will be closely monitored. A small proportion of animals will undergo a surgical procedure but these procedures are expected to pose minimal risk to animal welfare. Furthermore, some animals used in this project will be immune-compromised but these animals will be carefully looked after to minimise chances of infection. Studies will be designed to ensure only the minimum number of animals required are used. Throughout studies, animals will be regularly monitored; if any animal causes concern, action will be immediately taken to alleviate this and if this is not possible the animal will be humanely euthanised. At the end of each experiment, all animals will be culled using humane methods and tissues will be taken for further analysis.

## Application of the 3Rs

### Replacement

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

#### Replacement

The respiratory tract is extremely complex, with interactions between various different cell types and processes. This cannot be adequately mimicked using *in vitro* studies and, although *in vitro* and *ex vivo* experiments will be used to establish whether *in vivo* experiments are necessary, studying the respiratory tract in health and disease in a whole animal is crucial.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will plan our studies so that only the minimum number of animals required is used. We will also make use of minimally invasive imaging techniques to enable us to monitor changes in a process longitudinally in a single animal, which will eliminate the need to use more animals at different time points. Finally, wherever possible we will make use of alternative methods to genetically modify mice rather than by doing this through mouse crosses, which will reduce the number of animals generated unnecessarily.

### Refinement

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

## **Refinement**

The mouse models we intend to use have been chosen because they are widely accepted to be the most appropriate and relevant to the human condition they mimic. We have extensive experience in each of the chosen models, which allows us to reduce the number of animals required, to limit the invasive procedures carried out and to limit the discomfort experienced by the animals. Throughout this programme of work we will continue to monitor our own practices and the literature to look for ways to refine our procedures; these will be incorporated into our protocols wherever possible.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 9. Safety and efficacy of hepatic regenerative medicines</b>
<b>Key Words</b>	Liver, Injury, Regeneration, Repair, Stem cell
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Liver disease in the UK is increasing in prevalence. Novel medicines have the ability to promote tissue repair and aid liver regeneration (to re-build and gain function). However, they need rigorous testing before being applied to humans. The aim of this work is to assess the safety and mechanism of action of novel therapies to promote hepatic regrowth by finding out 1. How liver disease occurs, 2. How the liver repairs itself and 3. What happens when regrowth goes wrong? By understanding these events we can better design and use safely drugs that can prevent liver 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 benefits of this work primarily focus on liver disease and the treatment of such. However many of the processes that occur during liver disease also occur during disease in other tissues so our work in the liver will contribute to disease progression in a range of organs. The impact of techniques for the development of tracking regenerative medicines (cells, biologics) by imaging has a far wider benefit as these therapies have the potential to be utilized in curing a vast number of diseases. The translation of the novel imaging methods to assess liver disease can also be applied to other diseased tissues so our work will contribute to disease progression in a range of organs

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice and rats over the five years. We will use a maximum of 7900 animals

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

As we are modelling disease our animals they will progressively demonstrate symptoms of the disease. However, using imaging and non-invasive blood based

biomarkers we can closely manage these signs and generally can define disease at a much earlier stage and therefore we ensure that the animals do not undergo any undue suffering

## Application of the 3Rs

### Replacement

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

#### Replacement

Regeneration (or regrowth/re-establish function) in the liver is a complex, multi-staged process in which there are any different cell types interacting with one and other. It is impossible to model such complexity without using animal models and an understanding of potential safety implications for human health must be established in animal models first.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

It is possible to calculate the numbers of animals required for experimentation based on data from previous data. Imaging and non-invasive blood-based biomarkers lets animals be used as their own control, allowing paired comparisons. Moreover, imaging is inherently sequential, increasing statistical power and using fewer animals to achieve the same statistical power as conventional designs. In all cases we ensure that we have calculated the minimum number of animals required for the experiment to give us useful data. This approach reduces the animal numbers required, and also reduces the likelihood that the animal experiment would have to be repeated.

### Refinement

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

#### Refinement

Using imaging non-invasive blood-based biomarkers we regularly refine the disease models we use to reduce animal harm and to improve the effectiveness of our models. We can also stage disease and stop experiments before external clinical signs appear, thus limiting disease severity. Because of this we can ultimately use fewer animals per procedure to and still generate meaningful and clinically relevant

data. We also regularly monitor body weight, body condition, food and fluid intake of animals as a measure of disease; we set strict limits to ensure that there is limited harm to the animals used.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 10. Staphylococcus aureus and other pathogens: Disease to therapy</b>
<b>Key Words</b>	Infectious disease, antibiotics, vaccines, pathogens
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Infectious disease is still a major cause of human suffering and mortality around the world. This is made worse by the spread of antimicrobial resistance. Thus, there is a pressing clinical and societal need to understand the basis of infectious disease and to develop new prophylactic and therapeutic regimes. The aim of the project is to understand how pathogens cause infections within a host and how this knowledge can be exploited to reduce the burden of disease within the human population. Our primary disease causing organism for study is the bacterial pathogen *Staphylococcus aureus*, most well known in its antibiotic resistant form as the superbug MRSA. To achieve this aim we have 4 linked objectives to:

- Assess the role of host and pathogen factors in disease progression.
- Analyse how other microorganisms effect the outcome of *S. aureus* disease.
- Determine how microorganisms interact to lead to a disease outcome
- Use the findings from the above objectives to develop new intervention strategies to prevent or treat infectious 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 programme of work will lead to the significant advancement in the understanding of infectious disease and the identification and optimization of candidate molecule/s and therapies able to prevent or treat infections. Understanding the basis of infectious disease and the ability to develop new prophylaxis and therapy will be of a huge benefit to human healthcare. This is also set against the backdrop of the spread of antimicrobial resistance making the development of new translational opportunities extremely beneficial for human health. The project will take a wide-ranging approach to understand how *S. aureus*, and other pathogens, cause disease and how this knowledge can be utilised. The knowledge gained will be directly useful to researchers more working on infectious diseases and the host immune system. Translation of our findings will allow existing treatments and prophylaxis to be used more wisely and the development of new

regimes. This will be useful to the pharmaceutical industry, vaccinology and ultimately patients. The benefits will be manifest at multiple levels and timescales.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

During the 5 years requested within this project we estimate to use a maximum total of 11,000 mice. There is a breadth of knowledge on mice as models in biological and medical research, specifically in immunology and infectious diseases. Mice have comparable anatomy, genetics, and body functions -including immune responses- to humans.

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

Mice will be infected with *Staphylococcus aureus*, and other pathogens, by injection or inhalation, mirroring human disease processes. The injections alone are unlikely to cause more than momentary pain or distress. Where infection occurs, mice may lose body weight, change in fur quality, change in posture, reduced inquisitive behaviour, arthritis in joints and general decreased mobility. The onset of significant infection of major organs can lead to organ failure and death, but mice are culled before they experience such extreme symptoms. Procedures will be performed by closely supervised, highly trained and competent personnel. Pathogens, or substances, to be administered will be applied in conditions such that have minimum possible impact in the animals, and the protocols will be kept under regular review. In the course of the procedures, the animals will be very carefully monitored for signs of pain, suffering or distress. Animals giving any cause for concern will be more frequently assessed and the advice from professional animal care staff, including the veterinary surgeon, will be sought as appropriate. Health and welfare scoring schemes have been developed and applied, and animals whose health measurably deteriorates will be culled without delay. At the end of every procedure the animals will be humanely culled and multiple parameters measured to give optimal readouts from the subjects.

## Application of the 3Rs

### Replacement

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

### Replacement

We aim to replace protected animals as much as possible but ultimately determination of the role of host and bacterial factors in disease and the development of novel interventions requires the complex interactions given by the murine models. We use a combination of *in vitro* and *ex vivo* cellular models to

evaluate hypotheses before they are tested in animal models. In particular we then use the zebrafish embryo model of infection to inform studies in the mammalian system. This allows us to replace the use of mice in preliminary work.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

Experimental studies will be designed to maximise the data obtained from the fewest animals with the help from professional statisticians and experimental design experts. Preliminary dosing experiments have reduced the numbers of animals required. Our discovery that non-pathogenic organisms augment *S. aureus* infection has led to much more robust data, which in turn has led to a new statistical analysis allowing the number of subjects to be reduced by half. This is an extremely significant reduction and we will evaluate if this can be translated to other pathogens.

### **Refinement**

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

#### **Refinement**

Protocols will be regularly reviewed for opportunities to reduce severity whilst still achieving the scientific goals. Particular attention, are given to developing predictive health scoring schemes which could be used to identify early signs of developing disease and which will provide opportunities for applying early endpoints.

Mouse models of *S. aureus*, and other pathogen associated, infection are well established and have been refined to allow the most useful information to be gained with the least possible pain, suffering, distress or harm to the animals. Suffering of all animals is minimised by a stringent monitoring of health status, and frequent measuring of body weight. The range of models used is a testament to the variety of important pathologies for which *S. aureus* is responsible. Our objectives are to understand the basis of bacterial infection and to use this knowledge to develop new ways to prevent and treat disease. Determination of the underlying principles of disease will allow the number of models to be subsequently reduced. Only personnel proficient on the procedures, the discrimination of pain, suffering and distress, and excellence in husbandry and care conditions will implement the protocols.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 11. Characterisation of Skin Cancer Stem Cells</b>
<b>Key Words</b>	Cancer, Genetics, Therapeutics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Deaths from cancer are the leading cause of death in the UK, and the development of novel diagnostics and therapies remains an unmet clinical need. This project is aimed at increasing our fundamental understanding of the causes of cancer and the development of experimental preclinical models of human skin cancer that will accelerate the translation of novel therapies into the clinic. It is now clear that genetic status is fundamental to altered predisposition to cancer, cancer progression and response to therapy. Understanding the biological mechanisms, such as the role of cancer stem cells, associated with these genetic changes will be vital to facilitate new treatment strategies for human disease. In this project we wish to approach these needs using existing and new mouse models of cancer available to achieve two primary goals. First, we wish to better understand the fundamental biological mechanisms underlying skin cancer development. Second, we wish to explore how this knowledge might be used therapeutically, especially in the broader context of specifically targeting cancer stem 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 potential benefit relates to new knowledge about cancer stem cells, in cancer initiation, growth and metastasis. The aim is to publish the findings in academic journals. The information is likely to be of interest to pre-clinical scientists interested in tumour biology. The second potential benefit relates to the value of the results to clinicians, in particular oncologists, and to the possibility that new molecular targets and therapies maybe identified for which new pharmaceutical products could be developed.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mouse, approximately 7,000 over the five-year course.

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

This project will not exceed moderate severity, mostly mild but in some cases will be moderate, with safeguards of a structured approach to monitor clinical signs within the protocols. Mice will be generated that develop skin cancers, in some cases this will involve painting a carcinogen onto the back skin of mice, the subsequent growth of these tumours will be monitored to not exceed 17mm diameter. In addition, using established protocols that we have developed mouse and human derived skin cancers will be grafted on the skin of recipient immune deficient mice, the subsequent growth of these tumours will be monitored to not exceed 17mm diameter. Ultimately the growth of these skin tumours and response to therapeutic intervention will be evaluated using a variety of approaches (including blood sampling, cell proliferation assays and radiological imaging). Procedures involving pain will be mitigated by the prior use of anaesthesia and continuous analgesia. All animals will be killed at the experimental end point.

## Application of the 3Rs

### Replacement

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

### Replacement

We aim to determine the genetic control of cancer development and the responses of cancers to a range of agents including therapies. The extremely complex nature of such *in vivo* responses makes it impossible for these studies to be completely recreated in artificial systems *in vitro*. However, we have been pursuing the establishment of alternative procedures that have the potential to at least partially replace the use of animals. Most prominently we have been developing 3-dimensional culture systems for both normal tissues and tumour counterparts. To date we have established these for two skin cancers and have begun to develop this approach for melanoma tissues. Where possible and appropriate, we are using these *in vitro* approaches to inform our *in vivo* studies, with a view to replacing some *in vivo* studies and reducing and refining others, for example by establishing more precise hypotheses which can be directly tested *in vivo*.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The project incorporates a number of strategies to limit the number of animals required:

1. Preceding cell culture experiments will be used to determine many parameters and animal experiments will only be used where there is no other alternative.
2. Use of high resolution live animal imaging modalities reduces the number of animals.
3. Statistical approaches and good laboratory practice will be used to ensure the optimal experimental design and so limit the number of animals required.

### **Refinement**

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

### **Refinement**

The transgenic approaches we will use in most cases the phenotype will be restricted to the organ tissue we are interested in, namely the skin. To minimise suffering, aseptic surgery will be conducted in a laminar flow hood under general anaesthesia. For tumour bed preparation, protocol 4, gelatin foam will be preferentially used because it is more conformable and therefore less likely to ulcerate than glass disks. Our strategy to determine the minimum number of cells that can propagate a tumour ensure that typically the tumour burden is small at the experimental end point. Long-acting analgesia will be administered during the surgical procedure, which typically lasts less than 30 minutes, to ensure post-operative pain relief. Holistic assessments will be conducted using a specific assessment tool, for otherwise well mice (Appendix A: Clinical Signs) and those in post-operative care (Appendix B).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	Project 12. Nervous system modelling, protection and repair
<b>Key Words</b>	Peripheral nerve, cell culture, CNS
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to develop improved methods for the repair of the nervous system. This involves (1) enhancing peripheral nerve repair and (2) creating advanced cell culture models to facilitate research into cellular responses in the central nervous 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) Peripheral nerve damage is frequent and current surgical approaches often fail to achieve functional repair. This can have debilitating effects on patients, in particular leaving hands without sensation or movement. The development of artificial tissue that can be implanted by surgeons or drug treatments to improve nerve repair is a promising and realistic opportunity. This work will serve as a final stage for assessment of therapies which have the potential to improve outcomes in patients following nerve injury. (2) Advanced cell culture models allow neuroscientists to understand the molecular and cellular changes that occur in brain cells following damage and provide a powerful tool for developing new treatments for nervous system damage and disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats or mice may be used for peripheral nerve repair and maintained for typically 4-16 weeks (up to 200 per year). Newborn rats or mice may be culled humanely and used immediately as a source of cells for culture models (up to approx. 10 litters per year).

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

Adverse effects from nerve damage in animals are minimal and mainly associated with the loss of sensation and movement in one paw. The ability of animals to eat,

drink, groom, interact and move around their cages is not impaired. The severity level may reach moderate since anaesthesia and surgery are involved. Animals will be killed humanely at the end. No adverse effects are expected from the use of newborn animals as a source of brain cells.

## Application of the 3Rs

### Replacement

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

#### Replacement

(1) Before a treatment can be considered for clinical use it is essential to assess it in a living mammalian system. Initial development can be conducted using non-animal systems, but it is not possible to progress a new device or drug to the clinic without conducting tests in animals. There are currently no alternatives which model all the body systems that would potentially influence outcome.

(2) Cells derived from freshly culled animals are essential for CNS models, however, it is important to note that the resulting cell culture systems are an alternative to live animals in many experiments. Alternatives include fresh human cells, cell lines and various stem cell sources, but in some cases these options are not suitable; cell lines and differentiated stem cells can fail to resemble natural brain cells, and fresh human brain cells are difficult to obtain and grow in culture.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Therapies are developed to a late stage and optimised using non-animal models, including advanced *in vitro* and *in silico* systems, to minimise animal use. Many outcomes can be measured in one animal over time. Statistical calculations are performed to ensure the minimum number of animals are used in each experiment.

Many millions of cells can be obtained for use in cell culture allowing multiple experiments to be conducted using a small amount of animal tissue hence reducing the use of live animals.

### Refinement

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

## **Refinement**

Nerve repair in rats and mice is the least severe and most widely accepted animal model that mimics the human clinical situation. Harm will be minimised by causing the least amount of nerve damage necessary in each experiment and ensuring that the animals are able to behave normally (e.g. feed, drink, groom, interact) following injury. Animal welfare will be monitored and recorded.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 13. Investigating cell viability/integrity in vivo</b>
<b>Key Words</b>	Cancer, Cell Viability, Cellular Integrity, Tumour therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to use mouse models of human cancer to understand how factors and pathways that control tumour cell viability and integrity lead to cancer development and, thereby, identify new treatment options for 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)?**

Work from this licence will lead to new knowledge in fundamental cancer research and increase our understanding of cancer initiation, progression and spread. It is likely that this will uncover potential new targets for cancer therapy, which will be assessed using mouse models of human cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

All studies in this licence will use mice. We expect to use approximately 50,000 mice over 5 years. We estimate around 70% of these will be for breeding and colony maintenance and will not undergo any scientific procedure.

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

Approximately 70% of the animals will be used for breeding or are surplus from breeding and will not experience any adverse effects except for ear notching (for identification and genetic testing). These will be humanely killed when no longer required for breeding. Control or test groups of animals that arise from the breeding will develop or be predisposed to developing cancer. A small proportion of animals will develop tumours because cancer cells have been implanted and allowed to

grow. Some of these animals will be administered substances/agents (which will affect cancer development/progression, change aspects of their metabolism, modulate specific cell viability/integrity factors, or used for monitoring/analysis), therapeutic treatments, or fed altered diet (eg. high fat diet). For some procedures, anaesthesia will be used to minimise discomfort (eg. imaging). All animals on treatment or anaesthesia will be carefully monitored for discomfort, recovery or development of clinical symptoms (eg. weight loss, abdominal swelling, hunching). Any animal with signs of illness such as weight loss of reaching 20%, excessive weight gain, immobility or hunching will be humanely killed. At the end of the study all animals will be euthanized and tissues collected at post-mortem to gather as much information from the study as possible.

## Application of the 3Rs

### Replacement

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

#### Replacement

Although some aspects of cancer research can be conducted using cancer cell lines cultured in the laboratory, the complexity of genetic changes, accumulation of multiple pathological events and effects on the immune system can only be properly assessed within the context of the whole animal. The mouse models that we use closely resemble the features of human cancer progression and, therefore, serve as useful models for pre-clinical testing. We are however using more advanced, 3-dimensional tissue culture systems and the Beatson Institute is also investing in simpler animal systems (eg fruit fly) as well as mathematical models) to use alongside the mouse models.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The number of animals used per experiment will be decided based on experience within the Applicant's laboratory or Beatson groups, previous published literature and advice from the in-house statistician. We will use inbred strains of mice that are genetically very identical, to minimise experimental variability so fewer mice will be required to obtain a statistically significant result. We will perform pilot experiments with a few mice before scaling up for a full study if promising observations are made. The ongoing monitoring of animals ensures experiments will be terminated as soon as sufficient data has been obtained, thereby minimising suffering and animal numbers. Non-invasive imaging techniques will allow monitoring of tumour growth/spread without sacrificing the animals. We often share common mouse

strains within the Beatson groups and freeze sperm from lines not immediately required to avoid unnecessary breeding.

### **Refinement**

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

### **Refinement**

The mouse is a mammal and warm-blooded, sharing many features of human not found in cold-blooded species like flies, fish and worms. The ease and success of manipulating the genetics of the mouse also makes this animal the lowest and best model organism to study the genetic changes leading to cancer. We will always use the the most refined model with the least severe adverse effects to address a question. For example, most of the models we use only target genetic changes to specific tissues/organs, negating the adverse effects of genetically altering the entire animal.

We will mostly use established and widely applied techniques and treatments as the adverse effects and clinical symptoms of these techniques/treatments are known. Occasionally, when a group is given a treatment for the first time, we will perform a pilot study (3-6 mice) which will be closely monitored before extending to a larger number. Regular monitoring of weight/welfare will allow us to complete studies at the earliest endpoint in which we observe a significant result to prevent unnecessary suffering due to prolonged study duration.

Our animal facility is serviced by highly-trained staff dedicated to husbandry, breeding and technical procedures. All Users will be fully trained in monitoring clinical signs for each of our models and will be signed off as competent prior to initiating their own tumour cohorts.

The animals will receive the highest standard of care, housing environment and anaesthesia/analgesia as appropriate. All mice on procedure will be regularly monitored for signs of altered health status and humanely sacrificed when exhibiting moderate adverse symptoms.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 14. Characterizing the neural basis for the perception of speech in noise and evaluating new treatments for hearing loss</b>
<b>Key Words</b>	Hearing, Hearing loss, Rodent, Speech
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The proposed research is focused on understanding how single neurons and neural networks contribute to auditory function and dysfunction, with an ultimate goal of improving the treatments that are available for hearing loss.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The results of this project will provide us with a deeper understanding of the features of neural activity that are critical for the processing of speech in background noise. This will allow us to identify the key features of neural activity that are distorted by different forms of hearing loss and to evaluate the degree to which new hearing loss treatments can correct them.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use no more than 500 mice, 750 gerbils and 420 guinea pigs 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 levels of severity? What will happen to the animals at the end?**

A majority of animals will be used only for breeding, terminal experiments, or pilot behavioral studies, and will therefore experience no more than mild discomfort (e.g., from ear-punching for genotyping or injection of terminal anesthesia). The remaining animals will experience procedures of moderate severity, such as recovery from surgeries involving implantation of neural recording devices. The most common moderate-severity adverse effects expected are post-operative discomfort which will

be managed with analgesics given during and after surgery. At the end of all experiments, the animals will be humanely euthanised.

## Application of the 3Rs

### Replacement

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

#### Replacement

Many of the brain mechanisms that allow us to understand speech in noise are not yet understood well enough to be simulated effectively in computer models. These brain mechanisms can only be studied in intact animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will minimize the number of animals required by maximizing the number of brain recordings we obtain from each animal.

### Refinement

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

#### Refinement

We will primarily use gerbils, which are one of the most common model species for studies of human auditory processing. We will sometimes use guinea pigs when size or other physical constraints preclude the use of gerbils. We will sometimes use mice when genetic tools are required to answer a specific scientific question. Most animals will experience no more than mild discomfort, as from an injection of anesthetic. Some animals will undergo surgery with recovery, and will be given analgesics to ensure that any post-operative discomfort is minimized.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 15. Breeding and maintenance of genetically altered mice</b>
<b>Key Words</b>	Transgenic, Mouse, Breeding
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

To breed and maintain genetically altered animals to be used in neuroscience research.

Mice have been shown to be of great value in elucidating how sensory information is processed by specialised neural circuits in 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)?

Mice are sufficiently close to humans to reveal principles of information processing in the brain. Mice bred under this licence will be used to understand the function and connectivity of neuronal circuits in the normal and genetically altered mouse brain. This work will enhance and advance knowledge on how the brain processes information from the outside world and converts it into behaviour. This information could lead to the design of new highly selective drugs for treating neurological diseases such as epilepsy, Parkinson's Disease, Alzheimer's Disease, depression, schizophrenia and autism.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice only. In order to produce sufficient mice of the required genotypes for use in experiments, it is expected that approximately 20,000 will need to be bred and maintained annually.

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

Genetically altered animals will be maintained under this licence in order to understand processes involved in neural circuits and behaviour. The various steps involved will be: 1: Injection of hormone to increase egg production in female mice. 2: Female mice may have embryos implanted. 3: Vasectomy of male mice to allow

these to be used to induce phantom pregnancies in females so they will receive embryos generated in other females. Each new strain generated will have a well described and expected profile; however, animals will be monitored for unpredicted adverse effects and profiles will be monitored. Surgical procedures will be performed under anaesthesia, using pain relief and following aseptic methods to minimize risk of post-surgical complications. Anaesthesia will be carefully and regularly monitored to ensure that an adequate depth is maintained throughout any surgical procedure. Mice will be monitored regularly for their health status throughout all procedures. All procedures will be undertaken by trained, competent people. Mice that are no longer going to be used will be humanely killed following the accepted protocol. Animals that are fully recovered at the end of procedures may be kept alive at the establishment (with the agreement of a veterinary surgeon), with a view to their re-use on procedures if appropriate and licensed. No mice with genetic disabilities exceeding mild severity will be bred on this licence.

## Application of the 3Rs

### Replacement

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

#### Replacement

The different animal models maintained and bred under this licence will integrate the complete range of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic modifications result in normal or abnormal processes, focusing on neuroscience .

#### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Breeding programmes will be agreed in advance and regularly reviewed to optimally meet anticipated demand.

Breeding programmes will be optimised wherever possible to produce only the required genotype.

Freezing of eggs / embryos and sperm will be carried as routine. Archiving of lines will avoid wastage from the need to maintain colonies by continuous breeding.

#### Refinement

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

### **Refinement**

More is known about mouse genetics than any other mammal. The choice of the mouse as a model system enables the use of existing published data sets thereby reducing the overall number of control experiments required. Furthermore, by harnessing the power of mouse genetics, we are able to refine and target experiments to specific populations of neurons and circuits. Again, this reduces the number of mice required.

The mice will be cared for by dedicated, experienced animal technologists who have the expertise and skills required to breed mice. Welfare problems that may occur at an early stage will be monitored carefully to determine appropriate end points in consultation with experienced animal husbandry technicians and veterinary surgeons.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 16. In vivo imaging of inflammatory immune responses</b>
<b>Key Words</b>	Imaging, Inflammation, Diagnosis, Therapy, non-invasive
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Inflammation is an adaptive response made by the body, which affords protection to the host during tissue injury or infection. However, its chronic expression underlies a wide variety of pathologies in autoimmunity (such as rheumatoid arthritis, psoriasis, Crohn's disease). Conversely, induction of an acute inflammatory immune response in response to vaccines can be beneficial if the correct immune response is induced (e.g. generating high levels of protective antibody). The ability to identify and track the different components of an inflammatory immune response would help us to understand the efficacy of treatment (both for preventative and therapeutic interventions). It would also aid monitoring of treatment in the patient to make informed decisions about changes to the type or purpose of their treatment.

Molecular and cellular imaging can detect specific inflammatory processes, allowing patient-specific treatment decisions to be made. Molecular and cellular imaging is possible by virtue of techniques such as positron emission tomography (PET), single photon emission tomography (SPECT) and magnetic resonance imaging (MRI). These techniques employ contrast agents targeted towards specific molecular processes.

This project aims to develop **non-invasive imaging methods and agents** to quantify inflammatory immune responses, as found in various conditions such as autoimmunity, transplant rejection and infectious diseases. The programme spans “bench to bedside”, covering a variety of novel imaging agents and methods, including combinations of imaging technologies to enhance diagnosis, monitor treatment and assess outcomes.

**What 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 develop the requisite new contrast agents (including improved chemistry and improved biology for their production), and the imaging instrumentation and methodology, for use in quantifying, monitoring and evaluating inflammatory processes. We will develop agents for molecular and cellular imaging of processes that hitherto could not be imaged; develop improved agents so that known processes can be imaged better. The potential benefits are that patients will have greater access to new and improved imaging techniques and agents to enhance their clinical management; e.g. to see if an inflammatory immune process is present, predict whether a treatment will work (thus avoiding debilitating and expensive treatments in patients who will not benefit); monitor how well a treatment is working by non-invasive correlation of the magnitude of the immune response with serum biomarkers (such as antibody levels generated in response to a vaccine). Thus, a deeper understanding of the underlying immune responses to inflammation may lead to new and/or improved interventions in man. Novel contrast agents and non-invasive imaging methodology would also have applications in clinical trials, to measure the effectiveness of new drugs and vaccines. New imaging methods developed will refine future animal imaging experiments, improving the quality and quantity of data per animal.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice 5500 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 levels of severity? What will happen to the animals at the end?**

Symptoms of inflammation development are expected to occur in the models described herein and range from mild to moderate severity models. In a genetically modified mouse model of diabetes, pancreatic inflammation occurs prior to diabetes emergence, but no adverse symptoms are expected as this phase of disease development is transient and short. However, we wish to capture this underlying event with imaging modalities to enhance prediction/understanding of diabetes onset. In other cases, localised inflammation will be induced such as mouse ear (dermatitis) or inflamed paw models. In these models the inflammation cycle is known to be transient and naturally resolve over time following induction of inflammation. On the other hand, we will also study more systemic models of inflammation such as arthritis and neuro-inflammation which could result in multiple inflamed sites and consequently more discomfort and pain. However, in all cases, careful monitoring and where appropriate, use of painkillers, will minimise as much as possible, any pain, suffering and distress. Animals will be imaged using a variety of imaging modalities which are not expected to cause adverse events. Any adverse events expected are related to induction and maintenance of anaesthesia (animals

may die from respiratory depression <1 -2 % and/or hypothermia). Mild discomfort may ensue from injection of substances, however, where possible, this will generally be done under anaesthesia together with other procedures such as weighing the animal, blood sampling and inflammation site measurements. Other procedures such as urine sampling or withholding of food prior to imaging (as done in the clinic) or keeping certain animals on a gridded floor for a short time (to facilitate collection of urine /faeces for analysis – not applicable to arthritic models) may be carried out but are not expected to cause adverse events. However, where possible, efforts will be made to optimise anaesthesia, administration of substances, refine animal monitoring, refine pain management and avoid unexpected adverse effects &/or deaths. All animals will be humanely killed at the end of the experiments and tissues taken for further analysis. However, if at any point during the studies the animals reach a predetermined end-point at which pain, suffering and distress can be avoided or minimised, then these animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

#### Replacement

Animals have to be used in this project because: 1. Data generated from this body of work may be used to inform whether to go forward to human clinical trials/studies; 2. To validate the mode of action of novel compounds, experiments are required which 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 or with realistic models of inflammation.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Preliminary screening (i.e. immune cells or tissues used in a controlled environment outside a living organism) will eliminate unsuitable test substances/imaging agents at an early stage and thus these substances will not progress to animal studies, thus reducing the numbers of animals used in this project.

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. The use of imaging to determine bio-distribution of novel contrast agents in the life of the same animal rather than conventional killing at sequential time points, with removal of tissues for analysis is a major contributor to reduction of numbers. 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).

### **Refinement**

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

### **Refinement**

Species: Mice are the species of lowest neurophysiological sensitivity that provide the necessary size compatible with the scale of resolution or movement associated with the 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.

Pilot studies are small experimental groups 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. Generally, inhalation anaesthesia will be used to minimise any transient pain or 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/measures will optimise the animal's welfare whilst undergoing these procedures. By the very nature of the work involved in this project animals will develop inflammation. Therefore careful monitoring will occur and pain relief will be administered as required and under veterinary direction. Animals will be humanely killed at the end of the experiment or before then, if the humane endpoint is reached and tissues used for further examination.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 17. Gene regulatory networks in development and disease</b>
<b>Key Words</b>	Gene function, zebrafish, myopathy, neural crest, cancer
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We are trying to understand how the body reacts when certain genes do not function correctly. For example, are other genes switched on to compensate, and if the faulty gene is normally active in one tissue, are others also affected? We wish to extend our previous work and focus on four main areas:

- 1) In which tissues are genes, that we have previously characterised, expressed during normal embryonic development.
- 2) What role do previously uncharacterised genes play in the development of neural crest, an embryonic structure that forms many different tissues, such as nerves in the gut, but also pigment cells in the skin that can transform into the aggressive cancer melanoma.
- 3) How do genes respond to skeletal muscle disorders such as muscular dystrophy. The same hereditary diseases can affect individuals in different ways, even within families. This may be due to different gene interactions.
- 4) How does the loss of a specific gene, *kdm2aa*, make animals more susceptible to cancer, especially melanoma.

**What 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 obtain: 1) new fundamental knowledge about the genetics of embryonic development in vertebrates 2) new information about genetic involvement in neural crest dysfunction 3) new insights into the effects of skeletal muscle disorders on gene function 4) new understanding of the way absence of a known gene makes cancer more likely

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will need 77,500 zebrafish 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 levels of severity? What will happen to the animals at the end?**

Genetically-altered zebrafish will be produced by natural mating and in vitro manipulation and fertilisation. For the in vitro manipulation and fertilisation we will extract gametes from fish no more than five times per year per animal. Most of the study procedures will be done on embryos up to 5 days post fertilisation which are unable to feed independently. This is therefore non-regulated work. A minority of lines will grow to adulthood for further study. The adult fish which have the abnormal genes being studied may have physical or functional defects. If we observe distress such as lethargy, abnormal swimming or failure to feed or thrive, these animals will be humanely killed immediately. The fish carrying abnormal genes or fluorescently labelled tissues will have their genotype assessed by analysis of tissue from fin clipping or microscopy, respectively. The fish grown to adulthood will have their phenotype assessed by being weighed in water, or by imaging or microscopy. Some will undergo a second fin-clipping to assess the effects of a gene alteration on natural fin regeneration. None of the phenotypic procedures are invasive, we therefore do not expect any adverse effects. The only adverse effects are expected from the anaesthesia necessary for all procedures on adults. Potential adverse effects are poor recovery or non-recovery. Any fish showing poor recovery will be humanely killed. Based on previous experience we do not expect more than 5% of fish to show any adverse effects after anaesthesia. We will undertake a pilot study for 6 months to see whether we can extract gametes every 2 weeks, in order to reduce the total number of fish used. The work will move to a new laboratory during this study. Animals will be transported in special containers designed to minimise the adverse effects and their condition will be checked before and after transfer. Social groups will be kept together as much as possible before, during and after transit. Animals will be humanely killed at the end of the procedures.

## Application of the 3Rs

### Replacement

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

## **Replacement**

Our work focusses on understanding systemic, in vivo, responses to loss of gene function in the context of heritable human disease and development. This work would not be possible in non-vertebrate systems.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

We have developed a sperm freezing and IVF procedure that allows us to split individual sperm samples into up to eight aliquots. This reduces the number of animals needed for line archiving.

Experiments will be conducted to enable REDACTED and in accordance with the NC3Rs' ARRIVE guidelines. Sequencing or genotyping data will be archived at EMBL-EBI's European Nucleotide Archive (ENA) which is openly accessible to any researcher around the world.

Any new animal models we create will be archived in international repositories and made available to other researchers around the world. This will help reduce the number of animals used to make these models by other scientists.

## **Refinement**

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

## **Refinement**

The zebrafish is the most refined vertebrate animal system as most of the analysis can be carried out on non-regulated embryonic stages while producing data that are relevant for human health. We continually strive to implement the most up-to-date protocols to minimise harm to the animal. For example, we provide specialised spawning conditions that mimic the shallow river areas preferred by the animals for mating. The health of the fish is closely monitored by highly trained animal technologists.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 18. Improving the housed environment for farmed dairy cattle</b>
<b>Key Words</b>	Dairy cow, Housing, Environment, Health and welfare, Productivity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this project is to generate evidence based recommendations to enhance the housed environment of the UKs farmed dairy cows to optimise animal health, wellbeing and productivity. Specifically the project will address two key area:

1. How does the type and area of loafing space (the amount of space each animal has in a barn which isn't used for lying down) affect an animals health, well-being and productivity
2. How does the stocking density (the number of resting stalls in a barn relative to the number of animals present) affect an animals health, well-being and productivity

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

More than 95% of the UK dairy cows are housed during winter (when no grass is available for them to graze) and increasing numbers are housed year round. This research will lead to advances in how to design and manage the housed environment of dairy cows to maximise their health, welfare, productivity and the sustainability of dairy farming. Key beneficiaries of the research will be the UKs 1.8 million dairy cows and dairy farmers; there will be direct benefits to animal welfare when our findings are implemented on farm. Consumers will benefit from the assurance that dairy products in the UK are produced under acceptable conditions. Finally we expect our finding to influence policy, legislation and industry guidelines at a national and international level.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use up to 420 adult dairy cows over a 5 year period of experiments

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

We aim to test the impact of different environments on dairy cows in a flexible dairy cow housing facility which has two identical mirror imaged pens which can be set up with different layout. Animals will be housed in either a test or control environment, designed to replicate pen layout and conditions present on commercial farms. Typically these layouts will exceed normal on-farm conditions, i.e. typically, our objective will be to test the potential benefits of increases above minimum industry standards, which will be used as control conditions. The health, behaviour, physiology and productivity of cows in each group will be monitored and compared. In order to assess the impacts of the different environments we need to monitor and assess the animals and collect a range of samples from them to understand how their health, behaviour, physiology and productivity is affected. Overall, we expect the work we conduct to have a mild impact on the animals; as a result of a number of the procedures we will conduct, animals may experience short-term mild pain, suffering or distress (although it is of note that much of what we will do is likely to lead to no significant impairment of well-being in most animals). The potential for suffering in the worst case scenario would be the cumulative effects of a number of mild procedures over the maximum duration of an experiment (up to 13 months). At the end of the study, the animals will be examined by a vet; if deemed healthy and unaffected by the study they will be returned to our main dairy herd. Because of the nature of the research we are conducting, we would expect this to be the case in all but exceptional circumstances. To reduce the total number of animals subjected to experimental procedures, the same animals may be re-used on this project or on other projects. We consider that animals are suitable for re-use as they will have been subjected to only mild procedures and will have fully recovered before they are considered as suitable candidates for further experiments.

## Application of the 3Rs

### Replacement

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

### Replacement

This work aims to investigate the health, well-being and physiological responses of dairy cows kept under differing environmental condition in order to optimise housing for farmed dairy cattle. Dairy cows are the subject of, and direct beneficiaries of the research; as such it is not possible to conduct this work without using animals and no other alternatives are suitable.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

REDACTED We will design and conduct all of our experiments carefully and in accordance with best research practice to ensure we minimise the number of animals required, whilst at the same time ensuring our results are robust so we do not use animals unnecessarily.

## **Refinement**

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

### **Refinement**

Dairy cows are the subject of, and direct beneficiaries of the research. The effect that changes to the housed environment has on lactating dairy cows cannot be assessed in another species. Holstein cows have been chosen as this breed makes up the majority of milk producing cows in the UK so the results of this study will be applicable to most animals in the UK.

The welfare costs to study animals will be minimised by conducting only those procedures necessary to gain the information we require. As experiments progress protocols will be critiqued and further refined to reduce the number and frequency of procedures to those absolutely necessary for the validity of the study. This will reduce an individual animals cumulative exposure to the minimum required to generate robust results.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 19. an necrosis-induced inflammation: mechanisms and mediators</b>
<b>Key Words</b>	Necrosis, inflammation, skin, alarmins, inhibitors
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The proposed project arose from previous studies on the role of programmed necrosis in animal disease models as well as in human inflammatory diseases.

Programmed necrosis pathways are new molecular cascades responsible for tissue damage in models of diseases such as septic shock, viral infections, ischemia-reperfusion induced diseases (stroke, myocardial infarct, acute kidney injury, retinal degeneration), and inflammatory diseases (hepatitis, pancreatitis, atherosclerosis, encephalitis, intestinal inflammation or skin inflammation). While the ground-breaking discovery of programmed necrosis opens brand new perspectives in diagnosis and treatments of such diseases, the molecular triggers and regulation of programmed necrosis *in vivo* remain poorly understood.

Using a well-characterized model of necroptosis-dependent skin inflammation, this project aims at identifying the pro-inflammatory mediators triggering necroptosis *in vivo*, as well as interactions of necroptosis with other programmed necrosis pathways in order to develop innovative therapeutic strategies for inflammatory 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 anticipate that these studies will further clarify the contribution of programmed necrosis to inflammation. Skin inflammation will serve as a model to unravel the molecular mediators of necroptosis-dependent inflammation *in vivo* and the role of other forms of programmed necrosis in inflammation, in order to develop innovative diagnosis tools and therapeutic approaches for human inflammatory diseases. The potential benefits are large: to date, there is no diagnosis tools nor therapeutic

strategies targeting necroptosis in human diseases. However, given the numerous potential implications of programmed necrosis in human pathology, including some of our own results, we anticipate that the results obtained from this study will pave the way to innovative therapeutic strategies in a broad range of diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Species: mice Numbers: 35,000 30,000 animals are predicted to be used for breeding and maintenance of GAAs, and 5,000 for experimental procedures. Period of time: 5 years

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

Adverse effect: Skin inflammation The proposed model of necroptosis-induced skin inflammation has been well characterized. The onset and disease development, as well as endpoints, are thus very well described, including from my own previous work. This will allow us to minimize suffering and/or distress in the animals. Thus the level of severity is considered as moderate. Animals will be humanely killed at the end of the experiments (schedule 1 method or appropriate method listed in the procedures).

## Application of the 3Rs

### Replacement

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

### Replacement

While it is possible to investigate *in vitro* the effect of specific agents on cell culture, the variety of cells involved in an inflammatory response and their recruitment from different organs requires the use of a whole organism in order to study the onset of inflammation. Thus, the use of animal models is unavoidable if we are to understand the pathology of complex inflammatory diseases and to develop innovative diagnosis tools leading to earlier and more personalized treatment of diseases.

Although data from several non-protected species have proved very helpful considering innate immunity, the skin is a very specialized organ in mammals, with no equivalent in non-protected species. Thus, non-protected species could not be used for this purpose.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Rigorous *in vitro* work and study of the literature may ensure that the proposed experiments have a clearly defined aim and best possible experimental design.

Our experimental design and data collection is based on a well-characterized disease model of necroptosis-dependent inflammation, evidenced by immunohistological data and genetic rescues, which is, to date, the most accurate model to fulfil the aims of this project.

Effective colony management will be performed to minimize excess animals. Adequate statistical analysis will be performed to keep the animal numbers at a minimum. GAA lines that are no longer required will be frozen down for storage.

Finally, the choice of a skin inflammation model for our study allows easy macroscopic monitoring of disease progression in the animals on a daily basis, avoiding sample collection at an inadequate disease stage, thus reducing the number of animals required.

## Refinement

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

## Refinement

Choice of animal models and methods:

The murine model used in this application is the most accurate model of *in vivo* necroptosis-induced inflammation. The choice of the skin inflammation model compared to other organs is justified by:

- The very rapid disease development, avoiding maintenance of sick animals on a long period of time.
- The easy macroscopic monitoring of lesion development in the skin, requiring minimal invasive procedures.
- Our ten years of expertise with this model, allowing us to predict outcomes and minimise discomfort for the animals.

We are aware that no animal model can fully reproduce its human equivalent and recognise the dangers of too readily extrapolating from animal data to human diseases. However, there are abundant evidences that results from appropriate animal models can greatly benefit to the understanding of human diseases and support the development of innovative therapeutic strategies.

Refinement and endpoints: The proposed model of necroptosis-induced skin inflammation has been well characterized. The onset and disease development, as well as endpoints, are thus very well described, allowing us to minimise suffering and/or distress in the animals.

Our protocol defines a strict daily monitoring of skin inflammation. However, most of the crosses and treatment indicated here should improve cutaneous inflammation in the animals. In addition, the protocols encompass only minimally invasive procedures.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 20. Investigating Cellular and Molecular Mechanisms of Cardiac Remodelling</b>
<b>Key Words</b>	cardiac remodelling, heart failure
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Remodelling of the heart following ischaemia or higher pressures can be beneficial however adverse remodelling can eventually lead to heart failure (HF) which often represents the clinical endpoint of many cardiovascular diseases of different aetiologies. In HF patients, profound abnormalities have been shown both in the sympathetic and the parasympathetic control of the cardiovascular system. This is characterized by increased sympatho-hormonal activation, which predisposes HF patients to myocardial electrical instability (arrhythmia) and death due to progressive HF and attenuated vagal activity. Given its prevalence around the globe, the ageing population and the financial burden involved in treatment improved understanding of HF pathophysiology is essential in order that more effective anti HF therapies can be developed. The objectives of this project are to improve our understanding of mechanisms driving the remodelling process occurring in the cardiac ventricles following damage to the myocardium or pressure overload. Improved knowledge of these mechanisms will help lead to the development of new 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)?**

It is estimated that between 1-2 million people in the UK suffer from heart failure. Survival rates for heart failure patients are variable, dependent on the age and severity of disease, and the quality of care they receive. Around one in ten patients die in hospital, and of those who survive between one-quarter and one-third die within a year of their admission. At present there is no cure for heart failure because we do not understand how the disease progresses. The aim of this programme is to determine the complex interaction between the diverse cardiac cell types so that we

may better understand the remodelling process and determine sites in the pathways susceptible to intervention to slow or potentially reverse the process.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

5220 mice in total. 450 rats in total

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

Most of the animals will be used to examine tissue samples collected after humane killing. In other cases, we can study animals using scans similar to those in humans (for example ultra-sound or magnetic resonance scans (MRI)) whilst they are under anaesthetic. Some animals will undergo surgery to create diseases similar to those in humans (for example heart attacks or high blood pressure). Animals will be under anaesthetic for surgery and will receive pain-killers as they recover. Most animals are then humanely killed within 8 weeks to study the effects of disease. However, as seen in humans, a minority of animals might die suddenly.

## Application of the 3Rs

### Replacement

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

### Replacement

Whilst where possible we carry out our work on cells in the laboratory, heart failure is a complicated process that involves hormones and nerves. It is not possible to properly replicate the disease process in a cultured cell in a dish.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We careful design of our studies to minimise the number of animals used. Reduction is also achieved by the use of the latest imaging and telemetry techniques to allow longitudinal studies that detect progression of adverse changes in the heart much earlier and produce reliable and repeatable data which is helpful in the reduction of number of animals used.

### Refinement

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

### **Refinement**

Mammalian species are required to properly replicate the process of heart failure seen in man. Rats and mice have similar genes and similar cardiovascular function to man. Much work has been previously performed in these species. Suffering of the animals will be minimised. All surgery is performed under anaesthesia. Pain relief will be given during the post-operative period. Animals will be monitored very closely. If any animals show symptoms which cannot be alleviated they will be humanely killed

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 21. Perceptual Decision Making in the Primate Brain</b>
<b>Key Words</b>	temporal cortex, predictive coding, decision making, vision
<b>Expected duration of the project</b>	2 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Vision begins with our retina decomposing the visual scene into individual pixels that must then be reconstructed by our brain. Our current understanding of how the visual scene is reconstructed by the brain is somewhat limited by the fact that to date, most investigations have focussed on a single brain area. We know, for example, that neurons in early parts of the visual pathway respond preferentially to spots and bars of light, whereas neurons in later regions respond preferentially to simple and complex shapes. What we *don't* know is what are the “mathematical” calculations necessary to transition from one simple representation (spots, bars) to the next (simple shapes), and so on (complex shapes). Furthermore, we don't know how our visual pathway is influenced by “higher level” cognitive factors, such as expectation or attention. Most importantly, we don't fully understand how we then use these reconstructions to guide behaviour (e.g., decision-making behaviour).

This project will examine how sensory areas in the brain contribute to decision-making. Specifically, we aim to understand the transformations taking place between adjacent regions in the visual system; and how sensory activity is influenced by cognitive factors involved in decisions, such as expectation. We will accomplish these objectives by measuring brain activity from multiple visual regions while animals perform complex behavioural tasks.

**What 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 data will be of considerable interest to research groups interested in visual processing, particularly those relying on neuroimaging experiments in humans, as

our data will provide the necessary evidence to confirm/refute current theories in visual processing (e.g., the role of feedback connections in visual transformations) as these are not testable in humans. We further expect our data to be of considerable interest to those interested in the theory of predictive coding, which is a continually evolving theory regarding generalised brain function. Evidence for predictive coding at the neuronal level is extremely sparse. There are many outstanding tenets of this theory that are not testable in humans (e.g., laminar distribution of prediction signals). Our approach is specifically targeted at resolving several of these outstanding questions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We must use non-human primates (rhesus macaques) for this experiment (2), as they are the only laboratory species that feature the necessary brain complexity and behavioural sophistication. We expect this project to take a maximum 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 levels of severity? What will happen to the animals at the end?**

This application will allow for the completion of ongoing experiments initiated under a previous license. The most severe potential adverse effects, in terms of impact on physical health as well as welfare primarily derive from: a) the initial stages of training where it may have been necessary to use fluid restriction, neckplate training, or other forms of negative reinforcement and b) the neurosurgical procedures, which carry risks of adverse effects related to infection, seizures, and stroke. Under the previous license, these animals have already been successfully trained to perform all the necessary behavioural tasks without the use of fluid restriction or requiring pole+collar to neckplate train the animals. Both animals have been surgically prepared for chronic electrophysiological recordings. Thus, the protocol steps that incur the greatest risk to the animal have already been completed under the previous license. What remains are the risks associated with daily testing and recordings (which include mild infection around the implant edges, and potential for stress and discomfort during the training and testing phases) and the risks associated with any additional surgical procedures that are not expected but may become necessary should the current implants fail. These include risk of brain infection, haemorrhage, and/or seizures associated with chronically implanted arrays. Upon completion of the experimental objectives, the animals will be euthanized.

## Application of the 3Rs

### Replacement

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

## **Replacement**

Our project involves invasive procedures and therefore cannot be accomplished with human participants. All currently available techniques in humans (e.g., FMRI, PET, etc.) lack the spatial and temporal resolution necessary to achieve our objectives.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

Our experiments are based upon a “within-animal” design, which means that statistical comparisons are made between different behavioural conditions, not across groups of animals. We are also relying exclusively on modern electrophysiological techniques (i.e., arrays), which greatly reduces the number of recording sessions necessary to complete each dataset.

## **Refinement**

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

## **Refinement**

We require animals that can perform sophisticated decision-making tasks, and for which we already have a thorough understanding of the general visual pathway. Rhesus macaques are the most suitable species as their cortex is large enough that we can readily record from multiple brain regions. We know we can train macaques to perform the necessary tasks. We are continually refining our behavioural training procedures to emphasise positive reinforcement and voluntary engagement with the tasks. Finally, our animals are continually monitored – in consultation with the NVS and NACWO, to allow for early identification of behavioural and/or neurological problems.

We have taken advantage of a number of developments in the field such that our current proposal represents the most refined method for acquiring the necessary data. We have introduced a multi-step training programme for the initial chair training, which has allowed us to successfully train both animals without any pole-collar. We currently rely exclusively on fruit smoothie to motivate and reward our animal, which has meant that we have, thus far, not required any fluid control or restriction. Finally, we are using state-of-the-art recording devices that greatly increase data yield on a day-to-day basis. This means the overall time an animal will spend on project is substantially reduced.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 22. Sex chromosomal control of development and disease</b>
<b>Key Words</b>	Infertility, sex chromosomes, male-female differences
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Men and women are genetically the same, with the exception of their sex chromosomes. Men are XY and women XX. The sex chromosomes have a special role in making sperm and eggs. Abnormalities in these chromosomes are thought to be responsible for many cases of infertility, a condition that affects 15% of couples. Evidence suggests that sex chromosomes are also responsible for other differences between men and women, for example in their likelihood of developing cancer, their life expectancy and how they respond to medications. The overall aim of this project is to investigate how the sex chromosomes control these processes. More broadly, we also wish to understand how problems in chromosome behaviour in developing eggs and sperm give rise to conditions in offspring such as Down syndrome. Finally, many areas of medical research and agriculture require only animals that are male or female, and those of the opposite sex are therefore created needlessly. A good example is the dairy industry and egg industry, in which only female cows and chickens are required. We want to design a system for creating litters that contain only males or only females.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Ultimately, our studies of sex chromosomes could give insight into the causes of human infertility and of sex differences in disease. They could also help us understand why chromosome abnormalities are so common in humans, affecting 7-10% of all clinically recognised pregnancies. In turn, the discoveries could lead to new ways of diagnosing or treating these conditions. Finally, a method to create single sex litters would have a huge economic impact on the medical and agricultural industries, and would be a major step forward for animal welfare.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

For most of our experiments we will use mice. These are an excellent model system because their genetic make-up is similar to that of humans. Also, egg and sperm formation in mice occurs in a manner similar to that in humans. We also use a marsupial, the laboratory opossum, which diverged from mice about 180 million years ago in evolution. The rationale for using both model organisms is that mechanisms common to both are likely to be of highest importance for understanding the diseases in which we are interested.

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

Our objectives require the creation of genetically altered mice and opossums. In the majority of cases, the effect on the animal will be observed only in the testis or ovary, and so the severity level will be mild. Also, most of our experiments will be performed on material obtained from animals post-mortem.

## Application of the 3Rs

### Replacement

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

### Replacement

Our studies require the use of animals, because currently it is impossible to make germ cells (i.e. eggs and sperm) in a dish. This is probably because in order to form properly, germ cells require two-way interaction with other cell types in the gonad, as well as changing levels of hormones provided via the bloodstream. Nevertheless, one of our aims will be to try and make eggs and sperm types in the laboratory. If this succeeds, it could help replace the use of animals in the longer term.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We implement a number of approaches to reduce animal numbers. This begins with experimental design. Genes that we think are important for the processes we're studying are chosen based on published literature and data generated both by us and by other scientists. This vastly reduces the number of "false-leads". Genetically altered animals are only created if they not available from existing sources. If this is the case, the genetically altered animals are created in-house by highly trained personnel, and are usually maintained as small colonies. We plan our experiments so that each animal provides the maximum amount of material for analysis, and that tissue harvested post-mortem from a single animal can be stored and repeatedly

reused in different experiments. This approach, together with statistical approaches, means we use few animals to address a specific scientific question. A major focus of our work is to design a system for creating single-sex litters. Many areas of medical research and agriculture require animals of a defined sex, and those of the opposite sex are therefore created needlessly. If successful, this approach would dramatically reduce the numbers of animals needed for our experiments, and for those of the international research community as a whole.

### **Refinement**

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

### **Refinement**

Our work focuses on sex chromosomes. Mice and opossums are ideally suited for this work, because unlike other model organisms, their sex chromosomes, and the mechanisms by which their eggs and sperm are formed, are similar to those of humans. The mouse is also useful because it is the most tractable mammal with respect to genetic manipulation. In the majority of our experiments, the genetic alterations we create impact only fertility, and thus do not appear to cause pain or distress. Furthermore, in most cases material will be acquired post-mortem. We use highly trained personnel to carry out protocols with moderate severity limits, e.g. induced ovulation, in order to keep animal suffering to a minimum. We cannot always predict the effect of a new genetic alteration. However, animals exhibiting any unexpected or detrimental effect will be killed by a Schedule 1 method, or in the case of new lines or individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 23. Genome Regulation in Health and Disease</b>
<b>Key Words</b>	genome regulation, chromosome stability, germline, infertility, aneuploidy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Most cells in a human contain the same genetic information but different types of cell are highly specialised to carry out their different roles in the body. These specialisations are crucial for normal health and are achieved by cells switching on and off thousands of different genes in the DNA in a highly co-ordinated and regulated way. Mutations that disrupt this genome regulation can cause human genetic disease. For example inheriting the wrong number of chromosomes, each of which contains thousands of genes, causes imbalances in genome regulation, infertility, miscarriage and Down Syndrome. The aim of this project is to understand the how developing eggs and sperm ensure that chromosomes and genomes are correctly inherited and regulated, how defects in genome regulation in these and other cells in the body can cause disease, and how we might correct these defects to develop new treatments for disease. Experimentally we will use cultured cells and test tube systems to develop insight into aspects of chromosome inheritance and genome regulation that influence health and disease but are not well understood. We will use normal mice to work out how chromosomes are inherited and how the genome is regulated in healthy animals. We will then use genetically altered mice to alter these new aspects of chromosome inheritance and genome regulation in animals so that we can work out how important they are, and how they might contribute to human genetic diseases. The mice used in this project will be used at embryonic, juvenile and adult stages to provide cells and tissues or measure chromosome inheritance or genome regulation in test tube studies. Some adult mice will also be used to measure the effects of disrupting genome regulation on fertility.

**What 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 will improve our understanding of how the information in our genes and chromosomes is accurately passed from parents to their children, and how it is decoded to cause different cells and tissues to have distinct properties. These studies will also identify candidate genes that cause human genetic diseases such as infertility, and will help us understand why some chromosomal abnormalities, such as those that cause Down Syndrome in humans, arise.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will use around nine thousand mice over a period of five years.

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

The majority of these animals used will have been genetically altered to carry mutations that disrupt chromosome inheritance or genome regulation. Most mice used in this project will not experience any detectable adverse effects. The most common expected defect in these genetically altered animals is likely to be infertility, which does not have significant adverse effects on the health of the animal. To allow chromosome inheritance to be studied in eggs, some mice will be injected with hormones to stimulate egg production and will experience transient discomfort during the injection. Experimental animals will be humanely killed in order to provide post-mortem tissues and cells that can be analysed in the test tube using cellular and molecular techniques.

## Application of the 3Rs

### Replacement

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

### Replacement

The proposed project is strongly linked with extensive replacement of animals with cell culture and test tube models being the primary tools that are used to generate and investigate hypotheses upstream and downstream of animal use. Generation of genetically altered mice will primarily be used to confirm these test tube findings and test if and how they apply to cell types and tissues that have not or can not be studied in culture. Cell lines and tissues obtained from small numbers of genetically modified and normal mice will typically be analysed extensively using sophisticated molecular techniques over a period of years to generate and test new hypothesis. The suitability of alternative cell line and in vitro models will be reviewed throughout the duration of the project, and used to replace animal use where appropriate.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The number of animals used in this project will be reduced by optimising breeding strategies. The experimental designs in this project typically involve cellular and molecular analysis of post-mortem tissues obtained from genetically altered animals, and determining whether the genetic alteration has caused abnormalities in chromosome inheritance or genome regulation. Sample sizes and planned statistical analyses for individual experiments vary somewhat depending on the phenotypic parameters being scored, but aspects of altered genome regulation or abnormalities in paternal chromosome inheritance are each generally assessed using 3-5 genetically altered mice. Aspects of abnormal maternal chromosome inheritance are typically assessed using 5-10 genetically altered mice.

### **Refinement**

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

### **Refinement**

The mouse is the experimental animal of choice for this project due to the relative ease by which it can be genetically altered, the existing wealth of data in the field for this species, and its proximity to humans. Some of the biological pathways investigated in this proposal are mammal-specific and mouse is the most appropriate experimental mammal that can be used for this work. Where applicable we will use conditional strategies to introduce mutations in a tissue-specific, time-controlled or drug-inducible manner to limit adverse effects caused by these mutations. Mutations that affect germ cells do not cause pain, harm or suffering, minimising welfare costs to the animals. Welfare costs for mutations that cause more widespread effects will be minimised by humanely killing animals before they reach a stage where they would be likely to exhibit symptoms of pain or distress. Experimental data will typically be obtained from molecular and cellular analyses on cells and tissue isolated from animals post-mortem, therefore repeated measurements are not performed on each animal and low severity procedures and humane end-points are natural components of the experimental strategy. Mice analysed for reproductive parameters are typically young (6-16 weeks) and healthy, and mating these animals has minimal welfare costs. Environmental enrichment will be used routinely during animal husbandry.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 24. Frontal cortex: learning and decision making</b>
<b>Key Words</b>	prefrontal cortex, cingulate cortex, decision making, learning, behavioural change
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The main aim of the project is to investigate how the areas in the frontal lobe of the brain operate and interact with one another and mediate our ability to learn and to make decisions and adjust our behaviour. An additional aim is the assessment of the welfare impact of the procedures themselves on the animals.

**What 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 continually learn from experience and adjust our behaviour so that it is appropriate for the current environment. For example, we assess how successful it has been to take a course of action in a particular context or how well we have managed in a certain situation. We are so adept at doing this that we rarely notice what we are doing until something goes wrong with the process. For example, in some psychological illnesses, such as depression, our ability to assess the success of our choices and behaviour is diminished. Our intention is to understand better how such mechanisms for learning, assessment, and behavioural change operate in the healthy brain but we think that our findings are likely to have implications for understanding what goes wrong in psychological illnesses.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

In a series of experiments over five years we expect to use approximately twenty-five macaques.

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

We measure the activity and structure of macaque brains before, after, and while they learn and make simple decisions between objects or actions to obtain food and juice rewards. Measurements are made with magnetic resonance imaging (MRI). MRI is non-invasive. Nevertheless macaques may potentially be distressed by the confinement in and noise of the MRI scanner. However, this is mitigated by training sessions in which the animals are very gradually acclimatized to the features of the MRI scanner such as its visual appearance and the noise it makes. For example, recordings of the sound of the MRI scanner are played during training sessions that are conducted in a mock MRI scanner that looks like the real MRI scanner. The animals are restrained with a head post – an implanted device for holding the head still – while data are collected and there is a risk that this may cause stress. This is not only a problem for the animals' welfare but for the science if it prevents them from engaging in the learning and decision making behaviours we investigate. We therefore train our animals carefully so they are gradually familiarized with the head-restraint procedures. We use animals because we also want to examine what happens if interventions are made in the brain that change the way small parts of it operate. In many cases we intervene in the brains of human volunteers with a technique called transcranial magnetic stimulation (TMS). TMS can only be used to investigate a limited number of brain regions close to the scalp. To look at other areas and to examine longer term impacts on brain networks and behaviour we use animal models. In some cases we will make the brain intervention by making a focal lesion. The pain that might be caused by the surgery to make the lesion is minimized by the use of anaesthesia and analgesics. The risk of infection during the surgery is minimized by conducting the surgery aseptically. Postoperative pain is minimized by the analgesics. Because the brain lesion is small and circumscribed it does not usually cause an impact on the animal that is detectable by normal observation. However, the role of the brain area in which the lesion has been placed can be ascertained in carefully designed behavioural tasks that the animals are trained to perform. These allow us to measure small but important changes in their behaviour. Similarly, anaesthesia, aseptic techniques, and analgesia are employed if any other surgery is required (for example, for a head post). In other cases we think that it might be possible to make the brain intervention in a less invasive way by using focal ultrasound neurostimulation. This technique alters activity in a circumscribed part of the brain. We can measure the effect using MRI scanning and careful behavioural testing. This is a very new technique but we think that the effects are transient and only last for a period of several hours. We think that the stimulation itself is not painful. Veterinary guidelines are followed in all the work that we do.

## Application of the 3Rs

### Replacement

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

## **Replacement**

Much of the laboratory's work is REDACTED Nevertheless sometimes we use animals because we also want to examine what happens if interventions are made in the brain that change how it functions. Some techniques, such as TMS, are available for carrying out tests with human volunteers but they can only be used to examine brain areas close to the top and sides of the head and their impact is short lived. To look at brain areas that are some distance from the scalp and to examine the longer term impact of an intervention in one area on brain networks and behaviour we use animal models.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

To obtain reliable results it is rarely sufficient to examine a single animal. If we examine data from more than one animal we can be more sure that our findings have a general significance. Usually data from three or four macaques can be shown, using statistical procedures, to provide an indication of whether brain signals are reliably correlated with behaviour or whether a brain intervention affects behaviour.

## **Refinement**

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

## **Refinement**

We use macaques because they, like humans, possess a prefrontal cortex. Although the brains of most mammals contain frontal cortex, only the brains of primates contain prefrontal cortex. We focus on macaques because not only are the connections and activity patterns of the macaque's brain better documented than those of any other primate species but macaques can perform simple decision making tasks in an MRI scanner. Because the macaque brain is approximately 5cm long it is possible to obtain meaningful data about its function using MRI.

As already explained, where possible, we use non-invasive methods to disrupt brain activity and to record brain activity. When it is necessary to use an invasive approach then it is only undertaken with appropriate anaesthesia, analgesia, and veterinary advice.

We examine brain activity while animals perform behavioural tasks in order to understand the role that the brain areas play in mediating the behaviour. We

motivate the animals to perform the tasks by rewarding them with small juice rewards or food rewards. Every day, however, we ensure that the animals have a period of free access to water and they are given additional food when they have finished performing the tasks.

In some cases, the animals are restrained while data are collected and there is a risk that this may cause stress. This is not only a problem for the animals' welfare but for the science if it prevents them from engaging in the learning and decision making behaviours we investigate. We therefore train our animals carefully so they are gradually familiarized with the procedures.

To assess the impact of the procedures on the wellbeing of the animals we can take measures of behaviour and physiology. We hope that these measurements can be used to help us identify the least stressful ways to carry out the research.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 25. Development of new treatments and novel pathways in pulmonary arterial hypertension</b>
<b>Key Words</b>	Pulmonary Hypertension, Treatment, Novel Pathways
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Pulmonary arterial hypertension (PAH) is an incurable disorder characterised by high pressures in the lungs. This high pressure is due to thickening of the pulmonary arteries which prevents the vessels from relaxing. This process is referred to as pulmonary vascular remodelling and involves the growth of all 3 cell types in the pulmonary artery. This vascular remodelling leads to enlargement of the right side of the heart and ultimately heart failure which is the most common cause of death in these patients.

We need to understand why these vessels in the lungs become remodelled and develop a cure. We also need to understand why the heart fails and try to prevent this to prolong the life of 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)?**

There is no cure for PAH. Current therapies can only prolong life by a few years. There is an urgent need to develop new drugs that can reverse the disease in the lungs but also target the heart as this is ultimately what the patients die of. These projects are to trial new novel therapies and to help understand the mechanisms of how they work.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats (1000) and Mice (4000) 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 levels of severity? What will happen to the animals at the end?**

We propose to use the following procedures: Breeding and maintenance of genetically altered mice (mild procedure) Induction of PAH in mice and rats via exposure to a reduced oxygen environment (hypoxia) with an additional single injection of SUGEN (a VEGF receptor inhibitor) (moderate procedure) MRI scanning (moderate procedure) Right heart pressure measurements carried out under a general anaesthetic (non-recovery procedure) These procedures are routinely carried out our facility and adverse effects are rare. However, animals are closely monitored for adverse effects such as weight loss, abnormal behaviour or physical changes and if this occurs advice will be sought from a named veterinary surgeon. Drugs may be given to animals to investigate whether they can reverse or prevent disease progression (moderate procedure) via various routes of administration such as orally, IP, intravenously or inhaled. Only one route will be used in any given animal. Drugs are always administered by an experienced person and animals closely monitored. At the end of these procedures, animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

PAH is a very complex process. Our research into this disease started by looking at molecular pathways at the cellular level but now this work needs to be translated into living animals to determine if the findings on a cellular level hold true in life. Non-animal alternatives already used will focus the animal studies and reduce the number of animals required.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The number of animals required for each experiment will be derived using a mathematical calculation to estimate the lowest appropriate group size which will enable us to achieve statistically significant results. In all experiments appropriate statistical test will be chosen to determine the significance of the observations.

Cardiac MRI is non-invasive and will allow disease progression or regression to be determined within the same animal with minimal stress and discomfort to the animal. This will allow for a reduction in the amount of animals used and will also lead to

higher quality data as it will reduce the amount of variability in response to disease modifying drugs between animals.

In addition, multiple organs and blood vessels will be collected from animals upon completion of the terminal procedure. These organs will either be snap frozen, embedded for staining or be used to obtain cell cultures. This will serve to maximise the information derived from each animal and reduce the need for further animals to be used.

### **Refinement**

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

### **Refinement**

#### PAH models

The hypoxic (low levels of oxygen), sugen/hypoxic (low levels of oxygen with an injection of a vascular endothelial growth factor inhibitor) models will be used to study the development of PAH in mice and rats. The hypoxic model is extremely well characterised and these species tolerate hypoxic conditions very well. The hypoxic/sugen model is a relatively new model which has the advantage that rodents develop many of the hallmarks of human disease hence use of this model may increase the relevance of our results to human disease. Genetically modified mice will also be used to allow the determination of the role of individual genes thought to be key factors in the pathogenesis of pulmonary hypertension.

#### Choice of species

We have chosen to use rats and mice as our studies will focus on pre-clinical investigation. Both rodent models have been widely studied with respect to PAH, and thus the development of these diseases in response to various stimuli in these animals is well characterised. This will help us to design our studies in order to achieve the maximum information from them.

#### How animal suffering will be minimised

- a) Animals will be housed in comfortable conditions with environmental enrichment.
- b) Where possible animals will be group housed.
- c) All procedures will be carried out by, or under the supervision of, an experienced competent person.
- d) For all aspects of our work we will refer to the NC3Rs website for guidance ([www.nc3rs.org](http://www.nc3rs.org)).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 26. Determining important regulatory pathways that control immune responses to infection</b>
<b>Key Words</b>	immune system, infection, parasite, bacteria, virus
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our immune system must be activated to fight infections, but at the same time be controlled so it does not attack our own body or harmless substance that we encounter every day. An incorrectly controlled immune system can result in devastating disease; for example, overwhelming, life-threatening infection if the immune response does not deal with the infectious organism, or so-called 'autoimmune' disease such as type I diabetes and rheumatoid arthritis if our immune system attacks the body. Therefore, understanding the cells and molecules that control the immune system in health and infection is crucial in identifying potential new drug targets for diseases of the immune system.

Our project will focus on the cells/molecules that regulate immune responses to infection. Specifically, we use mice that have specific cells/molecules altered to identify how they control the immune system during infection with viruses, bacteria and parasites. Our project aims to identify ways we can boost beneficial immune responses during infection.

Additionally, we aim to discover pathways that promote so-called 'immunological memory', the process by which our immune system remembers a previous infection and responds more efficiently if we are infected with the same pathogen 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)?**

Our work aims to identify pathways that boost protective immune responses when we are infected, and prevent unwanted self-harmful immune responses. Such pathways may be targets for therapy in the future, to promote clearance of infection.

Our work will also identify pathways that are beneficial in promoting immunological memory, to promote better vaccines.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice, and estimate that approximately 12,500 mice will be bred during the 5 year project, with 10,000 mice used in procedures.

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

Our work will involve using models of parasite, bacterial and viral infection. Parasite models: Infection is generally without symptoms or with short-lived moderate suffering to the mouse. Parasites are either removed by the immune system, or develop into a long-lived, symptomless infection. Bacterial models: Mice will develop a short-lived infection and some illness (e.g. weight loss, lethargy). For some pathogens used, this illness will fully cured (moderate severity). Some pathogens will cause severe infection after ~1 week, and mice will be monitored closely and culled immediately if symptoms reach a pre-determined threshold. This severe procedure is wholly necessary for our project, as it will allow us to determine important ways of stopping such symptoms by using certain types of cells or drugs. Virus models: In some viral models, mice will develop illness peaking ~1 week after infection. However, we will only use doses that mice are known to fully recover from, with return to health ~2 weeks post-infection. In some experiments, after animals have fully recovered from initial infection, they will be re-infected to identify cells and molecules that regulate 'immunological memory'- the process by which we respond better and faster to infection the second time round. At the end of procedures, all animals will be humanely culled.

## Application of the 3Rs

### Replacement

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

### Replacement

The use of animals is vital to the success of the project. The mammalian immune system is complex, with many different cells and molecules working in combination to produce a co-ordinated response. Thus, using lower organisms such as *Drosophila* or zebrafish is not feasible, as they do not possess a complex immune system seen in mammals. Similarly, in vitro models cannot give an accurate reflection of how complex the mammalian immune system is. Thus, use of mammals is essential, with mice proving an invaluable tool in studying the immune system for the past 40 years.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

From the outset of our project, we will consult with specialist statisticians to provide advice on designing experiments and statistics. Such advice will allow us to use the minimal possible mice to achieve statistically significant results.

All data analysis will be conducted according to a pre-specified plan drawn up with the statisticians, with statistical tests performed with their input.

## Refinement

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

### Refinement

#### Choice of species, models and methods.

As stated above, given the complex organisation of the mammalian immune system, it is unfortunately not possible to recreate these conditions using lower organism models (which do not contain a complex immune system) or *in vitro* cell models. Thus, the use of mice is crucial in the study of mammalian immunity. There are numerous examples of discoveries made in mice that have led to the direct identification of similar systems in the human immune system, with such cells and molecules now being clinically targeted in disease.

There may be opportunities to perform more focussed *in vitro* experiments if, during the course of mouse experiments, we identify types of immune cell that are directly affected during infection. We would then isolate these cells and determine their responses to different parasite/bacterial/viral products, and how different molecules/pathways affect their responses to pathogens.

#### Minimisation of animal suffering

##### *Intestinal parasite infection models*

All intestinal parasite infections are well established models of infection used by researchers over many years, with the majority causing no detectable suffering or distress to the animal. In a minority of cases (e.g. infection of mice with *Toxoplasma gondii*) mice will develop short-lived illness during infection. Here, animals will be closely monitored (with frequency increased leading up to time points where illness has previously been shown to occur) and should any unreasonable loss in condition be observed, the animals humanely killed.

### *Viral infection models*

The viral models used will result in short-lived infection with moderate weight loss and illness, but mice expel the infection and fully recover from symptoms. However, all mice will be monitored closely in peak times of infection, and if any unreasonable loss of condition is observed the animals humanely killed.

### *Bacterial infection models*

Some bacterial infection models (e.g. *Francisella tularensis* LVS) cause a severe infection. It is important for this level of infection to be reached, to determine cells/molecules and interventions that reduce the harm of infection. Thus, we need to reach a point in control animals where infection is established to determine whether any benefit has been achieved from gene/cell knockout. Other models (e.g. *Citrobacter rodentium*) cause modest weight loss and diarrhoea but resolve within 3-4 weeks with mice fully recovering.

In all experiments, we will closely monitor mice (with increased frequency of monitoring at time points close to when illness is known to occur), and have a detailed scoring system in which to assess the health of the mice during severe acute infection. If an agreed level of discomfort is reached (based on a robust clinical scoring system), the mouse will be immediately humanely killed.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 27. Nutritional requirements of cats and dogs across lifespan</b>
<b>Key Words</b>	Nutrition, Dog, Cat, Health
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We are primarily interested in the interaction between nutrients and/or foodstuffs and the health and wellbeing of dogs and cats. In this sense we investigate individual or multiple body systems, initially to understand the fundamental principles of nutrition and health and subsequently to determine the impact of varying nutrients and foodstuffs on maintaining health or preventing disease in these species. We are interested in all life stages and the life-span experience of nutrition on the animals involved in the studies. To support this we will analyse then store small samples of urine, faeces, DNA and blood taken from animals intermittently across their lifespan.

Studies will be carried out to determine effects of feeding diets containing different levels of the nutrient(s) of interest to groups of dogs or cats over time. Many measures of health will be determined without the need for regulated procedures e.g. through collection of faeces or urine, but others such as blood sampling will require the use of regulated 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)?**

Whilst research has been carried out over the years to establish nutrient requirements for dogs and cats in health and disease, there are still significant gaps in our knowledge. This project will support the setting of safe maximum and/or minimum levels for pet food ingredients, and seek to identify new health benefits for individual constituents or combinations. It also aims to find new ways to improve the palatability and enjoyment of pet food. In addition, it will identify early signals of ill health for pets, which will advance our understanding of disease and could be used by veterinarians to provide earlier treatment.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will involve the regulated study of both cats and dogs for five years. Approximately 540 cats and 350 dogs will be involved in total.

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

The majority of the animals will be fed defined pet foods with different ingredients and the impact on their health and wellbeing will be measured across their lifespan. Typical procedures to achieve this include blood, urine and faeces sampling, oral dosing, intravenous dosing, and anaesthetic for restraint purposes only. These are not expected to result in lasting harm, and are classified as mild severity. At the end of this project these pets will either be used for additional studies or rehomed. A smaller number of cats have been diagnosed with progressive kidney disease. These animals will be studied in the same manner as their disease develops. Due to the progressive nature of the disease, these cats are likely to experience moderate severity and will be euthanized before their symptoms become severe.

## Application of the 3Rs

### Replacement

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

### Replacement

For some aspects of this work it is possible to use an *in vitro* approach and where possible this route will be followed e.g. the study of metabolic energy expenditure in cell cultures; the development of an *in vitro* model of plaque and calculus development; computer modelling of taste receptor and tastants interactions. However, the ultimate aims are to understand the impact of nutrition on dogs and cats and establish whether health benefits are associated with intake of particular levels of nutrients and, therefore, the studies need to be carried out in these particular species of animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Whenever possible we will study change in the same pets over time, rather than compare one set of pets to another. This requires the use of fewer animals. As the impact of most procedures are mild, we will use the same animals in a number of different investigations. This means that, overall, fewer animals are needed to meet the objectives of the project.

### Refinement

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

### **Refinement**

We are specifically interested in understanding the impact of nutrition in cats and dogs, therefore the use of these species is necessary. We will store extra samples from each animal on a study, such that we can reanalyse them once more advanced methods become available. This means we can extract the maximum research value from each sample.

When animals are under anaesthesia for veterinary purposes (e.g. when having their teeth cleaned), we will endeavour to carry out as many necessary research procedures as possible. This minimises the number of anaesthetic episodes across the life of the animal.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 28. FARM ANIMAL TREATMENT EVALUATION AND DISEASE CONTROL</b>
<b>Key Words</b>	farm animal
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Evaluation of veterinary medicines in farm animals, farmed birds and horses. In the UK and Europe it is necessary to demonstrate that any veterinary medicine or vaccine is safe and effective in the species (cattle, sheep, goats, pigs, horses and domestic poultry) for which it is designed before permission is granted to sell the treatment. We offer an evaluation service to companies wishing to develop veterinary medicines. Some complementary tests are conducted outside of organisms or cells and we aim to keep abreast of scientific advances to implement alternative methods as they become available. Within development projects there are normally a number of *in-vitro* studies: on occasion we conduct these types of studies and will recommend *in-vitro* alternatives to animal studies if these are established.

Each evaluation is a study where, for example, the animals are infected either naturally or by artificial administration with an infectious agent and then some animals are treated with the test substance normally by mouth, pour on or injection. The other animals are left untreated so that the presence of infection can be demonstrated. Standard criteria are measured so that the success of treatment can be evaluated. The data are reported in a format that can be submitted to authorities either in the UK, Europe or elsewhere for registration of the treatment. The regulatory authorities have created guidelines for the minimum numbers of animals to use in these types of studies. We consult those guidelines and then conduct our own assessments to ensure that enough animals are being used to create a statistically valid study which then avoids potentially having to repeat the study. Typically a minimum of 6 animals per treatment group are recommended.

We work to ensure that we conduct studies where we have experience and we carefully plan the details of the study to ensure that the study generates valid data hence animals do not suffer unnecessarily. We then observe the animals closely to ensure that any symptoms are monitored and managed as necessary

Despite having many treatments available, veterinary infections continue to cause health and welfare problems in millions of animals and birds annually. This work is aimed at helping in the development process for new and often more effective treatments for farmed animals, birds and horses. These diseases have economic significance for farmers so more effective treatments will have an economic benefit for farmers and animal owners, and reduce the risk of transmission of zoonoses to 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)?**

Despite having many treatments available, animal diseases (production related or otherwise) continue to cause health and welfare problems in millions of animals and birds annually. This work is aimed at helping in the development process for new and often more effective treatments for farmed animals, birds and horses and therefore improve the welfare of animals through improved treatments for parasitic diseases. These diseases have economic significance for farmers so more effective treatments will have an economic benefit for farmers and animal owners, and reduce the risk of transmission of any diseases at risk to transmission to humans.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Maximum numbers to be used over the course of the 5 year project Cattle - 9600  
Sheep - 5500 Goats - 5000 Pigs - 4600 Horses - 945 Birds - 31700

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

Typically animals would be monitored for their weight gain, and sometimes blood and dung samples are collected at intervals. Normally the animals become accustomed to being handled after the first one or two occasions. Where possible we habituate the animals beforehand to a procedure and avoid handling them where we can obtain the information in an alternative way. Normally we expect to see few, if any, abnormal signs in the animals, thus the severity is usually mild. The exception is an infection of farm animals and poultry caused by a particular group of animal disease-causing organisms (*Coccidia* spp.). When working with these infections we have to monitor the animals closely to make sure that we treat them or euthanase them if their symptoms appear to be approaching the upper limit of moderate, the permitted severity limit. At the end of a study the animals will be humanely euthanased or released if this is permitted.

## Application of the 3Rs

### Replacement

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

#### Replacement

Veterinary medicines, biologicals, feed additives and vaccines must be trialled in the target species for initial safety/tolerance and efficacy before taking forwards to larger trials. Unless otherwise recommended or if there is an established and validated tissue model available, the target animal will need to be used in these studies.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Expert statisticians will be involved in study design as well as consulting guidelines set by regulatory bodies and any relevant literature to ensure that the minimum number of animals is used that are needed for a valid statistical result.

### Refinement

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

#### Refinement

The animals used are the target species for the veterinary medicines and vaccines on trial. Any models will have welfare at the centre of their design. In addition, animals on these studies will have a heightened level of observations immediately after and in the days following administration of any substance. If observations are observed that are approaching or have breached the severity limits in place, then the Establishment Licence Holder, Project Licence Holder, NACWO(s) and potentially the a member of the Animals in Science Regulation Inspection Unit will be consulted to decide the course of action. This could be either, immediate alleviating of suffering if irreversible clinical signs via euthanasia, or treatment with, for example, analgesics and anti-inflammatories in order to relieve suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 29. Sleep Behaviour of Zebrafish</b>
<b>Key Words</b>	Sleep, behaviour, drugs
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

How the brain works to regulate diverse functions, including eating, sleep, arousal, motivation, and other behaviours is still poorly understood. In particular, sleep problems occur in almost all neurodevelopmental (such as autism), psychiatric (such as depression and schizophrenia), and neurodegenerative (such as Alzheimer's) disease, and often poor sleep exacerbates the symptoms of the disease. Therefore, understanding how the brain controls sleep may help to develop treatments that will impact a wide variety of clinical cases. We will observe the behaviour of wild type and mutant zebrafish larvae and will monitor the activity of neurons in freely behaving zebrafish larvae.

**What 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 how the brain turns sleep on and off will allow us to develop better sleep aids and will help us understand how sleep is disrupted by developmental disorders, such as autism, and aging-related disease, such as Alzheimer's disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use an estimated 150,000 zebrafish (*Danio rerio*) over 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 levels of severity? What will happen to the animals at the end?**

In most cases, the only adverse effects on the animals will be alterations in sleep, such as sleep deprivation or alternatively, too much sleep. The imaging of the brain of larvae is non-invasive; however, the animals are restrained and they find this mildly stressful, as they engage in natural behaviors, like normal swims, responses to videos, hunting behavior, and even interest in other fish. The likely level of severity for almost all procedures is mild, and any accidental harm can be swiftly detected to

terminate the experiment. In less than 10% of the cases, animals exposed to small molecules or imaging protocols will exhibit moderate levels of severity, for example the induction of hemorrhage or seizure, at which point the experiments are terminated. All animals are killed at the end of the protocols and prepared for analysis of genetics and brain histology.

## Application of the 3Rs

### Replacement

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

#### Replacement

Sleep is a complex behaviour that only occurs in intact animals. Some non-protected animals, such as fruit flies and worms exhibit sleep states, but the brain circuits that regulate sleep in these animals is not identical to humans. Since zebrafish is a vertebrate like humans, the regulation of sleep is more relevant for human disease.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We use sophisticated statistical techniques to analyse large behavioural datasets to make predictions of what drugs will do before we undertake the experiment. This helps us to greatly reduce the numbers of animals that are needed.

### Refinement

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

#### Refinement

We have chosen zebrafish because they are the least evolutionarily complex vertebrate species with clear sleep states, and sleep happens at an early developmental stage. Because we use video monitoring to track the animal's behaviour and whole brain microscopy to watch neuronal activity, any adverse effects can be quickly noted by the researcher, who will then terminate the experiment.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 30. Environmental Effects on Fish Physiology and Behaviour</b>
<b>Key Words</b>	fish, environment, climate change, physiology, behaviour
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

To determine how aspects of environmental change affect fish behaviour and physiology, and to understand how populations may show evolutionary responses to human-associated environmental disturbance.

**What 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 animals respond to environmental change
- An understanding of links among traits and consequences for evolutionary processes
- An increased understanding of how fishing practices may impose selective pressures on wild fish populations. In the longer term, this latter benefit may translate into altering fish capture methods to reduce evolutionary effects

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Fishes, predominantly minnows, sticklebacks, Atlantic salmon, zebrafish, and gadoids – up to 13000 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 levels of severity? What will happen to the animals at the end?**

The severity experienced by the great majority of fish used in this project will be mild since they will only be subjected to benign procedures such as anaesthesia (for purposes of measuring and/or photographing), manipulation of environmental conditions (e.g. diet, temperature) or measurement of metabolic rate or swimming performance. The majority of the procedures are expected to be mild in severity. Protocol 2 will involve infecting fish with a natural parasite but depending on the

reaction of the individual fish, this may constitute moderate severity. For Protocol 3, fish may be subjected to simulations of procedures that commonly occur during fishing practices including trawling, air exposure, and handling. The extent of these simulations will not be as extreme as that which occurs in an actual fishing scenario but will be sufficient to elicit variation in the physiological and behavioural responses of interest. Nonetheless, this Protocol may involve procedures of moderate severity. At the end of procedures throughout Protocols 1, 2, 3 and 4, fish will be humanely killed. An exception to this is a proportion of the fish used within Protocol 3 (approximately 250 fish), which will be released into the wild after being fitted with an acoustic transmitting tag.

## Application of the 3Rs

### Replacement

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

#### Replacement

The research programme described here addresses questions about the behavioural and physiological responses of whole fish to their environment and so the objectives cannot be met without conducting field and controlled laboratory experiments using fish.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Both field and laboratory experiments will be designed to minimise the number of fish used. This includes *a priori* power analyses to ensure a sufficient sample size to address the questions of interest. In addition, multiple measures can be performed on the same individual fish, thus acquiring data on a broad range of traits while reducing the total number of fish used.

### Refinement

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

#### Refinement

Freshwater fish exhibit pronounced behavioural and physiological variation, thus making them ideal for this kind of study. Moreover they live in simple environments, the essential features of which can be replicated in the laboratory; this has the

combined benefit of reducing stress on the fish while generating results that are applicable to the real world.

Fish will be monitored by qualified personnel during all procedures. During routine holding, all animals will be supplied with appropriate environmental and social enrichment. During any procedures that may involve transient pain (e.g. routine tagging procedures), animals will be treated with analgesics in consultation with the NVS.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 31. Safety and efficacy of cell-based hepatic regenerative therapy</b>
<b>Key Words</b>	liver, safety, regenerative medicine, stem cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Liver disease in the UK is increasing in prevalence. Novel regenerative medicines have the ability to replace damaged cells and tissue but need more rigorous testing before being applied to humans. The overall aim of the work outlined in this application is to assess the safety and efficacy of novel therapies to enable hepatic regeneration.

The underlying philosophy of this project is that by understanding the potential risks associated with regenerative medicine therapy, we can improve the safety of such treatments for liver disease, and ultimately other diseases amenable to regenerative therapy. Specifically, in order to do this, we must address the main worry, or concern, regarding the use of cells in regenerative therapy, namely the risk that such cells

can migrate to other organs, and in the case of stem cells that their capacity to proliferate, differentiate and form teratomas and even tumors, could cause serious health problems.

In our project, we will use an integrated quantitative assessment of the whole body biodistribution of cell therapies using non-invasive imaging methods, in relevant healthy and diseased animal models coupled with the measurement of liver biomarkers - molecules that are found in the blood, that can inform us as to the health and disease of the liver (and which are commonly used in humans). Specifically, we will investigate the teratoma potential and tumourigenic properties of relevant cells – these will initially be stem cell-derived hepatocytes, but we may also use macrophages and other non-hepatocyte liver cells - that are likely to be the cells that will be able to repopulate a damaged liver, or stimulate the regeneration of a damaged liver. The cells will be administered into mice with acute liver injury elicited by a single dose exposure to a chemical, that is very well-described in the literature by many groups around the world, and with which we are very familiar. For example, we will test the safety and efficacy of cells with a specific genetic abnormality that is

commonly observed in many stem cell lines. Tumour growth will be monitored using non-invasive imaging and liver function will also be assessed. This way, through imaging, we will be able to use as few animals as possible in our studies, in order to better understand potential safety issues of regenerative cell therapy, coupled with restoration of healthy tissue function, in the same animal.

**What 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 primarily focus on liver disease and the treatment of such. However, the hazard associated with a particular cell therapy used for treatment of a specific tissue injury (in this application, the focus is on liver damage) will likely translate to other therapeutic indications for cell therapy. The impact of techniques for the development of tracking regenerative medicines (cells) by imaging has a far wider benefit as these therapies have the potential to be utilized in curing a vast number of diseases. The translation of the novel imaging methods to assess safety of liver cell therapy and liver disease can also be applied to other diseased tissues.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use normal and immunodeficient mice. We will use a maximum of 300 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 levels of severity? What will happen to the animals at the end?**

As we are modelling disease, our animals will progressively demonstrate symptoms of this. However, using imaging and non-invasive blood-based biomarkers we can closely manage these signs and generally can define disease at a much earlier stage and therefore we can ensure that the animals do not undergo any undue suffering.

## Application of the 3Rs

### Replacement

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

### Replacement

Safe regenerative therapy in the liver is a complex, multi-staged process in which there are many different cell types interacting with one another. At present, it is impossible to model such complexity in order to understand potential safety implications for human health without using animal models.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

It is possible to calculate the numbers of animals required for experimentation based on data from previous data. Imaging and non-invasive blood-based biomarkers enables animals to be used as their own control, allowing paired comparisons. Moreover, imaging is inherently sequential, increasing statistical power and using fewer animals to achieve the same statistical power as conventional designs. In all cases we will ensure that we have calculated the minimum number of animals required for the experiment to give us meaningful data. This approach reduces the animal numbers required, and also reduces the likelihood that the animal experiment would have to be repeated.

### **Refinement**

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

### **Refinement**

Using imaging and non-invasive blood-based biomarkers, we regularly refine the disease models we use, to reduce animal harm and to improve the effectiveness of our models. We can also stage disease and stop experiments before external clinical signs appear, thus limiting disease severity. Because of this we can ultimately use fewer animals per procedure and still generate meaningful and clinically-relevant data. We also regularly monitor body weight, body condition, food and fluid intake of animals as a measure of disease; we set strict limits to ensure that there is limited harm to the animals used.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 32. In Vivo Function of Proto-Oncogenes in B-Lymphocytes</b>
<b>Key Words</b>	Cancer, Immunology, Immune system development
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Cancers of B-cells, a type of white blood cell that protects us against infections, often have an unfavourable prognosis and their treatment represents a major challenge for medicine. In the U.K., ~18,900 new cancers of B-cells were diagnosed in 2013, with ~7,700 reported deaths. In many of these cancers, particular signalling molecules or transcription factors are abnormally active because of accidental genetic mutations. In normal B-cells, these factors control cell growth and survival. The uncontrolled activity of these factors due to genetic mutations promotes the formation of B-cell tumours, thus identifying them as potential drug targets. Here we propose to determine the functions of certain cancer-associated signalling molecules or transcription factors in normal and cancerous human B-cells, and to investigate how these parts contribute to the development of 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 knowledge obtained from these studies is expected to help us better understand how certain cancers of B-cells arise by identifying the precise tumour-causing mechanisms underlying the cancer development. Importantly, our work will potentially establish new, more specific targets for drugs aimed at treating cancers of B-cells with abnormal activity of certain signalling molecules or transcription factors that have fewer side effects, paving the basis for personalized treatment of these cancers that have an unfavourable prognosis.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Our studies exclusively use mice and we anticipate using approximately 5500 mice over the 5-year time-frame 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 levels of severity? What will happen to the animals at the end?**

All procedures to be carried out are associated with a 'mild' or 'moderate' severity rating. Under the terms of this licence, animals will be euthanized at the end of all experiments.

## Application of the 3Rs

### Replacement

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

#### Replacement

The immune reaction that is so important for protecting us against infections, but that inherently bears the risk of developing cancers, is incredibly complex. It has been impossible to reproduce this reaction outside of the body in a culture system, which is due to the fragile nature of these cells that do not survive in culture systems. None of these complex individual events occurring in the living organism over a large time-window are available via other cellular systems. Therefore, and particularly because the information gained from the experiments with animals is expected to be directly relevant to the development of novel anti-cancer therapies against B-cell cancers, the use of animal models remains the only rational approach to study their role in the context of the complex living organism.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Our previous extensive experience in REDACT has allowed us to develop robust protocols involving the minimum numbers of animals required to provide reliable and informative results. Importantly, we seek advice from statistician colleagues to optimise our experimental design in addition to using well-established statistical methods (power calculations) in determining sample sizes.

### Refinement

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

#### Refinement

The mouse is the model organism of choice to study the biologically highly complex vertebrates by directed genetic manipulation, and the generation and use of transgenic mice to analyse the function of genes associated with human disease has over the last decades led to tremendous insight into the precise mechanisms of

disease development and has formed the basis for a great number of clinical applications in humans. Of exceptional importance for the planned studies is the fact that the immune system of mice is well characterized and closely resembles that of the human. Moreover, a wealth of commercial reagents and techniques for analysing the lymph system of mice are available. For this reason and since mice require a relatively short period of time for tumour development, this species is most appropriate for an analysis of body cells in health and disease.

Mice will be monitored regularly and routinely for signs of ill health or distress throughout all aspects of the project. Where appropriate, anaesthetics and pain-killing drugs will be used whilst advice from local veterinary surgeons will be sought in any situation where animals are showing unexpected signs of ill health or suffering. In all cases, experimental protocols have pre-determined "end-points" that when reached, animals will be removed from the study. For all procedures, we will apply the least invasive methods of dosing and sampling appropriate to the objectives of the experiment, including the use of anaesthesia for humane restraint where necessary.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 33. Biological controls of Campylobacter and Salmonella in chickens</b>
<b>Key Words</b>	Chicken, zoonosis, biocontrol, probiotic, bacteriophage
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this project is to identify mechanisms by which the food poisoning bacteria *Campylobacter* and *Salmonella* colonise the intestines of chickens. To use this information to examine how biological controls can be used to prevent or reduce levels of colonisation by these pathogens such that the risk of foodborne disease to the consumer is reduced. *Campylobacter* and *Salmonella* represent the two most common forms of foodborne disease, and poultry meat is the major source of contamination for *Campylobacter* with 46.7% of broiler chicken meat samples positive compared to 6.5% for *Salmonella*.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Gut health is important for the welfare of fast growing broiler chickens, and for their agricultural productivity. Biological controls of foodborne pathogens coming from poultry sources are desirable as they generally have the least environmental impact, maintain the quality of the meat, and are critically unaffected by antibiotic resistant bacteria. The ultimate beneficiaries will be the consumer, chicken producers and retailers of poultry meat.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Broiler chickens are reared for meat and are therefore the most appropriate animals for use in these studies. It is estimated these studies will require 1268 birds per year over the five year 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 levels of severity? What will happen to the animals at the end?**

Chickens will be reared in groups housed in pens suitable for normal movement from chicks with feed and water available. At 6 or 20 days of age the birds may be placed in single pens but in sight and hearing of birds from the same group. The birds will be orally administered with either *Campylobacter* or *Salmonella* bacteria that are causative agents of human foodborne illness, which often arise from poultry sources. Chickens may experience mild intestinal effects that can result in a temporary reduction in feed intake. Unlike humans most chickens show little or no sign of disease and the microorganisms are tolerated. Biological treatments (probiotic microorganisms or bacteriophages (viruses that only kill specific bacteria) or bacteriocins (proteins that kill bacteria) may be given in feed or administered orally or administered through the cloaca by natural reverse movement of the gut, with the aim of reducing the numbers of the *Campylobacter* or *Salmonella* in the gut of the animal. These treatments are targeted to have no other impact. A few drops of blood may be taken from the wing tips to assess immune responses to intestinal colonisation. All birds will be killed at the end of the study before the collection of tissues and intestinal contents for analysis post mortem.

## Application of the 3Rs

### Replacement

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

### Replacement

There is no other way to relate gut microbial communities to complex healthy gut structures and the growth of broiler chickens than to use birds.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The majority of the work to be undertaken in this project will be preceded by laboratory experiments and computational studies to reduce the testing required in chickens. Optimum group sizes for each experiment will be calculated using power calculations, and where possible small groups may be used to validate protocols and provide data as a basis to plan larger experiments. Where possible we will utilise characterised *Campylobacter* and *Salmonella* strains with a reproducible ability to colonise the guts of chickens that will enable meaningful differences to be determined.

## Refinement

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

## Refinement

Broiler chicken meat is the most popular source of meat and therefore must be the animal of choice to assess if sustainable biocontrol can reduce the intestinal carriage of the bacterial foodborne pathogens *Campylobacter* and *Salmonella*. Whenever possible chickens will be group housed to minimise stress of social animals and provided environmental enrichments to promote natural behaviour (for example, strings, reflective surfaces, and deep litter wood shavings in which to scratch and bath). Birds will be monitored at least twice a day for any signs of ill health or unusual behaviour.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 34. Initiation and resolution of inflammation in wounds</b>
<b>Key Words</b>	Immune cell, wound, inflammation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Wound healing complications (including scarring and chronic wound formation) affect millions of people worldwide and can result in death, limb amputation and disfigurement. To date there remains little in the way of effective treatment strategies to target chronic wounds and scarring. Consequently, there is an urgent unmet need to develop such treatment strategies and lessen the disease burden on society and healthcare systems. We have developed a new mouse model of human chronic wounds in order to discover how wound repair goes awry and whether we can rescue this process. We will study the skin during inflammation, wound repair, and chronic wounds to ascertain what kinds of immune cells, especially macrophages, that are present throughout the diverse phases of normal repair, how they behave, precisely how do they change in wounds which fail to heal and how does this impact repair outcome? We will study pathways which help terminate the inflammatory response to determine whether we can harness them to accelerate wound repair or rescue chronic wounds which have become 'stuck' in the inflammatory phase. The overall goal of this research is to achieve an improved understanding of the events that determine whether a skin wound heals acutely or develops into a chronic wound.

**What 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). A greater understanding of the cell biology of physiological and pathophysiological skin inflammation and repair, which will allow us to develop more effective treatments for animals and human patients with wounds or skin inflammation. 2) Identify the events which govern whether wounds will heal acutely with scarring or develop into chronic wounds which will enable the identification of basic molecular pathways regulating immune cell behaviour and persistence at wound sites which could potentially be targeted to improve healing outcome. 3) Establishment of and increasing experience with mouse models of acute, impaired and chronic skin wound models, and models of skin inflammation, which may allow

other researchers throughout the wider research community to study the cell/molecular biology of tissue injury and repair. 4) The effects on chronic wound treatment are potentially far researching. Effective therapies for non-healing wounds might avoid the need for amputation and the resulting risk of death or disfigurement altogether. This would lesson the burden of these wounds both on the afflicted patient and the healthcare systems. 5) Identification of new anti-inflammatory and pro-resolving pathways could impact the treatment of a wide range of inflammatory disorders, including, but not limited to wound healing, for example arthritis, heart disease and cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We estimate we will use approximately 8000 mice in the lifetime of this license. We estimate using 4000 of this total 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 levels of severity? What will happen to the animals at the end?**

We plan to use different mouse of skin wounds, skin inflammation and a model of sterile peritonitis. These models are of moderate severity and therefore we expect minimal adverse effects for the animals using these models. The animals will be culled humanely at the end of the experiment.

## Application of the 3Rs

### Replacement

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

### Replacement

We need to use mice to study skin wound repair mice because we cannot study complex multicellular interactions nor the impact of disease states on immune cells or wound repair, in cell cultures. Human skin explants cannot be used as the blood vessels and lymph vessels are disconnected and we need to be able to see how immune cells in vessels arrive and leave wounds. Important differences between fish, fly and mammals mean we cannot replace mice with invertebrates, although we will continue to explore their potential use. Zebrafish, for example, are unsuitable as the skin structure is very different to mammalian skin due to the presence of scales and water environment, it is also not currently possible to model chronic wounds in non-mammalian systems at present which is a vital component of this program of work and offers reduced ability to translate our findings into the clinic to benefit patients. We will continue to use *in vitro* approaches with human tissues and skin cells where possible to add value to the animal studies performed and to minimise the need for in vivo experimentation as much as possible.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

When experiments are planned we receive input on experimental design and power calculations from in-house statisticians. We also attend training courses on experiment design to ensure. This ensures that we use the most appropriate group size and include appropriate control groups so that experimental data is scientifically interpretable. We carefully store samples from experimental animals such that additional future can be undertaken on archived material wherever possible. This material will be made available to other research groups on request so that additional experiments involving animals will not be required. The models we use will be refined to reduce variability and therefore enable us to use the minimum number of animals possible. We will continue to explore the potential of small animal imaging to allow collection of data at multiple time points to reduce numbers and will maximize and refine the collection of multiple pieces of data from individual mice.

## **Refinement**

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

### **Refinement**

Mice will be used in light of the ready availability of genetically modified mice available for study of disease pathways and processes. We have selected models of skin repair and inflammation that equate to human disease and we are familiar with these models.

Breeding mice with genetic modifications to study the working of the immune system does not involve the creation of major defects or illness and mice do not have features of ill health. If the mutation makes them susceptible to naturally occurring infections, these are prevented by filtering air and the use of antibiotics.

The wound repair models used are the most refined approaches to study both normal and pathological healing responses, under conditions of altered inflammation and with minimal distress to the mouse. Most of the models we use have been very well defined and progressively refined over many years, both within my lab and internationally, meaning the likelihood and types of adverse effects are minimal but known so that animals can be appropriately monitored and precautions taken to minimise harm. For example, the wounds are made under anaesthesia, with pain killers used to minimise adverse effects including wound discomfort and we routinely house mice at 30C following wounding which improves recovery following

anaesthesia. We have identified clear monitoring procedures and humane endpoints which enable us to minimise adverse effects whilst maintaining our scientific outputs. We always seek veterinary input in the event of any concerns about the condition of the mice under protocol to ensure that signs of distress are not missed.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 35. Novel Treatments for Primary Ocular Cancers: Retinoblastoma and Uveal Melanoma</b>
<b>Key Words</b>	Retinoblastoma, Uveal Melanoma, chemotherapy, Rabbit, intraocular tumour
<b>Expected duration of the project</b>	3 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this project is to setup a suitable model to further research into the treatment of rare eye tumours such as retinoblastoma (RB) and uveal melanoma (UM). These models will be used to test the effectiveness of the leading four candidate drugs from an earlier in vitro screen.

**What 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 date there are no good models of RB and UM in which to test novel therapies. However, we now bring together the required components to generate in vivo models for RB and UM in which to test novel therapies. This is backed up by a systematic in vitro screening of candidate drugs and a direct route through to clinical trials. This should ensure that we give this project the best start to find novel therapies capable of treating these devastating eye cancers

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use a maximum of 360 New Zealand White Rabbits over the lifetime 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 levels of severity? What will happen to the animals at the end?**

Cancer cells will be injected in to the animal to develop eye tumours, which will be left to grow for 2 weeks then treated with novel drugs for 3-6 weeks. During tumour growth, various measurements will be made using what is known as fundus photography (eye photography) and intraocular pressure will be recorded. The

surgery is very minor and should result in minimal discomfort after a relatively short period of anaesthesia. The main side effects will be loss of appetite (and hence weight) due to the immunosuppression and vision may be affected. We propose to run short 5-8 week experiments which are long enough for tumour initialisation and to observe drug effects but not long enough to see serious complications from tumour growth. After the treatment regime, the animals will be killed. All efforts will be made to use other organs after termination, such as corneas being used as an ex vivo corneal infection model.

## Application of the 3Rs

### Replacement

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

#### Replacement

It is not possible to mimic the growth of these tumours in vitro or ex vivo. It has also been documented how the unique eye environment affects the use of drugs rendering both of these tumours relatively resistant to current drugs. By modelling these tumours in their natural environment using relevant cells we hope to provide as accurate a model as possible.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Bilateral injections of cells will double the number of tumours available as the primary output for histology. We will also be measuring the total tumour mass, cross sectional area and proportion of live vs dying cells within the tumour. This will increase the accuracy of our primary output thereby reducing the number of animals required.

### Refinement

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

#### Refinement

The rabbit is currently the best animal model for precision placement of therapeutic drugs into the eye space. The encapsulation process of the drugs will ensure a slow steady release in locally high doses while avoiding the toxicity of systemic chemotherapy. Rabbits were chosen due to the medium size approximating a human

child's eye allowing accurate placement into specific layers of the retina. There is also a documented history of using this model but not with either the rare tumour cell lines or the novel therapeutics that will be employed. Animal health will be monitored by bi-weekly weighing and physical examination of each animal with weekly fundus photography and intra-ocular pressure measurements to track the progress of the tumour.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 36. Repair and regeneration of the injured heart</b>
<b>Key Words</b>	heart, mouse, fish, myocardial infarction, regeneration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Heart transplantation remains the only viable cure for adult cardiovascular disease (CVD), as such there is an urgent need for alternative therapies to replace and restore damaged heart tissue either following birth defects or heart attack. Cell transplantation has been rapidly progressed to clinical trials over the last decade, but the outcome has been disappointing to-date. We are adopting an alternative strategy for treatment, based on stimulating resident cells within the heart towards repair. To this end we seek to determine how neonatal mice and adult zebrafish can regenerate their hearts so we can stimulate similar processes to repair hearts in adult mice and ultimately human patients (objective 1) and how to control the level of inflammation and scarring in the heart after injury to enable tissue restoration to occur (objective 2). By combining insights from these two main areas of work we hope to ultimately develop therapeutic approaches to stimulate heart muscle and vascular repair and regeneration and to dampen inflammation and fibrosis (objective 3), thus preventing adverse remodelling and heart failure.

**What 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, as outlined, will help identify changes that occur in the heart in the first few minutes following the loss of oxygen and nutrients after a heart attack, thought to contribute to the early death of muscle cells in the heart, and will also provide insight into mechanisms underlying progression to abnormal heart function and heart failure. This knowledge will help us manage patients who have suffered a heart attack in the first instance and, secondly, may lead to the development of new treatments drugs to stimulate the regeneration of lost cardiovascular tissue and to modulate

inflammation; thus reducing the risk of further heart attack and progression to heart failure.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The expected approximate usage of animals per annum is as follows: Mice- 2400 for generation of genetically altered lines and breeding and maintenance to supply the project and, usage of 1540 940 adult mice and 600 neonatal mice in surgery and therapeutic agent testing. Zebrafish- 2200 for the generation of genetically altered lines and breeding and maintenance (includes embryos and adults). 2300 adult zebrafish for surgery (heart and tail fin/flank) and cell and compound testing. Medaka – 660 for generation of genetically altered lines and breeding and maintenance (includes embryos and adults). 1200 adult medaka fish for surgery (heart and tail fin/flank) and cell and compound testing. These apply over the 5-year lifetime of the project.

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

We are breeding genetically altered mice and fish, the majority of which will be used for breeding and some of which will be used in experiments as adults. In the adult experiments we will need to injure heart muscle in living animals; in these cases, either a blood vessel in the heart will be tied-off to block the blood flow, or a piece of heart muscle will be removed or injured by freezing under general anaesthesia. With heart surgery there is a risk of death, but this is minimized, in our hands, to less than 10%. We will test whether administering cells and/or drugs can induce optimal repair of the heart via new tissue growth and/or reduced inflammation and scarring. In the case of mice, animals will be allowed to recover and given pain-killers; for zebrafish, we will test whether pain-killers are effective without altering outcome. The function of the heart will be monitored in the ensuing days (or weeks) by ultrasound imaging in conscious animals, or by studying the function of the heart in anaesthetised animals. At the end of the study, animals will be humanely killed and tissues removed for further analyses.

## Application of the 3Rs

### Replacement

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

### Replacement

The heart is a complex organ containing many cell types of which arguably the most important are the muscle cells, responsible for the pumping function of the heart and the endothelial and smooth muscle cells, which make up the blood vessels of the

heart. Many of the experiments we propose will be carried out on isolated pieces of cardiac tissue or cell cultures of heart muscle, blood vessel and epicardial cells studied in the laboratory. However cells in a test tube or in a tissue culture dish cannot be used to study the complex changes occurring in the complete heart, nor how it functions in a living animal. Equally, isolated cell populations in tissue culture transform to adopt different functional characteristics, compared to the equivalent cells as they reside in the heart proper, which confounds any experiments to determine the effect of externally-added factors on heart injury and repair.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Power calculations (80% power at 5% significance to show a 25% point difference in any one parameter) provide a minimum number of animals. Use of between 8-11 per treatment group ensures statistical significance, given the inherent variation between animals in response to heart injury. An important reduction in number will be by restricting control (sham-operated) animals to the first set of experiments to determine the baseline response of the heart to the surgery itself, in the absence of the final injury insult; once this is standardised sham animals will not be required.

## **Refinement**

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

### **Refinement**

The choice of species is based on the need to study regenerative animal models, such as the adult zebrafish and neonatal mouse as compared to a non-regenerative model such as the adult mouse and medaka fish which recapitulates the response to oxygen-deprivation and cardiac injury in humans. Moreover, the genetic tools (transgenic and knockout lines/strain in both fish and rodents), the ease of manipulation of individual cell populations and proteins both in circulation and resident within the heart make these models relevant for translating findings into humans. For all surgical procedures in mice pain killers will be administered routinely for the control of post-operative pain and aseptic techniques will be used to minimise the risk of post-operative infection. For teleost fish including zebrafish and medaka fish, pain sensitivity is unclear and no recommended pain killers exist, so we will test those used for routinely elsewhere for effects on fish and on the outcome of our experiments. We have also introduced ECHO as an imaging modality for assessing cardiac function in fish (in addition to MRI), that does not require injection of contrast agents (as for MRI) and moreover is conducted over a much shorter

timeframe thus reducing the length of time of exposure of fish to anaesthetic and risk of over-anaesthesia. Animals will be routinely monitored after surgery for signs of discomfort in recovery and any infection treated with veterinary advice. General anaesthesia will be used for mouse models requiring heart surgery. For the neonatal mouse model this has recently been refined across early stages to ensure that ice-induced anaesthesia is combined with a suitable inhalation agent to ease potential discomfort upon recovery and body warming; working closely with an in-house anaesthetic expert.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 37. Principles of human development and germ cell program</b>
<b>Key Words</b>	Germ cells, Totipotency, Epigenetics, Stem cells, Embryo
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Primordial germ cells (PGCs), the precursors of eggs and sperm emerge within the human embryo on day17 of development, and in the mouse on day 6.5, while the surrounding cells develop into rest of the bodily tissues. We know relatively little about the development of the human germ cells, which may have diverged from the mouse. Tracing the origin and development of PGCs will reveal the organisation of the early embryos. At fertilisation, germ cells pass on approximately 20,000 genes, the blueprint for development, and non-genetic information that can regulate gene expression. Together they are critical for how the brain and other organs develop and function. The transfer of this information from parent to offspring by the 'immortal' germ cells is repeated for every generation and has consequences for human health and disease. Germ cells undergo extensive reprogramming which rejuvenates the lineage and is essential for their distinctive potency. By contrast, bodily tissues become prone to age-related diseases. Detailed understanding of the mechanism of germline reprogramming may provide insight into possible approaches to address age-related diseases in bodily tissues

**What 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 how the human body develops from a fertilised egg to adulthood, functions, and ages are critical for advances in medicine. Recent progress in our work provides unprecedented opportunities to study how human germ cells develop into sperm or eggs, as well as very early human development. The study may potentially reveal causes of infertility, germ cell tumors, and mutations affecting fetal abnormalities. We aim to elucidate the origin and establishment of the germline precursor cells in early embryos that eventually develop into viable sperm and eggs.

At fertilisation, these cells pass on the instructions for the development of a whole new organism consisting of all the diverse cell types in adults. Understanding the nature of the instructions being transmitted by sperm and eggs, and how these instructions are interpreted during development is important; faulty instructions cause diseases and premature aging. With advances in knowledge from this research, it may be possible to prevent or overcome the causes of some human diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 4000 mice during this 5-year project overall for the project. Of these, the majority of the animals (approximately 1500) may be genetically modified, while nearly 2000 will be used to provide embryos for studies on germ cells or early embryos.

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

Most of the mice to be used in this project are not expected to suffer any adverse effects. They will be maintained as genetically modified animals, which rarely if ever show mild abnormalities. These animals will be used for the production of early embryos in short-term procedures or to generate pluripotent stem cells for in vitro models for germ cell development. We estimate the use of 250 animals in surgical procedures, which may receive transplants of early germ cells for their anticipated development into mature gametes. Occasionally, tumors may form from such transplants, but none will be allowed to cause more than minimal discomfort. All animals will be humanely killed at the end of experiments, and within one year if required for the purpose of breeding.

## Application of the 3Rs

### Replacement

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

### Replacement

We have most detailed knowledge of mouse development amongst all the mammalian species. Mice are therefore ideal for studies of germ cells that develop into sperm and eggs. We are able to mimic most aspects of the early mouse germ cell development with the help of pluripotent stem cells, which we can use extensively in many studies. However, it is essential to validate the key findings with observations on mouse germ cell development embryos *in vivo*. Our extensive studies on mice have now led to the models for early human germ cell development from pluripotent stem cells for the first time. This is important because very early human embryos are not accessible for such studies. The human

germ cells that develop from stem cells in a petri-dish are at very early stages of their development. These may, however, develop further, possibly into viable sperm and eggs gametes, if introduced into ovaries or testis of sterile mice.

Alternatively, aggregates of germ cells and gonadal cells if placed underneath the kidney capsule of adult mice may also develop significantly further, possibly into sperm or eggs. As a surrogate for human embryos, we also study porcine embryos since development of their embryos and germ cells is apparently like what occurs in human embryos. Altogether, the combined studies are necessary to provide detailed knowledge of mammalian germ cell development.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We have developed very efficient methods for generating germ cells from mouse embryonic stem cells. These methods are robust and reproducible and we use them extensively to study how early germ cells can develop outside the animal in a petri-dish. However, we require animals initially for obtaining embryonic stem cells. After that our model allows us to perform a large number of experiments without direct use of animals, which reduces the number of animals that would otherwise be required for these studies. The work carried out with cells in a dish must, however, be confirmed to be reflective of what actually happens within a live animal. Germ cells made from human stem cells may also develop further and potentially into sperm or eggs if introduced into adult mouse ovaries or testis.

### **Refinement**

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

#### **Refinement**

We have performed extensive work on mice since they are easy to breed, and we can determine the consequences of genetic modifications of essential genes on the development of germ cells. We have also developed many tools and reagents, which help us to design the best experiments with a minimum number of animals. We share our cell lines with others in the field, and in exchange, we obtain reagents from others, which also helps to reduce the overall use of animals in this field. The short length of gestation in mice, and the time required for germ cells to develop into sperm and eggs makes it easier to develop culture methods for studies on germ cells. Consequently, we only need to validate the key findings with minimum numbers of animals to ensure the authenticity of the results obtained from cells in culture. Less than 1% of our animals undergo regulated procedures and subjected to

surgery, which involves procedures for making mice receptive for transplantation of early germ cells. This is necessary since we currently lack methods for their full development of germ cells into mature gametes in culture. All animals undergoing surgery will be provided with pain relief. Adult mice receiving transplanted cells to be tested for development of germ cells into viable gametes will be carefully monitored daily for signs of ill health. If signs of ill health are apparent, or if there are signs of anything more than minor discomfort, the animals will be humanely killed.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	Project 38. Fracture Fixation Devices
<b>Key Words</b>	Orthopaedic, Infection, Implant, Trauma, Osteoplasty
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

**(a) Resorbable Balloon Stent Osteoplasty:** The active population suffering from osteoporosis (OP) contributes to the growing issue of fragility fractures, which are common in both men (1/5), and women (1/2) over 50 years of age. In more dramatic terms, an osteoporotic fragility fracture is estimated to occur every three seconds with some experts predicting that 30% of hospital beds will soon be occupied by these types of patients. Despite these market trends, implants for fracture treatment are designed for young, healthy bone, and have not shown equal success when applied in OP bone. A minimally invasive resorbable balloon osteoplasty could address this unmet clinical need by proving an alternative option for stabilising an OP fracture that could reduce pain, and increase mobility. If this device proves to be successful with these patients, it could also be an attractive option for extremity fractures sustained in (a) the younger patients who want to remain active during rehabilitation, and avoid the prospect of a permanent implant and potentially a second procedure, and (b) patients with impending or pathologic fractures caused by metastatic carcinoma. The primary aim of this project is to establish proof of concept by determining whether a resorbable (dissolving) intramedullary osteoplasty is capable of stabilising a fracture in a suitably designed in vivo bone healing model, which is representative of a human upper extremity fracture. If successful, further studies will be conducted to demonstrate product safety and whether the implant fully resorbs within a 2 – 3 year period.

**(b) REDACT** Patients with traumatic injuries are at increased risk of infection most notably in the tibia due to the low level of blood flow. Infections are difficult to treat even with systemic antibiotics especially in the presence of a foreign surface such as an implant where bacteria can form a protective biofilm and become immune to systemic antibiotic delivery. Further, the impact of trauma and other comorbidities may compromise the immune system where it is most needed, therefore making those patients more vulnerable to the risk of infection. Infections can lead to delayed healing, the need for further surgery to remove the implant, and in severe cases,

amputation of the infected limb. Over the past few decades, attempts have been made to prevent and cure orthopaedic-related implant infections by incorporating antibiotics in polymethylmethacrylate bone cements, in primary and revision surgery. However, the clinical efficacy of antibiotic-releasing bone cements is not accepted by all and the long-term exposure to low doses from antibiotic-releasing bone cements in patients is strongly related to the emerging threat of antibiotic resistance in medicine today. Consequently, there is a clinical need to reduce the number of device-related infections by developing a surface modification which protects the implant from bacterial colonisation. The aim of this project is to demonstrate that these antimicrobial coatings applied to either an intramedullary nail or bone plate do not have an adverse effect on fracture healing in a large animal model through radiographic and mechanical testing of the callus. REDACT

What 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) Resorbable Balloon Stent Osteoplasty: The primary aim of this project is to develop a pre-clinical model in a sheep metatarsus to investigate the potential application of a minimally-invasive resorbable inflatable balloon stent osteoplasty as an alternative option to traditional metallic fixation devices for stabilising upper extremity fractures while avoiding the need for open surgery. This would provide an attractive option for (a) younger active patients who want a more rapid rehabilitation and do not want a permanent implant and a potential second surgery, (b) osteoporotic patients where treatment is difficult due to diminished bone density and compromised biomechanical integrity, and (c) oncological or impending fractures due to metastatic cancer. Osteoporotic and oncologic fractures that require internal fixation represent significant unmet clinical needs and significant patient populations, i.e. >5 million OP and >450,000 oncological patients worldwide annually. Treatment with conventional internal fixation using intramedullary nails or plating is associated with poor clinical outcomes in the OP and oncologic patient populations. Subsequent fractures and complications such as screw pull-out necessitate additional interventions, prolonging recovery and increasing health care costs. (b) REDACT The primary aim of this project is to develop pre-clinical fracture models in sheep for screening the impact of a local delivery of an antimicrobial agent, e.g. silver on the progression of bone union. This will ensure that antimicrobial implants developed are safe and effective for clinical evaluation without compromising the primary function of the fracture fixation device. If successful, this would significantly reduce the occurrence of infection following implant surgery leading to higher success rates and less risk, pain and inconvenience to patients, with all that entails, and reduced economic burden to already stretched healthcare systems. The average cost of combined medical and surgical treatment of an infection related to a trauma fixation device is estimated to be £10,000 but can be as high as £500,000 if multiple surgeries, amputation, and rehabilitation is required.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the five year duration of the license it is estimated that no more than 600 sheep will be used across the six 19b protocols.

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

The project license will involve the surgical implantation of either an intramedullary nail, bone plate or a resorbable balloon stent osteoplasty into a bone canal, which may lead to some degree of discomfort following surgery. However, this will be reduced by the use of minimally invasive surgical techniques. Any discomfort will be minimised with the use of appropriate pain relief administered before, during and after surgery as advised by the NVS, by either subcutaneous, intramuscular, intravenous or oral routes as required to control post-operative discomfort or infection. Any local or systemic reactions to the materials are very unlikely due to the known biocompatibility of the materials when implanted in bone but if problems arise then the animal will be promptly and humanely killed. Significant surgical sepsis is unlikely but if the condition is suspected (appearance of malaise, pyrexia, pain, redness, swelling) then the animal will be promptly and humanely killed. If the IM nail, bone plate or stent osteoplasty is dislodged in such a way that it compromises the welfare of the animal then the animal will be promptly and humanely killed by a schedule 1 procedure regardless of project requirements. This decision will be made by the NACWO or personal license holder and reported to the project license holder and NVS. At the end of the studies the animals will be humanely killed. In addition any animal showing severe signs of suffering whilst on study (e.g. excessive weight loss, signs of uncontrolled pain, significant lameness) will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

The project consists of an extensive laboratory based testing regime to select the most appropriate formulation(s) for development. While this testing will allow accurate selection of prototypes, it cannot replicate the complex biological interactions at work within the body, thereby making performance testing in animals an essential part of the plan before human trials can commence. The sheep model offers some unique advantages over other species that makes it particularly feasible for testing fracture fixation devices in terms of anatomy, surgical technique and post-operative care.

### **(i) Anatomy:**

- Bone (cancellous, cortical, and plexiform) will usually heal in 10 -14 weeks depending on the type of fracture created.
- Body weight of adult sheep is similar to humans (approx. 70-80Kg) and their long bones have a similar surface to volume ratio.
- The histomorphological and anatomical characteristics of sheep bone are comparable to those of human.
- The position of the sheep tibia during locomotion as being similar to the position of the tibia of a human who is running on the toes with partially flexed knees

### **(ii) Surgical technique:**

- The mechanical characteristics of the implant and instrumentation are usually identical to those used in human patients.
- Surgical approaches and anatomical positioning of the fixation device are also similar between sheep and human given their similar bone dimensions.
- Minimal complication rates are observed in this model after IM nail surgery (cf. 5 to 10% maximum).

### **(iii) Post-operative care:**

- The activity/noncompliance of the animal can/may be controlled if necessary during the post-operative period by the use of casts or Schroeder-Thomas Splint, which limits the peak forces exerted on the healing bone. A cast will limit shear, rotational and 3-point forces but not compressive. Schroeder-Thomas splint will also limit compressive forces by transferring the loading to the hip joint.
- The physical activity levels of the animal can be quantified using Force Plates to determine when the operated limb reaches full weight bearing.

In summary, the biomechanical and bone healing characteristics of sheep are more similar to those found in humans than of other, smaller species. The use of a human implants, surgical techniques and post-operative care techniques also justify the use of this species for assessing fracture healing in this license.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Prior to studies covered by this project license, extensive laboratory based testing will be used to screen out the most promising technologies that will be put forward for testing in animals. Consultation with a statistician at the planning stage will be actively used to optimise study design, minimise the number of animals required, and meet the study objectives. This will comprise setting clear study objectives, and

ensuring appropriate output measures are collected and analysed using appropriate statistical methods. Historical data will be used wherever possible to determine the appropriate sample size to achieve the required study power.

### **Refinement**

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

### **Refinement**

Animal suffering will be minimised by proactively refining the models using the following approaches, (a) consultation with people with expertise in either orthopaedic surgery, animal welfare or biostatistics, (b) cadaver sessions, which will refine surgical procedure and hence, reduce the level of pain, suffering distress or lasting harm experienced by the animals and (c) pilot studies to monitor animal behaviour to a particular surgery/implant design. Other animal models or experimental approaches will be investigated by appropriate consultation of the background literature.

Other steps to reduce animal suffering will include performing the surgery aseptically under general anaesthesia. Antibiotics and analgesics will also be administered before, during and after surgery as advised by the NVS, to control post-operative discomfort or infection.

The operated limb will also be either cast or splinted for up to 6 weeks to minimise the risk of implant failure and secondary fractures.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 39. Production, maintenance and analysis of genetically altered livestock</b>
<b>Key Words</b>	Sheep, pig, editing, disease
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aims of this project relate to the creation of specific changes to the genes of pigs or sheep. They include experiments to make pigs that are resistant to diseases that blight the commercial pig industry, the creation and analysis of sheep models of human diseases, and refinements to the way we make genetic changes to animals.

Diseases of livestock are a huge economic burden on the farming sector, and can result in considerable uncertainty in production systems. One of the diseases we are working on is caused by Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). This global disease causes abortion in pregnant pigs, death of newborn piglets and a failure to thrive in older animals. There are currently no effective vaccines or treatments for this disease. It is estimated that in the EU alone PRRSV is responsible for approximately €1.5 billion of production loss every year. A second disease that will be addressed in this application is caused by African Swine Fever Virus (ASFV). This virus causes a disease in pigs that is similar to Ebola in humans, and currently there is no good vaccine or treatment. Until recently this virus was restricted to Africa, but in 2012 there was a case of ASFV in Ukraine, which has since spread throughout the Balkan states and Russia and this year has spread as far west as the Czech Republic. For both of these viruses one must not only consider the financial losses of farmers but also the significant suffering experienced by infected animals.

Animals are often used by scientists as models of human diseases, allowing us to better understand disease processes or to test new treatments. Mice are commonly used for this purpose, but either the biology of mice or their small size can sometimes make them inadequate models. We are currently investigating the use of sheep as models of two different human diseases. Cystic fibrosis (CF) is a disease

associated with repeated chest infections and a significantly shortened lifespan. Mouse models of CF do not develop the lung disorder experienced by humans. A pig model of CF has proven useful for better understanding the human lung disorder, but also develops an intestinal blockage requiring corrective surgery as soon as piglets are born. We think that the genetic change we are making in sheep will result in a model with lung but not intestinal disease, allowing us to test new therapeutic approaches without the welfare concerns associated with additional surgery. The second model is of Batten disease. This is actually a group of closely related genetic disorders that result in the death of cells in the brain resulting in death of affected patients. Mutation in a gene called PPT1 cause the most severe form of this disease, with affected children dying before puberty. There are good mouse models that have greatly increased our understanding of this disease. However, a larger model with brain structure more similar to humans is now required to investigate the application of therapeutic approaches.

Most biological traits (e.g. height or intelligence) are influenced by many interacting genes. Small differences in the sequence of the genes inherited from each parent contribute to the nature of the offspring. Standard livestock husbandry involves breeding the “best” animals together, but it can often take many generations and therefore many years to reach a desired goal. We previously used genome editors to make precise changes to single genes of pigs and sheep. We now want to try to change several genes at the same time to test how efficiently this process could be used for agricultural improvement.

**What 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 on disease resistant animals should benefit agriculture (both farmers and REDACT).. The main uncertainty at present is not the value of disease resistant animals in agriculture (everybody wants healthy animals) but the way the technology we use to manipulate livestock genomes will be regulated by authorities. As such, we are also working on ways to minimise the amount of genetic change while at the same time maintaining the observed benefits. Our models of human diseases aim to allow pharmaceutical companies or charitable organisations to better evaluate therapies that they have developed in other systems. Both of the proposed models of human disease have the potential to overcome limitations of existing animal models and provide a tool to both evaluate therapies and further improve our understanding of these diseases. The ability to manipulate several genes at the same time will initially benefit the scientific community through a better understanding of applications (and limitations) of the molecular tools we use. In the longer term we anticipate that such approaches will become increasingly common as part of commercial livestock husbandry practices.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The work set out in this project will involve approximately 330 sheep and 650 pigs over the next 5 years. Our experiments involving PPT1 require approximately 250 sheep. Our CF experiments will involve approximately 80 sheep. For experiments involving disease resistant pigs we will use approximately 350 pigs to supply animals for pathogen challenge studies under other licenses. In order to refine our methods of genetic alteration we will use a further 300 pigs.

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

Pigs and sheep are housed on our research farm. Pigs are housed indoors all year, but most sheep in these studies (with the exception of those involved in disease progression monitoring) will have access to pasture for most of the year. The majority of animals used in this project will be involved only in breeding, with no ill effect anticipated as a result of either the procedure or their genetic alterations. Some of the animals involved in human disease modelling (those carrying 2 copies of the altered gene) are likely to become sick. Because these are new models we do not know how fast disease progression will occur, so will monitor animals closely as they age. Once the first few animals (3-5) reach a pre-defined point (e.g. the first sign of significant disease) they will be killed and samples taken for analysis. Subsequent animals will be killed before they reach this point. Sheep will be anaesthetised by single injection of a suitable drug into a vein in the neck. Pigs will first receive an intramuscular injection of a sedative then anaesthetic via an ear vein. Both species cope well with anaesthesia. Sheep recover to standing within 10-15 minutes after cessation of anaesthesia, while pigs return to performing normal behaviours within several hours. Bronchoalveolar lavage of both sheep and pigs will be used to take samples from the lung. Animals will be anaesthetised throughout and it is our experience that they experience no apparent adverse effects from this procedure. For MRI imaging (to look at the brain or lungs) animals will be anaesthetised and remain unconscious throughout the scanning process. A sample of spinal fluid may also be taken at this time; there is a small risk of infection as a result of this procedure. This is avoided by careful use of hygienic methods. In creating new lines of animals some will undergo surgery. For pig embryo recovery donors are sedated then given an anaesthetic overdose to kill them prior to surgery. Recipients are anaesthetised and ovaries exposed through a small surgical incision in the abdomen. Genetically altered embryos are inserted into the uterus, and then the abdominal wall is sutured closed. One risk that all of the above procedures have in common is infection. However, our good practice means that this is very rare. At the end of these procedures some animals will be retained for breeding and some may be moved to other projects, but most will be killed at the end of their use, for example to provide tissues for analysis.

### Application of the 3Rs

## **Replacement**

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

### **Replacement**

Where possible we do use alternatives to animals. For example, we can use computer analysis of the large amounts of genetic information that is now available to identify which genes are involved in eg disease resistance in farm animals. Also, there is a lot of work in cells to refine methods of genetic manipulation before they are used to create genetically altered animals. However, to fully understand how an animal responds to an infection there is no alternative to using whole animals.

In modelling human diseases, much earlier work has been done in cell culture and in mouse models. However, for both of the diseases outlined in this project the mouse model either fails to replicate the human condition or is not appropriate, due to its size and physiology, for adequate testing of novel therapeutic approaches.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Every new experiment is carefully evaluated by experts in statistics, ethics and animal care and requires official approval before it can proceed. As part of this process we must set out clearly the goals and the experimental design we will apply to answer our questions. This is an ongoing process throughout the project that subjects every experiment to rigorous expert evaluation and ensures the minimum number of animals is used to meet our objectives.

## **Refinement**

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

### **Refinement**

The pig diseases we work on only infect pigs, so “lower” species cannot be substituted. This license is restricted to production, maintenance and breeding of these animals; any infection studies will be carried out on separate license authority.

Sheep have been chosen as a model for CF because they are large animals whose lungs are anatomically and physiologically similar to humans. They are amenable to bronchoscopy, and tolerate repeated interventions in this manner with no discernible clinical effect. Measures of toxicity relating to bronchoscopic interventions show

consistency with related work involving human CF patients. Sheep are frequently used animal models and are widely accepted as a key element in the process of developing drugs to combat respiratory disease.

Similarly, sheep have been chosen as a model for Batten disease because they are large animals whose brain architecture is similar to that of humans (while mice are not). Sheep are amenable to handling for regular behavioural and neurological assessment and have good tolerance for the repeated anaesthetic required for consecutive MRI imaging over time.

Pigs have been chosen to test methodological improvements in the application of genome editors to livestock because they have multiple offspring in a single litter (typically more than 10). This means that fewer animals are required to undergo surgical procedures to generate statistically significant datasets.

Initial observations are usually of a small number of animals, enabling us to refine subsequent studies with larger numbers. Such a progressive approach allows us to better understand the outcomes of our genetic alterations and identify experimental and humane end points that minimise any suffering experienced by the animals.

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<b>Key Words</b>	Sheep, pig, editing, disease
<b>Expected duration of the project</b>	5 year(s) 0 months

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### Application of the 3Rs

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Initial observations are usually of a small number of animals, enabling us to refine subsequent studies with larger numbers. Such a progressive approach allows us to better understand the outcomes of our genetic alterations and identify experimental and humane end points that minimise any suffering experienced by the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	Project 41. The effect of <i>H. pylori</i> infection on colitis.
<b>Key Words</b>	Immunosuppression, <i>H. pylori</i> , Colitis, Microbiome
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

*H. pylori* is a bacterium that causes life-long infections of the stomach by suppressing the ability of the host's immune responses to clear the infection. *H. pylori* is well known as the causative agent of stomach cancer, however, this occurs in only 1% of all infected patients. Recently, *H. pylori* has been associated with beneficial effects. Epidemiological studies suggest that people who carry *H. pylori* in their stomachs are at a lower risk of developing auto-immune disorders, such as asthma and IBD. However, the mechanism for this association is not understood. We aim to study how *H. pylori* infection in the stomach affects the diversity of bacterial species in the intestine (by analysing faeces) and intestinal inflammation (by analysing a subset of white blood cells known as T lymphocytes) during colitis to understand the mechanisms underlying this epidemiological link between *H. pylori* infection and protection against IBD.

What 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 mechanism of how *H. pylori* manipulates host immune responses to prevent auto-immune disorders may provide the rationale for drug design of novel anti-inflammatory therapeutics, which can be used to tackle auto-immune disorders such as asthma and IBD. Furthermore, our human clinical studies suggest that *H. pylori* infection in the stomach has an impact on the types of bacteria found in the intestine in IBD patients. Thus, exploring this phenomenon in mice will help us to unravel the hugely complex issue of the intestinal microbiome in colitis and how *H. pylori* affects this, which may lend itself to a protective mechanism against IBD.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

All of the studies proposed in this licence will use mice as the chosen model. The numbers of mice to be used in the next 5 years will be approximately 1000

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

*H. pylori* in humans is largely asymptomatic. Only a very small percentage (<1%) of infections lead to gastric cancer. However, in nearly all infections, *H. pylori* shapes the development of our immune system and the microbiome in the stomach and potentially, in the intestine. In mice, infection with *H. pylori* is also largely asymptomatic and severe gastric disease will only be seen after 2 years of infection. In this licence, we will not be infecting mice with *H. pylori* for longer than 12 months and so the mice will not suffer from morbidity or mortality during this period. However, *H. pylori* will have more subtle effects on the immune system and microbiome of mice and we are interested to see how this manipulation affects the development of colitis. Colitis will be induced in mice using well established methods. Mice are likely to suffer adverse effects from colitis such as diarrhoea, intestinal inflammation/bleeding and weight loss. These effects are closely monitored on a daily basis using score sheets to monitor and record disease severity. These experiments are likely to lead to a moderate level of severity. With careful monitoring, mice will be humanely killed when they reach the end of the experiment or they reach the clinical end point as defined by the score sheet. Mice will be terminated via a schedule 1 method and organs harvested for further study.

## Application of the 3Rs

### Replacement

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

### Replacement

*In vivo*, adaptive immune responses to bacterial infection develop through the interaction of multiple cell types including one or more DC subsets, T cells and B cells. We, and others, have repeatedly shown that these interactions *in vivo* are not fully replicated *in vitro*. However, we will be using immortalised cell lines and both human and mouse gastric and intestinal organoids to investigate epithelial and T cell responses to *H. pylori* and other members of the gastrointestinal microbiomes. Understanding the effect of *H. pylori* on T cells *in vitro* will inform the cell types of interest in *in vivo* studies.

In order to study the gastric mucosal immune response to the infection, and how it influences the community of bacteria in the intestine, we require intact gastrointestinal systems. Mice provide an established and verified means of studying immunity with tractable systems that allow detailed analyses of these complex environments.

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. To this end, we will use the NC3R's systematic review tool to ensure we are using the best model for our scientific objectives. This tool can be found at [www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf](http://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf)

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

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. For instance, after experiments are concluded, tissues such as the spleen are often sectioned for immunohistology and archived. This archived tissue can be revisited by other workers at a later time.

Power calculations and group sizes

The minimum group sizes required to obtain statistically significant differences have been calculated using power equations with a 2-sample t-test on previous experimental data. For a power of at least 80%, group sizes should be of no less than 6-8 mice when comparing *H. pylori* infected and non-infected mice. For experiments comparing colitis in *H. pylori* infected and non-infected mice, group sizes are likely to require 8-10 mice. Short-term experiments are likely to provide statistically significant data with groups of 8, as found previously. During long-term experiments, it is possible that a few animals may die prematurely from problems that could be unrelated to the regulated procedures. Therefore, to ensure that experimental data can be analysed statistically and that data from remaining animals can still be used, slightly larger group sizes will be used. In experiments longer than 6 months, groups of at least 8-10 mice will be used.

This website will be utilised to provide additional information on statistics and experimental design <http://www.3rs-reduction.co.uk/>

In order to ensure that high quality, reliable and valid data is extracted and reported from the minimum number of experiments, the ARRIVE guidelines (Kilkenny *et al.*, 2010) will be followed. <http://www.nc3rs.org.uk/page.asp?id=1357>

## Refinement

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

### **Refinement**

Mice are an appropriate model as their immune systems share many similarities to those of humans including lymphoid organization and cellular populations (e.g. lymphocytes, DC). GA mice, provided by collaborators, will be used in this project and provide a well-established means to study the immune system. For new protocols we work closely with REDACT to refine the techniques to minimise any suffering that might otherwise occur.

Bacterial and host response factors are likely to have an effect on the colonisation density of *H. pylori* in the stomach of mice. The relationship between the immune response and the density of *H. pylori* must therefore be assessed in detail. Furthermore, pilot studies will inform the shortest length of time and the lowest dose of DSS or TNBS that can be used to induce sufficient intestinal inflammation, whilst limiting any adverse effects to the mice.

Before conducting each experiment, it is discussed with named veterinary surgeon to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to achieve the scientific endpoints. We will also 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 the next experiment.

Additionally, animals that are infected with *H. pylori* for 6 -12 months and show a weight loss of greater than 5% will be weighed weekly.

The score sheet outlined in the Appendix will be referred to for every experiment to ensure the animal welfare throughout the experiment.

Furthermore, if the named veterinary surgeon or the NACWO on site are not familiar with a certain technique, we will visit external collaborators in order to be fully trained on new techniques prior to initiating pilot studies.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 42. The use of tissue engineering for reconstruction and regeneration in congenital heart defects</b>
<b>Key Words</b>	Congenital heart disease, Tissue engineering, Stem cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of the work to be conducted under this licence is to develop and test bioengineered grafts/devices for the treatment of children with congenital heart defects (CHD). The emphasis of the work is on assessing the safety and effectiveness of devices that have the potential to grow like normal tissues as the child develops.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

About 1% of children are born with a heart defect that requires surgical repair, which often involves the implantation of grafts. Current available graft materials do not grow as the child develops and consequently further corrective surgery is often required to repair or replace grafts, to enable the developing heart to function effectively. Corrective surgery carries inherent risks for the patient, involving a not insignificant suffering on the patient, and is costly and time consuming for the NHS. The development of a graft that would fully integrate with the patient's tissues and grow normally with the child's development would significantly reduce the number of patients requiring corrective surgery and therefore benefit both the patient and the NHS. In addition, the development of such device has the potential to advance the effectiveness of tissue repair in a wide range of medical conditions, such as the repair of heart tissue that has been damaged (such as by myocardial infarction or failing CHD therapy).

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We need to use 200 pigs and will assess the grafts for up to 9 months after the surgery.

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

We have optimised the procedures to cause the minimum level of suffering. The pigs will undergo surgical procedures that closely mirror those conducted routinely on patients within the NHS, and animals will receive comparable pain relief. We are very experienced in treating any adverse effects (eg bleeding, accelerated pulse) that may occur clinically or experimentally. The surgery undertaken is relatively major, but the pigs tolerate this well and recovery is rapid. Following surgery, the pigs are expected to return to normal behaviour within 24 hours. The animals will be killed at the end of the experiment in order to recover the graft and further assess its performance.”

## Application of the 3Rs

### Replacement

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

### Replacement

Determining the safety and effectiveness of our devices prior to clinical use requires testing in a relevant live animal model. The conduits being tested are ultimately intended for use in humans, consequently they can only be effectively tested in an animal model with a comparable cardiovascular anatomy and size to a human infant. There are no non-protected species that meet these criteria.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Wherever possible, we will use the pig’s own cells, to reduce the need for donor animals and thus avoid graft rejection. This also will reduce the number of animals required

### Refinement

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

## Refinement

The replacement tissues being tested are ultimately intended for use in human, consequently they can only be effectively tested in an animal model with a comparable cardiovascular anatomy and size to a human. The pig is an ideal candidate as it's cardiovascular system closely resembles that of humans and it is of a suitable size to receive human sized implants. In addition, the rapid growth of the pig makes it an ideal species for assessing the ability of tissue engineered device to integrate and grow in response to the animals' development.

We have improved and refined our pig models for the last 5 years. This has enabled us to optimise our surgical and pain-control therapies. All surgical procedures will be conducted following standard NHS practices including post-operative pain control.

We will assess device function at a number of time points, using non-invasive imaging techniques e.g. MRI scanning and echocardiography, which will enable us to detect any failing device and thus end the experiment without causing distress to the animal.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 43. Production of Blood Products for Scientific Use</b>
<b>Key Words</b>	Horse, Sheep, Blood
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of the project is to provide a regular source of fresh sterile donor blood products from horses or sheep. Clinical and Veterinary Laboratories use the animal blood as a nutritious supplement for the manufacture of culture media for the identification of microbial organisms.

Defined volumes of blood are collected from healthy donor horses and sheep at pre-defined 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)?**

Microbiology/Pathology laboratories have established methods for the detection of pathogenic organisms by recognition of the growth characteristics and colony morphologies of the different organisms on culture media containing defibrinated blood. These classical microbiology methods are well established and although new media types are more readily available culture media products containing horse and sheep blood are essential for the diagnosis in microbiology/pathology laboratories

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 300 horses and 900 sheep 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 levels of severity? What will happen to the animals at the end?**

The adverse effects will be mild. Procedures have been designed to limit the suffering to donor animals. This includes refinement of the bleeding equipment and facilities to ensure animals are less stressed, and ensuring the bleeding process is efficient and ensures as little physical damage to donor animals as possible.

## Application of the 3Rs

## **Replacement**

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

## **Replacement**

Classic microbiology methods rely on the availability of horse and sheep blood.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

Processes have been established to ensure the minimum numbers of animals are used. Each animal is logged within our database and closely monitored to ensure it can provide the correct amount of blood keeping animal numbers and cost to a minimum

## **Refinement**

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

## **Refinement**

There are no alternatives to the animals selected. The bleeding facilities and equipment are refined to ensure the animals are less stressed and prevent physical harm. The bleed process has also been refined to ensure efficiency.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 44. Regulation of glutamate transport and inflammation in neonatal hypoxic-ischemic encephalopathy</b>
<b>Key Words</b>	new-born baby, brain injury, treatment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The work conducted under this licence aims to identify treatments and diagnostic aids that can be used to improve the outcome for babies that suffer brain injuries at, or around, child birth as a result of being deprived of blood and oxygen. Such injuries are often fatal and for surviving babies invariably result in life long debilitating effects. Although the causes of these events are well understood current treatments are at best only partially effective and in many cases babies fail to respond to the treatments available. A major obstacle to the development of effective treatments and diagnostic aids is a lack of clear understanding of the pathological process involved at the molecular level. This work aims to address this lack of basic knowledge and to evaluate diagnostic tools and treatments that could be used to improve the outcome of brain injured babies.

What 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 estimated that globally around 800,000 babies die each year as a result of brain injuries incurred at, or around, birth and that over a million babies incur injuries that result in life long debilitating effects. Current treatments and diagnostics are limited and are at best only partially effective. It is to be expected that through a better understanding of the molecular mechanisms of injury it will be possible to identify interventions and diagnostic aids that can be used both to improve treatments, aid diagnosis and provide better prognosis for brain injured babies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 400 new-born rats over the course of 5 years.

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

In the attempt to simulate the brain injury that happens to babies around childbirth due to blood and oxygen deprivation, we will surgically impair the blood flow in the brain of new-born rat pups under anaesthesia and expose them to a period of low oxygen. In addition, some animals will undergo treatments aimed at restoring normal brain function. As a result of these procedures most animals will incur a degree of brain impairment and experience minimal pain or distress. Some animals may develop repetitive seizures that may cause distress. Pain associated with surgery will be prevented through the use of appropriate general and local anaesthetics. The expected level of severity for the project licence is classed as severe as a proportion of animals may die whilst being exposed to low oxygen levels. However, they will be unconscious and therefore will incur minimal suffering. Following the induction of a brain injury the animals will be killed at specific time points to enable the collection of brain tissue for assessing key genes involved in the transport of the neurotransmitter glutamate and in controlling inflammation.

## Application of the 3Rs

### Replacement

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

### Replacement

The aim of this work is to identify treatments and diagnostic aids that can be used to improve the outcome for babies that suffer brain injuries at, or around, child birth as a result of being deprived of blood and oxygen. Such injury cannot be studied using *in vitro* or *in silico* systems due to the complexity of the interactions that occur at the level of the brain. Although some data can be obtained from humans, this is limited as it is not possible to obtain actual brain tissue in all but a very few cases, consequently the only way to obtain the required data needed is to undertake animal studies using a species with a well-developed brain that models that of the human.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will use appropriate statistical techniques to calculate the minimum number of animals required to answer each objective. The experiment is designed in two phases with an initial pilot study that will inform the main experiment with regards to essential controls, group size and the most meaningful time points for tissue collection. Several measures are in place to minimise variability, including using a

single strain of rat, conducting the experiment at the same age on all animals, limiting the management of surgery to two experienced team members and ensuring that experimental conditions (e.g. duration and dose of anaesthesia and hypoxia; temperature; humidity) are equivalent for all study groups.

### **Refinement**

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

### **Refinement**

REDACTED Rats provide a more suitable model than mice as their brain development more closely resembles that of humans and their response to brain injury is less variable. The surgical procedures are performed under a surgical plane of general anaesthesia and post-surgical pain is minimised through the use of anaesthetics. Measures are in place to minimise the duration of the surgery and avoid adverse effects and involve expert management of surgery and careful monitoring. All other procedures are either pain-free or cause only transient pain. The least severe route of administration will be used to give drugs or substances and pilot studies will be performed if they have not been given to the animal before. All animals will receive the highest possible standard of care and we will continue to look for ways to minimise suffering. Markers developed from this study are likely to improve the validity of the animal model in the future potentially minimising the number of animals needed to induce brain injury.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 45. Genetic control of cardiovascular development</b>
<b>Key Words</b>	Heart development, congenital heart defects, genetics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Genes play a major role in the development of congenital heart disease, a major cause of illness and death in childhood. However, the genetic and developmental mechanisms underlying most congenital heart defects remain unknown. Some patients with congenital heart defects have syndromes where certain genes have become mutated or are missing but it is thought that the syndrome can be further affected by mutations in other as yet unknown genes. This research aims to identify genes that work together in a network to control normal cardiovascular development. This project will use genetically modified mouse models to uncover genetic pathways that control cardiovascular 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)?**

Congenital heart defects affect approximately 1% of all births and is a major burden for the patient as well as the health care system. Data obtained from this project will help us to understand the interaction of the genes that contribute to the development of congenital heart defects. Also, by furthering our understanding of the genetic pathways that control cardiovascular development we may be able to devise screening strategies for prospective parents that can highlight any potential risk to the child.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will breed up to 100 mice of each strain listed on the project licence per annum, of which 90% will be used for intercrossing with other strains. We expect to use

about 10,000 adult mice, neonates and foetuses over the 5-year duration requested on 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 levels of severity? What will happen to the animals at the end?**

In a typical experiment, overtly normal genetically altered mice are bred together and the pregnant female is humanely killed and the foetal forms removed for analysis. There are therefore no adverse effects during this procedure on the adult mice. The foetal forms obtained may present with developmental abnormalities but these will also be humanely killed prior to birth, and will therefore not suffer any adverse effects.

## Application of the 3Rs

### Replacement

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

### Replacement

Unfortunately, due to the complexity of the processes involved in cardiovascular development, and the many different cell and tissues that need to interact with each other during this process, there are no suitable cell culture systems that can be used to completely replace animal models. We use genetically altered mice in our studies, since there is no non-protected animal alternative.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

All experiments are designed to use the least numbers of animals. We regularly consult a statistician for advice so that experiments are properly designed and group sizes are the minimum number required to give an accurate answer. We also use a magnetic resonance imaging technique which allows for foetal forms to be imaged without destruction, therefore the foetal forms can be used after imaging for analysis with other techniques. Moreover, the datasets generated of the foetal forms can be shared with other researchers. Our use of embryonic stem cells in culture will mean that we can recreate certain parts of the developing embryo in a dish. This will give us a model in which we can look for genetic interactions within one specialised tissue type without using actual embryos.

### Refinement

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

### **Refinement**

Mice are being used in this project because this is the simplest organism that has a similar heart and blood vessels to human, and that can be used to investigate the roles of different genes in cardiovascular development. Although zebrafish are a commonly used animal model in cardiovascular studies they are not an appropriate model for this project as the development of their great arteries, which is a major focus of this study, do not develop in the fish as they do in mammals such as mice and humans. Genetically altered mice are also available from other sources and can be obtained for answering our research questions. In the majority of cases we use embryos or foetal forms for analysis thereby minimising any harms to adult animals. Most of our mice are used for maintaining genetic lines and have no abnormalities. As we are investigating the effect of genetic mutations on the developing embryo or foetal form, the main procedure we carry out is by inter-breeding different genetically altered strains so that embryos or foetuses can be collected from the pregnant dam. Because mating is considered a natural act, this does not result in any abnormal suffering for the mice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 46. Interactions between cancer drivers as determinants of tumour tropism, phenotype and response to therapy.</b>
<b>Key Words</b>	Cancer, Breast, Treatment, Resistance
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aims of the project are to understand the fundamental reasons why different cancers arising in the breast appear different to each other and also why a single cancer will have regions of different appearance within it. We will also try and understand why different genetic mutations cause some type of cancer but not others e.g. why do people who inherit a mutation in the BRCA1 gene tend to get breast and ovarian cancer but not liver or skin cancer, as BRCA1 is a gene important for protecting all cells from damage. Finally, we will build on our previous studies to test treatments aimed at specifically killing the different types of cancer and understanding resistance to 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)?**

If we can understand the fundamental reasons for the differences between cancers, we can identify potential targets for treatment specific to different cancer types and begin to develop treatments for them. This could lead to 'personalised cancer medicine' for patients. We have already identified one such potential target previously and are beginning to develop possible therapies against it. These will need to be tested in cells in dishes and, if they prove effective, in animal studies. Understanding the reasons for differences in appearance within a tumour will enable us to understand how the different regions of a tumour may respond to treatment i.e. can some regions of tumour be killed by a treatment but others survive and cause a tumour to regrow. If we can understand why some regions of a tumour, but not others, are resistant, we may be able to identify ways to overcome that resistance. Understanding why different genetic mutations cause some type of cancer but not others will advance our fundamental understanding of the biology of cancer and also

may lead to new therapeutic approaches if we can mimic the features that protect one tissue from cancer in another tissue that is sensitive.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice. We expect to use approximately 12,500 mice over 5 years. At least 60% of these will be used only for breeding.

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

Some mice will undergo surgery under general anaesthetic for implantation of cancer material. Others will develop cancers spontaneously. The adverse effects caused by the cancers will vary depending on the site of the cancer. For example, breast cancers, being located close to the body surface, have minimal health effects but if they get large or are close to the legs may affect walking. In contrast, gut cancers may cause digestive side effects and bleeding into the gut. This may be of varying severity. We will have a 'welfare score' system to assess the overall health of all animals carrying cancers as well as a number of absolute defined limits beyond which adverse effects will not be allowed to proceed. When the 'welfare score' reaches the prescribed limit the mouse will be humanely killed. The overall expected level of severity is 'moderate'.

## Application of the 3Rs

### Replacement

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

### Replacement

To eventually have benefit for patients, understanding what influences the development of cancer, its behaviour, interaction with the cells around it and response to treatment requires a mammalian whole organism system. Furthermore, development of new treatments requires proof that such treatments can work in animal studies.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We have calculated the minimum number of animals required for us to determine statistical differences in experiments.

### Refinement

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

### **Refinement**

Mice are the animals of choice for using genetics to study cancer and for the testing of new cancer therapies. Genetically modified mice are widely available, meaning that the biology of most genes can be studied in many organs in the body.

We will finish experiments at the first possible humane endpoint which enables the object of the experiments to be achieved with the least possible suffering.

We will increase our use of imaging techniques to enable non-invasive measurements to be taken of animals and allowing them to be humanely killed earlier.

Suitable anaesthesia and analgesia will be used under the guidance of a veterinary surgeon.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 47. Analysis of the tissue of origin of cell-free DNA</b>
<b>Key Words</b>	Cell-free, DNA, tissue, origin
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project is to investigate a substance called cell-free DNA, a molecule in the blood that is released by different organs and cells in the body. If we can understand the normal levels of cfDNA in blood then we may then be able to develop more sophisticated tests for non-invasive detection of 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)?**

Initially the main contribution is to improve our understanding of how different body organs and cells contribute to the cell-free DNA which is found in a part of the blood. It is necessary to understand this if we are then to understand changes associated with disease for example cancer. This project ultimately therefore will contribute toward the development of non-invasive and accurate medical testing for cancer. The blood-based medical testing developed from this project is an alternative to painful and invasive surgery or cancer biopsy and may allow earlier diagnosis with a better chance of effective treatments.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

1000 animals will be used over a period of two years. These animals will be genetically altered to allow us to look at specific cells and parts of the body but they will be normal animals in terms of behaviour and appearance.

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

Animals used in this project will be used for breeding and the offspring will be culled by humane methods around 12-16 weeks of age after which we will collect tissues for analysis. A small number of animals will be treated with a substance to activate the genetic alteration that these animals carry. Both protocols are of a mild severity with minimal impact on the animals.

## Application of the 3Rs

### Replacement

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

### Replacement

The nature of the study requires in vivo biological system to be used and cannot be replaced with simulations or modelling.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Minimum numbers of animals will be ensured by taking into account the amount of sample that can be obtained from each animal and the sensitivity of analytical platform to analyse the samples. Study design will be consulted by experienced doctorate scientist within the institute.

### Refinement

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

### Refinement

The choice of animal models used in this study is the most refined because they are all backed up by scientific literature that show no harmful characteristics observed in the animals. The harms of the animals will be minimised by ensuring that only trained individuals with the proper licenses work with the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 48. Pharmacokinetics, delivery and fate of dosed biopharmaceuticals and new chemical entities (NCE's)</b>
<b>Key Words</b>	Pharmacokinetics,, Pharmacodynamics, New medicines
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

When researching, and developing potential new medicines it is important to understand how potential new medicines will be affected by the body (pharmacokinetics) and whether the medicine is likely to have the desired effect (pharmacodynamics). New medicines have to be as safe and effective as possible before they are given to patients and this involves giving the medicines to animals before man.

The work on this project will provide information or 'data' on what happens to a potential new medicine after it has been given or 'administered'; how long does it remain in the body, what effect it has, which by-products are produced and where do they go after the medicine is given. The results will inform further development of more effective medicines and support development of new and improved ways of taking them.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The data produced by this project will contribute to development of potential new medicines to treat patients with a variety of conditions (for example, cancer, HIV and heart failure). Data generated will allow them to be progressed from the laboratory into early studies in humans. Data from some of the studies will be used to determine the dose that should be used in other studies (for example animal toxicity or clinical trials). Some of the work carried out will be used to develop dosing systems that are more convenient, easier to use, less painful and/or reduce the frequency of taking medication for patients

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The majority of the studies will use rodents as they offer appropriate anatomy/physiology to investigate the pharmacokinetics and pharmacodynamics of new medicines with results likely to give an indication of what will happen in humans. However, as pig skin is very similar to human skin some studies, particularly those investigating delivery to the skin, will use mini pigs. In the five-year course of this project licence up to 2000 on mice, 500 rats and 150 pigs will be used to test substances. (This is based on a typical study requiring 3 animals per dose group to be tested to obtain a statistically significant effect) Up to 200 mice, 80 rats, and 80 pigs will be used to provide samples from untreated animals which will be used to set up and validate tests or to use as controls. All studies will go through a scientific review where consideration of numbers, methods and design is reviewed with strong consideration of the 3Rs

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

Healthy animals will have potential new medicines administered by various routes but most often intravenously (directly into the vein), subcutaneously (just under the skin) or intraperitoneally (into the abdominal cavity). Blood samples will be taken either with a needle and syringe from a blood vessel near the surface of the body or a cannula which has been temporarily implanted into a vein. Adverse effects are not expected with these procedures with animals experiencing very little discomfort. Very rarely non-specific signs such as changes in behaviour or posture, weight loss, and reduction in food and water intake will be seen. If this happens action will be taken such as stopping study procedures and seeking veterinary advice. Where an animal cannot be treated, it will be humanely killed to prevent unnecessary suffering. The project has clear guidance on actions to be taken when animals experience side effects, including immediate euthanasia to prevent unnecessary suffering. Samples of organs and tissues are often taken after death to measure the concentration of a potential new medicine in them. During procedures, such as administration or blood sampling, animals are briefly restrained either by holding or placing animals in to devices such as slings or restraint tubes that briefly minimise movement to avoid injury to the animals and ensure success of the procedures. Most animals are housed in pairs or groups; for some studies, animals are on their own for short periods in special cages to collect urine and faeces for analysis to see how the body is removing the medicine from the animal. Animals are monitored for general signs (as mentioned above) that may be due to the potential new medicine being tested. However, the doses administered under this project are typically low so that no side effects would be expected following administration. At the end of the study most rodents will be humanely killed and terminal samples will be taken for further analysis. At the end of a study rodents and minipigs will have a health check and

provided the criteria set by vets are met, the animals will be returned to stock to be used again on a new study.

## Application of the 3Rs

### Replacement

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

#### Replacement

Research programmes have evolved substantially in the past decade and scientific areas are now heavily dependent on non-animal experiments. However, how the potential new medicine is absorbed and, distributed around the body and excreted from it still needs to be tested during the research of the potential new medicines. The mechanisms by which medicines are handled in the body are complex and cannot be adequately evaluated with non-animal tests, therefore currently the properties of potential new medicines can only be fully understood by using a combination of non-animal models and animal approaches.

Regulatory bodies that review and authorise new medicines to the market require data from this type of study before allowing potential new medicines to progress into human studies.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We are continually researching new technologies and processes that will aid reduction in animal use. Progress within these technical and scientific approaches required during animal experiments are constantly challenged by researchers across the world and published data is regularly reviewed and considered by our team. Our team have a history of developing and implementing methods to reduce animal usage and these will be used on our studies whenever possible. Such methods include: micro sampling methods, whereby multiple small volume samples are taken from an individual animal, thereby reducing the number of animals required for a range of studies.

Discussions with a panel of internal experts will advise on optimisation of any study design to ensure the right species and procedures will be used to maximise the chance of success.

We obtain statistical input into study design is to ensure the appropriate and minimal animal numbers are used to meet the scientific objectives.

Analysis of all samples will be via highly sensitive techniques that are more consistent with those used in later stages of research. This data will be reviewed by a panel of experts to determine if a substance can progress to the next stage of development or not.

Animals suitable for reuse will undergo a thorough health check by the vet, where there should be no lasting effects as a consequence of the previous use on the health, behaviour or welfare of the animal including those caused by compound, procedures or housing. There should be no signs of infection and bodyweight should be stable. There should be no other health, behaviour or welfare issues unrelated to the study.

### **Refinement**

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

### **Refinement**

Early phase drug discovery uses rodents as a primary model before any further species are considered where it is clear other species, such as fish, fruit flies, or worms for example cannot be used. Rodents offer the most appropriate biological anatomy to reproduce a biological/drug interaction that can be measured.

Pigs are considered to be one of the major animal species used in translational research, Pigs will be used for device delivery as the architecture of their skin is similar to humans and are increasingly being used in preference to the dog or monkey as the choice of nonrodent species in testing of pharmaceuticals.

Minimising welfare issues will be addressed by understanding the requirements, collaborative decision making/planning and using staff with experience at recognising potential issues should they occur. Close working relationships will ensure the study is required and that the study design appropriately meets the objectives.

Where blood samples are taken, micro sampling techniques will be used whenever possible, to make sure the minimum sample (e.g. microliters) volume is obtained using the least invasive techniques possible. For example, where possible the same needle stick wound will be used for more than one sample by wiping with a swab to remove the scab allowing the flow of blood for collection. This means fewer needle sticks are required. The frequency and volumes used when administering potential new medicines will also be minimised.

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Word limit; 1000 words

<b>Project Title</b>	Project 49. Molecular ecology of bats
<b>Key Words</b>	Bats, conservation, genetics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

<b>Yes</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>Yes</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project licence is to cover my research on the molecular ecology of bats.

Molecular methods have allowed great insights in animal behaviour and conservation biology. REDACTED Understanding how bats responded to past climate change can also be used to predict how bats may respond to climate warming in the future, and whether current areas of high genetic diversity may be lost under climate change. We are studying which areas of the genome are affected by natural selection in relation to environmental conditions such as temperature, to determine if animals have the potential for short-term evolutionary change in response to climate change.

Finally, we are attempting to understand how bats are able to live for much longer than other mammals of a similar body size. To this end we will be studying how telomeres (repeated portions of DNA that protect chromosome tips) change with age in bats. These typically shorten in other mammals, resulting in a lack of protection for chromosomes and ultimately cell death. We will also study whether genes are expressed differently in relation to age in bats, as some genes may become switched off due to a process known as methylation. These findings will hopefully help understanding of specialised genetic mechanisms that slow down ageing processes in mammals.

**What 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 obtain information that will improve conservation strategies for bats, many of which are threatened. For example, we can identify mating sites, determine inbreeding and genetic isolation in the wild. REDACTED By exploring genetic changes associated with longevity in a mammal with extreme longevity for its body size we may open up opportunities for identifying biomarkers associated with ageing, and identification of genes associated with longevity more generally.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will take samples from a maximum of 3000 bats over 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 levels of severity? What will happen to the animals at the end?**

We take small tissue samples (3 mm diameter) from the wings of bats, and sometimes take small blood samples. We have experienced no adverse effects resulting from the use of these methods in REDACT.

## Application of the 3Rs

### Replacement

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

### Replacement

The aim of this study is to better understand the ecology, and conservation needs, of native bats. It is not possible to undertake this work without the capture, marking, sampling and re-release of the bats we are studying. The capture, marking and sampling methods do not cause any long-term harm to the animals. REDACTED

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We sample the minimum number of individuals to achieve our aims. We follow protocols suggested in peer-reviewed scientific literature to determine the minimum number of individuals we need to sample in a population to obtain accurate measures of genetic diversity.

### Refinement

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

### Refinement

This study will use bats, as it is the ecology of these animals that is the basis of the work being undertaken. The handling methods used are considered best practice and are used extensively throughout the world. The sampling techniques used

provide the least severe way of obtaining reliable, high quality sources of DNA for our analysis. REDACTED

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 50. Developmental mechanisms of mammalian cortical development</b>
<b>Key Words</b>	Brain, cerebral cortex, development, stem cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The cerebral cortex is the brain structure regulating our higher cognitive function and sensory processing. Once the cortex has been damaged by neurodegenerative disorders or injury therefore, it greatly affects on our quality of life.

This is mainly because the adult mammalian brain is unable to regenerate due to its poor neurogenic capacity.

Our project aims to understand embryonic cortical development how the early brain expand the stem cells and subsequently generating a rich variety of neurons and glial types. We are aiming to decode molecular mechanisms of self-renewing stem cells in embryonic brains in order to apply the knowledge to the repair of the adult brains.

**What 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 is a basic science project that will primarily produce enhanced understanding of self-renewing capacity of embryonic cortex. Given the poor regenerative capacity of mammalian adult brains including human, therefore, it will be of enormous potential benefit for the aim to develop the strategies for brain repair of neurodegenerative disorders such as Alzheimer's disease, or trauma caused by stroke or injury.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The cerebral cortex is a brain structure which is unique to the mammals including humans. Since anatomical structures of the cortex are highly conserved across all mammals, we use mice as experimental model. We expect to use around 8000 mice

over the course of this 5-year project. The vast majority of these (~5000) will be used for breeding purposes.

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

For our study we will breed and maintain genetically altered mice, which we will subsequently use to perform experiments both *in vitro* and *in vivo*. This later set of experiments will include post-mortem analyses (e.g. immunohistochemistry, biochemistry), *in utero* and neonatal manipulations of embryos and postnatal mice.. These experiments are expected to have mild or moderate levels of severity. The most common adverse effects of these procedures are expected to be surgical complications such as post-operative infection, which will occur very rarely (<1% of animals), and will be closely monitored and treated in consultation with veterinary staff. All procedures will be of moderate severity at most. At the end of all protocols animals will be humanely killed; in many cases their brain tissue will then be used for experimental purposes.

## Application of the 3Rs

### Replacement

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

### Replacement

Our studies focus on the development of the cortex, which is a unique brain structure in the mammalian species. For example other well established model animals such as chick or zebrafish do not have “the cortex” as defined by anatomical/molecular similarities to those of the human cortex. Therefore the use of animals which have the “cortex” is absolutely essential for this study in order to understand how the cerebral cortex develops normally or abnormally.

However, we will use cell culture approaches in parallel (dissociation cell culture, slice brain culture as well as the usage of iPS/ES cell delivered neural progenitors) with proposed protocols to inform our experiments, thus minimising animals used.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Animal numbers will be minimised by mainly three approaches by

1) Using previous and preliminary experiments in *in vitro* systems to identify the most appropriate analyses and necessary sample sizes *in vivo* (see “Replacement”).

2) Combining multiple measures of brain structure and function within individual animals, such as multiple brain slices for different experiments.

3) Maximising data quality for each animal through stringent welfare controls and optimised experimental design.

### Refinement

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

### Refinement

Mice will be used throughout the project because they represent the simplest organism with appropriate neuronal maturation, and because genetically-modified lines allow us to ask powerful and crucial experimental questions.

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.

We do not propose any protocols with substantial severity.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 51. Genetic Dependencies of Renal Cell Carcinoma</b>
<b>Key Words</b>	Renal cancer, genetics, carcinogenesis
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and 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 spread outside the kidney. The study of renal cancer pathogenesis and the development of more effective treatments have been hampered by the limited availability of appropriate genetically defined animal models of the disease. The development of reproducible and accurate renal cancer mouse models will allow in depth investigations of the underlying tumour biology and the discovery of new methodologies for the detection, management and treatment of human 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 benefit relates to new knowledge about the initiation and progression of renal cancer. We will aim to publish the findings in academic journals and this information is likely to be of interest to pre-clinical scientists interested in tumour biology. The secondary potential benefit relates to the value of the results to clinicians and to the possibility that new therapeutic targets may be identified, for which new pharmaceutical products could be developed. Thirdly, any developed mouse models of renal cancer will be made freely available to academic collaborators and will represent an invaluable resource for the early evaluation of novel methods for the detection and therapy of renal cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

3750 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 levels of severity? What will happen to the animals at the end?**

Mice used for breeding and maintenance of colonies are not expected to develop any adverse effects. In the experimental protocol, the mice will receive gene-inducing agents either via food, water, or injections. Injections will be used if the transgene-inducing agent needs to be administered in few occasions only, for continuous administration the agent will be administered via food or water. After gene induction, the mice will be followed by general health checks as well as imaging techniques that allow the detection of internal tumours in living mice. During imaging the mice will be anaesthetized. The imaging procedures are not expected to cause harm. Some of the mice may also receive potential anti-cancer or cell labelling agents via food, water or injections. Some of the mice are expected to develop renal tumours and some may as a consequence experience abdominal distention, haematuria, weight loss and reduced activity. We expect these to occur in less than 15% of experimental mice. Following completion of experimental procedures mice will be killed, after which tissues are collected for molecular analysis.

## Application of the 3Rs

### Replacement

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

#### Replacement

The elucidation of the genetic events and mechanisms critical for the development of cancer requires investigation within model systems that replicate as close as possible the human disease (cell and tissue of origin, surrounding environment, immune system etc.). These parameters cannot be replicated in culture systems. The laboratory mouse represents one of the best available model systems for cancer owing to various factors including its extensive biological similarities to humans, and an entirely sequenced genome. Furthermore, genetic modification of the mouse genome can be easily and efficiently achieved.

An important aim of this project is to generate improved mutant mouse lines that are prone to the development of renal cancer. Such mouse models will be useful to determine the significance of various genes in the development of the human disease. In addition, a robust and reliable mouse model of renal cancer will be a useful system for the pre-clinical evaluation of diagnostic and therapeutic approaches. This model will be a valuable addition to the currently used systems (cell cultures, transplantation mouse models) that have proved of limited clinical prognostic value.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

We will aim to minimise the numbers of animals used by:

- (1) Investigating candidate genes and therapies in culture systems prior to generating mouse models
- (2) Reduce the amount of breeding required to produce experimental and control animals
- (3) Determining the approximate lifespan of the various renal cancer models in small pilot studies and collaborating with biostatisticians at our institute to determine the minimum number of mice that are required for any studies in order to reach conclusive results with suitable certainty
- (4) Creating a tissue repository from generated mice to use in future experiments and share with other researchers
- (5) Ensuring that none of our investigations duplicate work already performed

## Refinement

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

## Refinement

Mice are a well-studied experimental species whose genome can be easily and efficiently modified allowing the investigation of complex genetic diseases like cancer. The models used in our studies will allow us to control and direct genetic changes to the stage of development and tissue relevant to the purpose of our studies (i.e. the kidneys of adult animals) thereby limiting the effects of modified genes to other organs/systems. We will only use well established reagents and protocols and where novel methods need to be employed, potential harms will first be carefully characterised in small pilot studies.

We will aim to detect the development of kidney lesions as early as possible and therefore limit their effects on the health of the animals by performing regular health checks and imaging.

The staff of our animal facility has extensive experience in animal husbandry, welfare and disease and we will take advantage of their expertise to pick up signs of suffering early so that it can be minimised/alleviated.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 52. Investigating the dog as a naturally occurring model of epilepsy and its neurodevelopmental comorbidities</b>
<b>Key Words</b>	Epilepsy, EEG, MRI, Behaviour
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Epilepsy, defined by spontaneous recurrent seizure activity, is a common and serious disorder, affecting around 1 in every 26 people. The dog is a recognised model of human epilepsy, with striking similarities in the cause, clinical manifestation, and disease course when compared to human patients. Although seizures can be induced in normal dogs by electrical or chemical means, a large population of pet dogs with spontaneous recurrent seizures exist, with the dog considered to be affected by epilepsy more than other domestic species, making canine epilepsy a disease of considerable comparative medical interest. It is increasingly recognised that epilepsy is not simply a seizure disorder, and is a more global brain disorder with multiple manifestations. Neurodevelopmental disorders such as autism and ADHD are commonly seen in people with epilepsy, and are termed 'co-morbidities'. It is possible that altered neurobiological mechanisms involved in early brain development lead to the co-development of one or more these disorders. It is not yet known whether these co-morbidities are seen in dogs with canine epilepsy.

This project will answer the following questions:

- 1) Do dogs with epilepsy show increased levels of autism and/or ADHD-like behaviours in comparison to healthy control dogs?
- (2) Are there differences in the brain structure and function of dogs that show autism or ADHD-like behaviour compared to controls?
- (3) Is the presence of autism or ADHD-like behaviour associated with an individual's response to drug treatment?

(4) Does epilepsy, autism and/or ADHD have a negative impact upon the welfare of affected dogs?

**What 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-medium term, the results of this study will: • Produce objective screening tools to detect behavioural abnormalities in dogs with epilepsy, which clinicians will be able to use to screen for potential co-morbid behavioural problems in dogs they treat for epilepsy • Allow researchers in the field of canine epilepsy to more fully characterise dogs included in their studies, and lead to genetic research to advance our understanding of their genetic cause(s) • Enhance our abilities to use ambulatory EEG (brain activity monitoring) and MRI (brain structural scanning) to detect differences in brain activity and brain structure between dogs with epilepsy and healthy controls, and between dogs that show abnormal behaviour and those that do not, which may lead to an improved understanding of their underlying causes In the long term, naturally occurring canine epilepsy and its neurodevelopmental comorbidities may be used more widely in epilepsy research, potentially reducing the number of genetic or induced rodent models of epilepsy that are commonly used at present.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Around 60 client owned dogs will be used in the studies over a 3 year period, 30 of which will be 'case' animals that have been diagnosed with spontaneously occurring epilepsy, and 30 of which will be controls, which are matched to the case animals on breed, sex, age and neuter status (where possible). Each dog will be enrolled to the study for two days during this period.

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

All dogs will be returned to their owners at the end of the study. The expected level of severity is mild, with no significant adverse effects expected. Due to studying dogs with epilepsy, there is always a risk of seizure activity occurring during the study protocols; however, all will be carried out in a veterinary hospital with assistance available in emergencies.

## Application of the 3Rs

### Replacement

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

### Replacement

The dog has been chosen as a model for this study, as epilepsy is the most common chronic neurological disorder in this species. As such, the work carried out will be of direct benefit to dogs, but will also be of translational value to humans with epilepsy. Directly studying the behaviour of dogs in a naturally occurring disease model will lead to greater understanding of epilepsy and its comorbidities in dogs compared to using experimentally induced disease in different species.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Sample size calculations have been based on evidence from published studies to use the minimum possible number with sufficient statistical power. This sample size will allow for statistically significant differences in EEG, MRI and behavioural variables to be detected. To reduce variability only one breed of dog will be recruited from. Each case dog will be matched as closely as possible with a control dog based on age, sex and neuter status to further reduce variability between the study groups.

### Refinement

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

### **Refinement**

Selecting the dog, a naturally occurring model of epilepsy, negates the need to breed for animals with genetic epilepsy syndromes or induce seizures with electro- or chemo-convulsants.

Minimal distress is anticipated to be caused by this work; however, to minimise any potential suffering, local anaesthesia will be used where necessary for procedures where mild pain/discomfort may occur (e.g. placement of subdermal EEG electrodes), and sedation will be used in anxious dogs to avoid restraint and/or distress. Throughout the study procedures, the behaviour of the dogs will be closely monitored, and if considered to be distressed (e.g. excessive panting, excessive vocalisation, hiding), data collection will be paused, and if distress continues or is severe, procedures will be terminated.

Habituation to testing environments and equipment will be carried out where appropriate, and the owners of study dogs will also be present during procedures where this is feasible and beneficial to the dog's welfare. REDACTED dogs will be closely monitored by veterinary nurses, with additional observation of the epilepsy group to monitor for signs that may indicate impending seizure activity.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 53. Pattern recognition receptors against pathogens</b>
<b>Key Words</b>	Pattern Recognition Receptors, pathogens, immunity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our first line of defence against harmful bacteria is known as the "innate immune system". This system relies on a diverse group of proteins that are collectively known as Pattern Recognition Receptors (PRRs) and can recognise invading microbes or molecules produced by our body while under attack by such microbes. Such recognition results in their activation which initiates inflammation. The key role of PRRs in the development of the immune response suggest pharmacological manipulation of their activity could significantly help fighting infectious diseases and resolving chronic inflammation. This cannot be performed until we understand exactly how PRRs affect the development of immune response against bacteria. The overall aim of our project is, therefore, to determine the mechanisms by which PRRs drive the host inflammatory response to bacterial infection and characterise how these receptors contribute to the generation of protective memory immune response against the important food borne pathogen *Salmonella* Typhimurium.

**What 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 an urgent clinical need to develop new ways to fight bacterial infection because of the emergence of antimicrobial resistance, chronic inflammatory diseases are increasingly common and the current salmonella vaccines are far from ideal. We expect our work will help to improve vaccines against Salmonella and other bacteria with similar mode of action and identify novel drugs against excessive inflammation and infection. Our data will, therefore, be of interest not only to our research group, but also to basic and clinical research scientists, the pharmaceutical industry and, ultimately if we identify new therapies, patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will not use more than 8400 mice over 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 levels of severity? What will happen to the animals at the end?**

Mice will be administered with live bacteria or substances that activate certain PRRs and, in some experiments, will be also treated with compounds that can modify the immune response. In most experiments, such interventions will be performed by a single or multiple injections. In some trials, small amounts of blood will be collected from each mouse at regular intervals. The majority of mice will not experience more than a mild discomfort associated with the experimental procedure, such as injection or blood collection. However, it is likely that some mice will exhibit signs of systemic infection, such as weight loss of maximum 10%, staring coat and transient hunched posture. These mice will be assisted via careful and skilful husbandry that is typical of the culture of care present at our establishment. They will be monitored closely and, if not recovered within 24h, they will be humanely killed with no further delay.

## Application of the 3Rs

### Replacement

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

### Replacement

We cannot use invertebrates, such as fruit flies and worms due to fundamental differences in their PRR repertoire compared to that of humans. We always first perform experiments in mouse and human cell lines and human samples to identify whether a PRR is likely to be important in the host response to infection. However, cell lines and single cells lack structural complexity and particularly the interactions between the different cell types required to generate a full immune response to prevent spread of the bacteria in the tissues. If our research is to significantly advance our knowledge on how PRRs affect infection and inflammation, our findings will need to be confirmed in an animal, in this case in the mouse model.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We are supported by experienced biostatisticians who will provide valuable advice on all aspects of experimental design, including number of animals required to obtain reliable results, and statistical analysis. We will minimise variability by minimising the amount of stress induced on the mice, by following the codes of good laboratory practise during data acquisition, by randomising samples and by blinding certain types of analyses when possible. At the same time, we will reduce animal to animal variability by making sure that mice allocated to different groups will be age- and

sex-matched and housed in identical environments. Furthermore, we will make every effort to extract a large amount of data from each experiment and we will pair as many experimental groups/conditions as possible to a single control group of animals to avoid unnecessary repetition of experiments. Finally, we will investigate the use of novel technologies in order to address our objectives while reducing the number of mice required from an experiment.

### **Refinement**

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

### **Refinement**

Mice are the most suitable laboratory animals for our studies; first, their PRR repertoire is similar to humans, and, second, availability of several genetically altered strains is key for studying the interaction between PRRs and bacteria. This is because specific genetic alterations enable us to selectively look at pathways of interest in the context of the whole mouse.

Our infection model is the most refined; first, it closely resembles/models human typhoid fever which is a sublethal infection in man, second, *Salmonella* is a potent activator of several PRRs, and, third, as it is not lethal, it allows us to study the development of the full immune response against infection.

We will use husbandry procedures that support animals with mild signs of infection. These include, for example, the use of wet mash, supplementary heat and aids to reach more easily the water and food supply within the cage. Any mice showing clinical signs that cannot be promptly ameliorated, such as weight loss and hunched posture, will be immediately humanely killed. We will also use of the smallest possible size of needles for injections and minimal numbers and frequency of procedures to deliver the work of the project. We progressively refine our protocols to ensure that the smallest possible doses of bacteria are administered to the animals. For novel bacteria of unknown virulence and/or genetically altered mice of unknown susceptibility to infection, data generated from work not requiring live animal experimentation and small pilot trials will provide key information on how to further progress studies whilst minimising impact upon animals involved.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 54. Mechanisms of tumour development and treatment resistance</b>
<b>Key Words</b>	Cancer, Chemotherapy, Treatment resistance
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

It is not known why some children develop cancer; the impact of their environment and genetic changes play a role, but the specific timing and types of genetic alteration and environmental exposures are to be elucidated. In the case of paediatric lymphoma, a cancer of the immune system, it remains to be determined why some forms of lymphoma occur more in children than in adults. In this project, Genetically Altered (GA) mice will be used to identify genetic drivers of disease: Genetic changes detected in human tumour tissues will be engineered in mice to assess their contribution to tumour development in vivo in established GA model systems. The effects of environmental exposures such as viral infections will also be analysed in these model systems to determine whether these processes force tumour development in the lymphoid system. The established GA models will then be applied to investigations of novel therapeutic agents in order to develop less toxic treatments than the chemotherapeutic agents currently in use. We will also apply these findings to patient derived xenografts – mice propagating human tumours as ‘hosts’ or ‘avatars’ of their disease thus taking into account patient genetic variability. In doing so, we will also identify the tumour cells that are responsible for disease relapse.

**What 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 the course of this project we will not only shed light on why and how children develop lymphoma and other cancers but we will also derive new therapeutic approaches. Current therapy is toxic leading to life-long health problems. Our model systems will inform on new, less toxic therapies that not only lead to disease remission and cure but also prevent relapse from occurring. Specifically, this work will be conducted applying to certain types of childhood cancer including neuroblastoma and lymphoma that carry mutations in a gene called ALK.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 5000 mice will be used over the course of the 5 years of this project. This number of mice is required so that all of our experiments are conducted with sufficient replicates to provide meaningful data with statistical significance before we progress to first-in-man studies.

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

The mice used in this project are housed in a controlled environment in a barrier unit. They are tended to by qualified technicians who ensure their health and safety, clean housing by regular inspections and cleaning protocols. The mice are also provided with an enriched environment with stimulating toys such as tunnels and wood sticks. The large majority of work will be conducted with genetically altered mice that express genes previously shown to be prevalent in human patient cancer cells. These mice will be aged for tumour growth; once tumours have grown the mouse will be killed, tumours removed and studied *ex vivo*. As such, procedures conducted on the mice are minimal. All mice will be closely monitored for any clinical signs of ill health so that no more than transient harms are experienced. This will be conducted by daily monitoring of their health status and a score sheet to assess the cumulative effects of any treatment. At present, we are unable to grow these tumours in plastic dishes as they require a number of support systems that we cannot yet mimic *in vitro*. Sometimes, it is not possible to detect tumour growth in the mice and they may get sick very quickly once it reaches a critical mass (normally this occurs overnight). This can happen in up to 25% of our mice and in these cases, as soon as the mouse is discovered to be ill, it is killed by a Schedule 1 method. Some mice will receive injections of anti-cancer agents in an attempt to cure them of their cancer. Some of these agents may cause the animals to suffer ill health as do humans on treatment with chemotherapy but we will ensure that any clinical signs are detected and treated within 24 hours. If it is not possible to alleviate their symptoms, the affected mice will be killed by a Schedule 1 procedure. The injections themselves will give rise to minimal pain and suffering and will be transient. When we wish to grow human tumour cells in mice, we may first expose them to radiation to kill their immune system to essentially create a mouse that has no immune system. When lacking an immune system, the mice will not reject the human tumour cells. As these mice have a suppressed immune system they will be housed in isolator cages, provided with sterile food and water, and monitored closely for any signs of infection. These mice may also be exposed to chemotherapeutic agents at low doses to induce resistant tumour cells to form. All animals will be killed by a Schedule 1 method at the end of the procedures.

**Application of the 3Rs**

## Replacement

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

### Replacement

Cancer is a disease involving the whole body; tumour cells recruit a variety of other cells within the body to support their growth. This includes new blood vessels to support their increasing requirements for food, nutrients and oxygen as well as a stromal network to support its size, and inflammatory cells to provide growth factors and other growth-promoting signals. As such, cancer can only be fully mimicked in an experimental setting where all of these facets can be taken into account. At present, we have limited other model systems which at best can only replicate a few properties of these growth support systems. A second consideration are the effects of chemotherapeutic agents and their distribution within the body – their so-called bioavailability. Whilst some drugs may work very well in a tissue culture dish, when applied to a whole-body scenario, they often fail due to the above-mentioned facets. Therefore, to truly mimic cancer treatment, the whole body, its response to the tumour and to chemotherapy must be evaluated. However, we maintain close attention to the scientific literature for any techniques that might improve our ability to replicate tumour growth in plastic dishes and at the same time, every time we grow a tumour in a mouse, we also attempt to refine conditions for its growth in plastic.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Every experiment will be conducted following consultation with a statistician or the performance of Power calculations to ensure that we use the minimal number of animals to give significant and meaningful results. We will also use existing genetically altered mice rather than generating new models where practicable and monitor databases of genetically altered mice towards this aim.

## Refinement

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

### Refinement

Mouse models of cancer have been developed over many years to mimic the human disease in order to facilitate research into how and why cancer develops and therefore how best to treat it. By refining these models we have been able to further our understanding of cancer considerably – something that could not be achieved

with less sentient systems. Largely, this is because cancers are not autonomous growths of cells, i.e. they cannot grow on their own without the support of the microenvironment including, for example, a blood supply, the immune system, an extracellular matrix and a variety of proteins, chemicals and growth factors. Until we have determined what all of the support systems are, we will not be able to fully model human cancer in a petri dish. As such, we continue to work with a murine model whilst also developing *in vitro* systems. All techniques will be conducted by experienced staff that are trained to be competent and are regularly assessed, therefore reducing any suffering to the mice. All experiments will therefore be conducted in a timely manner and when necessary with the use of anaesthesia. Mice are housed in a designated facility under sterile conditions in enriched housing environments.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 55. Correction of cardiac arrhythmias and methods of resuscitation</b>
<b>Key Words</b>	Pig, CPR, Resuscitation, Heart, Cardiac Arrest
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this licence is to further our understanding of life threatening heart conditions as well as to optimise treatment of these potentially fatal conditions using an Automated External Defibrillator (AED). Providing lay user AEDs will help optimise patient care, particularly in the community and home environment. As almost 1.8 million people suffer from a heart condition annually, there is a significant need to improve treatments worldwide.

Treatment of heart conditions can sometimes create discomfort or cause residual issues for a patient. Optimizing treatments will help improve outcomes for the patient. The first objective is to reduce the amount of electrical energy delivered to a patient when a defibrillator is used to reduce damage to the heart muscle and discomfort during treatment which improves quality of life following return of a normal heart rhythm using an AED. Therefore, observing at basic science level if damage to heart muscle is occurring is necessary. As the number of young cardiac arrest patients increases every year, quality of life following a resuscitation is vital.

In addition, this project will aim to improve cardiopulmonary resuscitation (chest compressions) techniques through refining the optimal force to be applied to patients irrespective of size, gender and age by testing a wider range of models. For example patients may require more or less force depending on size and age due to the depth of the patient's chest, the diameter of their chest and the flexibility of their ribcage (for example, brittle bones in elderly patients). Furthermore, ensuring that chest compression force and rate feedback is optimised for patients irrespective of the surface the patient is lying on when receiving CPR.

If a patient collapses, often, a lay user struggles to confirm reliably whether the patient is not breathing. Therefore, a feedback feature which would let the rescuer know when a patient is breathing would prevent unnecessary delay to resuscitation or initiation of a resuscitation effort when it is not required. The end goal of this CPR research programme is to design AEDs which will provide CPR feedback which is 100% effective for every individualised patient to improve resuscitation outcomes worldwide.

Reduction of energies and increasing efficacy used to internally shock a patient with the treatable heart condition, atrial fibrillation (AF). AF is a condition affecting millions of patients worldwide. Drug therapies are often ineffective or have severe side effects which make quality of life for an AF sufferer low. Early research and use of defibrillation for the treatment of AF became under-utilised due to the advent of drug therapy (now proven to be less effective) and the discomfort caused by higher energy shocks. However, the technology now exists to miniaturise defibrillation systems and reduce the energy delivered to a comfortable level for the patient.

**What 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 seen in terms of improved treatments of serious and often life threatening heart conditions. Improvements in CPR efficacy for all patients is a vital step in improving the management of sudden cardiac arrest patients. Improving CPR feedback devices will ensure ease of use and increased effectiveness of resuscitation systems. Development of feedback devices which can be easily used by lay users. Wider availability of lower cost defibrillation devices which could be accessed in shorter times thereby reducing time to defibrillation. If time to defibrillation is reduced from 10 minutes to 2 minutes chance of survival increases from around 5-10% to up to 75%. Since present therapy, drugs and electrical cardioversion have limited effectiveness and adverse effects, improvements would come from either improving efficacy or reducing toxicity of AF treatments moving forward. Understanding the levels of damage caused to a patient's heart muscle is necessary to ensure that the ever-increasing number of young patients are provided with higher quality of life. Development of lower energy waveforms have the potential to make more portable defibrillation systems which save more lives in space restricted areas.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Domestic Pigs, 200-260 over 5 years and Adult sheep, 60 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 levels of severity? What will happen to the animals at the end?**

All studies are conducted under general anaesthesia, so the animals are not aware of any of the procedures and feel no pain. When administering anaesthetics to the animal it's windpipe could become blocked as it closes over (spasms). However, a breathing tube can be inserted straight into the windpipe to ensure the animal can continue to breathe normally while under anaesthetic. Pain during surgery will be controlled by ensuring adequate anaesthetic and pain relief is provided for each animal. The animal's blood pressure and oxygen levels, as well as heart rate will be monitored during the procedure to help ensure the animal remains under anaesthetic.

## Application of the 3Rs

### Replacement

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

#### Replacement

Prior to 2005, ethical approval (local IRB) would have given approval for studies which enabled the induction of atrial fibrillation (an abnormal but not immediately life-threatening arrhythmia) in patients with transient atrial fibrillation. The treatment would then be applied using a novel system to correct the abnormal rhythm. If it was unsuccessful the standard treatment would be used.

The novel system could not be used, however, without first being tested on an animal model. For this particular study, sheep were used.

Subsequent studies have not been approved for the induction of atrial fibrillation (an abnormal but not immediately life-threatening arrhythmia). The reason for this change was the potential risk of inducing an abnormal rhythm which is life threatening (ventricular fibrillation). Unsuccessful treatment of this rhythm can result in death. Only patients which were in persistent/chronic atrial fibrillation (an abnormal but not immediately life-threatening arrhythmia but chronic in nature and life limiting). Again, the novel system could not be used without first being tested on an animal model.

The impact of not testing on animals prior to a patient study is sub-optimal treatment, a significant delay or absence of treatment resulting in death.

The same rationale applies for CPR (chest compression) and ventricular defibrillation (correction of a life threatening abnormal heart rhythm). The impact of not testing on animals prior to a patient study is delay in treatment, inappropriate, sub optimal treatment or absence of treatment resulting in death.

Currently human medicine in this field could not progress without the use of an animal model.

Computer models of arrhythmia are in the process of development, and will benefit from the new information generated in animal studies and clinical cases in people.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

The aim of these studies is to support a decision to proceed to clinical trial of the intervention, or clearly rule out clinical use of the procedure. To this end the number of animals studied in any group will be the minimum necessary to achieve statistical significance.

In our experience relatively small groups of animals, some 6-8 animals per group, are needed for a preliminary evaluation of an idea. For statistical power, particularly if establishing that there is no benefit from an intervention, larger groups of some 10–15 animals are needed. A calculation is made before every study to ensure the right number of animals is used.

### **Refinement**

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

#### **Refinement**

It is only in the hearts of large animals that these abnormal heart rhythms (atrial and ventricular fibrillation) persist without spontaneous correction. Consequently, it is necessary to use the pig and the sheep as models for initiation and electrical correction of the arrhythmias.

Sheep and pigs are the preferred model for sustained atrial fibrillation as the arrhythmia causes relatively little upset to normal heart function. However, the pig copes better with the reduced heart function that will occur after ventricular fibrillation.

As all studies are conducted under general anaesthesia, the animals are not aware of any of the procedures and feel no pain.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 56. Discovery and development of anticancer therapeutics</b>
<b>Key Words</b>	Tumour, Preclinical Model, Toxicology, Drug discovery
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

- To establish animal models for human cancer by injecting or transplanting tumours, which have come from animals (mice or rats) or patients, under the skin or into internal organs of experimental mice or rats.
- To test new anticancer agents, which have previously been shown to stop growth and multiplication of cancer cells cultured outside of the body, for their: 1) suitability for experimental mice and rats (are they tolerated); 2) availability, possible accumulation in and removal from the animal body and transplanted tumours; 3) effect on molecular targets in normal and tumour tissues; and, 4) ability to reduce or stop growth, division and dissemination of human and animal-derived tumour cells in mice and rats.

**What 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 the leading cause of death worldwide. The need for new effective and safe anticancer therapies is enormous. Testing new drugs in animal (mouse and rat) models of cancer is essential for evidence-based drug development and is a regulatory requirement, i.e. agencies that approve taking a drug into the clinic would like to see that a new agent demonstrate a clear anti-tumour effect. All the available body of evidence published in the scientific literature suggests that the ability of a new agent to reduce or stop tumour growth in the animal model will often predict whether a given drug would also have an anti-tumour effect in patients. Testing drugs in animals therefore eliminates unnecessary clinical trials with patients, as the

drugs that are inefficient or toxic in mice and rats can be recognized and eliminated early.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

• Mice (*Mus musculus*) – 51200 • Rats (*Rattus norvegicus*) – 5520 • 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 levels of severity? What will happen to the animals at the end?**

• Tumour model establishment: Cultured tumour cells or tiny tumour pieces will be injected under the skin or into organs of tumour origin (e.g., pancreas, liver, or kidney) using a syringe fitted with a needle. Injections are expected to be of mild severity, comparable to injections of vaccines to human patients. Rarely, tiny pieces of tumour tissue will be inserted into the animal tissue. In such cases, a minor surgery will be required to expose internal organs for the ease of insertion (or injection) of tumour cells or tissues. The surgical procedure is consequently graded as moderate due to the surgical intervention. This is however a well-established procedure in mice and rats in which the animal receives full anaesthesia and also receives pain-killers during and after the surgery. • Testing drugs: Compounds that were previously tested in cells, cultured in test dishes, will be administered into mice or rats via different routes (for example, into a major vein, in the abdominal cavity or via the mouth). This will usually result in a minor discomfort and local tissue irritation. The expected severity level for one or several treatments with new drugs, which have never been previously tested in animals, will be designated as severe due to possible unexpected effects (for example, pain or even sudden death) that will however be closely monitored during the procedures. Once the compound has proven to be safe in animals, further procedures (e.g., pharmacokinetic or efficacy studies) will be of moderate severity due to the need of repeated handling of animals. • Sampling: At different time points after compound administration, blood and healthy tissue (such as hair plucking) can be performed to assess levels of compounds in circulation and peripheral organs. Piercing the skin by a fine needle and plucking hair is assessed to be of a low discomfort for the animal (mild severity). Alternatively, animals can be killed humanely to allow sampling of tissues (moderate severity). • End of procedures: All animals will be assessed for their well-being during the procedure and culled should any signs of ongoing pain, suffering or distress become visible. All animals will be euthanised humanely at the end of treatment. Blood and tissues, including the tumour tissue, will be taken after their death for analysis of biological markers (nucleic acids and proteins).

## Application of the 3Rs

### Replacement

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

## Replacement

- Animal tumour models can mimic human cancer with good fidelity by recapitulating genetic characteristics of cancer cells, their behaviour (e.g., spreading to other organs and interaction with normal tissues) and response to drug treatment. They also allow investigation of the drug behaviour in the body (pharmacokinetics) and drug's effect on molecular targets (pharmacodynamics), which presently cannot be fully reconstituted in a test dish. Thus, active proteins (enzymes) found in the liver and the gut progressively destroy the drug, so that only a fraction of it reaches the tumour. Also, tumour architecture in an organism (defined by its interaction with the body it grows in) is by far more complex than one can model in vitro.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

- We use sophisticated experimental systems in test dishes (*in vitro* functional assays) and well-established drug testing cascades to ensure that unsuitable drugs are eliminated prior to experiments with animals, so that the minimum number of animals is used throughout the project. We use "microbleeds" (tiny blood samples) and highly sensitive detection methods to assess circulating drug levels during therapy. This potentially reduces the number of animals in a standard 4-compound 9-point pharmacokinetics study from over 100 to 6. Where appropriate, we use surrogate tissues (e.g., mouse whisker follicles, serum or circulating blood cells) to measure cellular effects. In addition, as far as possible, tumour development (and target modulation) is monitored by non-invasive imaging.
- Solid tumours are rarely passaged in animals, except for tumours derived directly from genetically modified mice or patients, which reduces variance (compared with using the original hosts). Our experimental methods have been evolved to be as robust as possible. The animal models are designed to provide maximum information from the minimum number of animals, compatible with statistical requirements.

## Refinement

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

## Refinement

- Mice and rats are the lowest species that are appropriate for drug development studies and are widely used for this purpose. Most of our studies are carried out using well-characterised human tumours, grown subcutaneously (or in some cases orthotopically in immunodeficient adult animals to avoid tissue rejection). This enables us to study human cancers in the correct tissue microenvironment (albeit in a rodent host). The animals are maintained in individually ventilated cages using sterile food and bedding and all procedures are carried out in laminar flow cabinets to avoid infections.
- Suffering is minimised by keeping tumour burdens within tolerable and acceptable limits and using non-invasive imaging wherever possible for internal tumours. Test drugs will have been carefully selected prior to evaluation in animals. Tolerability studies in small numbers of animals use the minimum dose predicted to be active based on *in vitro* assays and low dose drug concentration (pharmacokinetics) studies before more detailed studies are undertaken. The drugs are generally of low toxicity (e.g., agents targeted to molecules selectively overexpressed or mutated in human cancers).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 57. Defining the Drivers of Immune Variation</b>
<b>Key Words</b>	mice, heredity, immune-variation, environment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this proposal is to quantify the amount of variation in immunological responses under natural conditions in wild mice and to establish the major causes of this variation

We aim

- **To establish if variability in immune responses among individuals is less driven by host genetic rather than by environmental factors**

We know that genetics plays an important role in susceptibility to disease and that disease is associated with variation in immune responses. This project will define in more detail how individual types of immune responses develop, are they programmed by genetic factors or do they vary in response to infections or other environmental factors that an animal experiences over its lifetime?

- **To determine if the predominant environmental factor driving variability in the expression of immune responses among individuals is infection by parasites**

The major factors that influence immune responses are expected to be infections but this project will allow us to assess the relative proportion the effects of infection have in relation to other environmental factors such as food supply, season, or adverse weather conditions.

- **To determine if the variability in animals' immune responses increases with age**

As an animal ages its exposure to environmental variables will accumulate, it will have experienced more infections, for example, so we expect immune responses to be more varied in older animals.

**What 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 research will be to identify the types of individuals, and the environmental circumstances, that make individuals more or less vulnerable to infection and disease. Within medical and veterinary settings it is important to understand why some individuals are predisposed to make an immune response that renders them more likely to develop disease following infection, or to fail to be protected after vaccination or to develop autoimmune pathologies. Doing so would be an important step in first identifying and then developing strategies to protect them from disease and to pave the way to more individualised treatments or vaccination protocols.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice 1200 over 2 years

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

1 Trapping: REDACTED We will set traps each night and then collect any trapped animals first thing the following morning by emptying into a plastic bag. Animals will undergo a health assessment and any pregnant or lactating animals will be released. There is an unavoidable harm if a lactating mother has left a vulnerable litter. REDACTED 2 Tagging: We will then assess if they have been caught previously - if they have they will be released, if not they will be tagged by injecting a passive integrated transponder (PIT) tag and ear punched for rapid identification of experimental animals the next time they are trapped. They are then scanned with a hand-held electronic tag reader to identify the animal. Tagging is a very rapid progress and animals will be subjected to minor discomfort. 3 Monitoring: For animals undergoing their first capture within the current trapping session (whether or not they had just been tagged), we will assess the sex, whether they are sexually mature, the condition of the coat and their body condition and they will be weighed with the animal held in a cloth bag. 4 Collection of blood: Animals will be bled once a month during the experimental period which could be a maximum of 7 times for animals followed over the course of the trapping season but for most animals this will be three times. Whilst being held, a small piece of skin will be cut off the end of the tail and blood collected. 5 Anti-parasite treatment: Some animals will be treated to reduce their parasite burden using a spot on anti-parasitic treatment which has been tested on mice and shown to have no adverse effects. This will happen once a month for three months after which they will be killed by schedule one methods and autopsied for detailed immunological, parasitological and genetic analyses. 6 Immunization: Some mice will also be immunized subcutaneously using an innocuous protein in a mild adjuvant such as alum (used routinely in humans). This protocol has been shown to be extremely unlikely to cause adverse effects. The

handling process will be stressful for the animal but this will be of a short duration (typically 15 minutes), we can undertake this process very rapidly. Great care will be taken to ensure animals released into the wild after undergoing these procedures are healthy, and we will assess each one against our detailed health and body condition scores and if deemed unwell they will be humanely killed. Similarly, there are unlikely to be any serious adverse effects apart from discomfort and all procedures are mild but if any adverse effects are observed we will humanely kill affected mice using an approved Schedule 1 method. At final trapping (3 months for groups 2-5 and 7 months/15 months for group 1) mice will be humanely killed allowing us to collect lymphoid tissues for in depth analyses of the body's defence system in addition to quantifying endoparasites, as well as ectoparasites.

## Application of the 3Rs

### Replacement

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

#### Replacement

This project aims to examine the longitudinal development of immune responses in animals exposed to natural infections and varied environments so replacement is not an option. Immune variation arises due to complex sets of interactions between intrinsic and extrinsic factors which cannot be modelled in vitro.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We aim to collect a total of 550 animals over the two-year period and repeatedly sample 1200 animals over the course of the project. This number will allow us to have a broad range of ages that have been subjected to vaccination and/or anti-parasite treatment. Whilst we are unable to do comprehensive power calculations because we are testing a large number of immunological parameters but for group sizes of 110 (550 /5) we should be able to obtain statistical significance of 5% at a power of 80%. This is a significantly larger power than in the only published study of immunological variation in wild mice.

### Refinement

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

## Refinement

House mice are excellent for studies of the role of infection and disease in how immune responses vary due to our knowledge of their genetics and the large number of immunological reagents available commercially. Using wild mice on a remote Island provides us with a population of mice that are not subject to significant pest control and are exposed to a variety of natural infections. They offer the unique opportunity to be able to monitor them by tagging them, releasing and recapturing them.

Traps will be baited with a good supply of food and hay to ensure warmth and they will be monitored regularly over the course of each day. At capture the health of the animal will be monitored via evaluating the individual's coat and body condition. Immunisation may result in some discomfort, which usually quickly resolves. Discomfort will be minimised by choice of adjuvant (typically alum), maximum volume injected, and number of injections (typically one). Blood collection after sampling will be controlled by application of surgical glue; blood sampling will not exceed 15% total blood volume in any 28 day period. Analgesia will be applied to the blood sampling site.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 58. Host-Microbiota Interactions Regulating Protective Immune Responses</b>
<b>Key Words</b>	Bacteriotherapy, microbiota, dysbiosis, infection, immune system
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Mammals are colonised by diverse and abundant microbial communities, termed *microbiota*, required for normal immune system development, sustenance and resistance to pathogens. Damage to the composition of the microbial communities, termed dysbiosis, are associated with, or cause a range of, significant physical characteristics, diseases and poorly understood syndromes, such as Inflammatory Bowel Disease and infection susceptibility. The aims of the project are to identify genes in the mouse that interact with the microbiota in their bodies to establish or maintain immunity, and to design and test bacteriotherapies - defined mixtures of beneficial bacteria - to correct dysbiosis, and treat infections and diseases involving the body's immune system attacking the body itself, in disease models in the mouse.

What 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 significant shorter-term output of this programme of work will be to generate data to identify a role of mouse genes in immune system balance, and to demonstrate bacteriotherapies that can be used to progress to pre-clinical development, in preparation for clinical studies in humans. In the longer term, the work outlined in this proposal is expected to lead to novel bacteriotherapies to treat intestinal dysbiosis linked to infectious diseases and chronic inflammation in humans, in order to reduce morbidity and mortality. The results of the research will be published in scientific journals and presented at scientific conferences. New mouse models will be shared with other researchers.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5-year period of the project, we anticipate using 13,500 adult mice.

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

We will give mice different mixes of microorganisms or a substance which causes inflammatory bowel disease by inserting a tube into their throats, by injection, by using infected bedding, or housing the mouse with another infected mouse, adding to drinking water or food, or by placing drops up their noses. We will then take blood samples and surface tissues and check the health of the animals. Some animals which will have been given the mix of microorganisms, will then be given disease-causing microorganisms to see whether they are resistant to infection. Some animals used for these procedures are expected to experience disease symptoms such as weight loss and inflammation. Most wild-type (not genetically altered) animals should control and clear infections but, if we see sickness, we will provide food (mash) in a dish on the floor. Mice will be humanely killed at the end of the experiments.

## Application of the 3Rs

### Replacement

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

### Replacement

The body and immune system of mammals is complex, with the surfaces inside the body and the organs interacting with each other, and also with health-associated bacteria. As it is not possible to use cells in a dish or computer modelling to discover what we need to know about these complex interactions for most of our work, we have to use mice in our experiments. However, where we can use cells instead of whole animals, such as seeing how microorganisms grow and compete with each other, we carry out experiments using cultures on nutrient agar plates. We carry out microbiological culturing, look at DNA and proteins, and grow cells (such as human or mouse derived intestine cells) in culture, to help us identify the bacterial genes we need to study, before we move on to using mice.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

REDCATED we are able to predict the number of mice we will use. We will use statistics to work out the minimum number of animals we need to use to get scientifically valid and reproducible results. To communicate our results and data to

the scientific community, we will publish them in scientific journals and present them at scientific conferences.

New strains of mice we create will be shared with other researchers.

### **Refinement**

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

### **Refinement**

We use mice for experiments as they represent an ideal model to study animal-microbe interactions, and serve as an invaluable pre-clinical model for human therapy development. Mice can be genetically manipulated to change genes relevant to human disease susceptibility and there are ways to scientifically monitor the mouse response to microorganisms. We closely monitor mice on a daily basis for signs of illness and suffering, scoring for physical signs of illness such as changes in their coat, hunched gait and mobility, along with weight loss. Any sick animals will be given food in a dish on the floor.

Our animal facility uses a sophisticated database to track the health status of every animal.

### **Group housing**

Animals will be kept in socially compatible groups.

### **Enrichment**

Mice will be kept in deep bedding and will be provided with nesting materials and 'fun tunnels'.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 59. Neuroinflammation, demyelination and degeneration</b>
<b>Key Words</b>	Multiple sclerosis, Diabetes, Small vessel disease, Neuroprotection, Therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project aims to reduce the suffering of patients with diseases such as multiple sclerosis, Parkinson's disease and Alzheimer's disease, namely diseases where inflammation within the brain causes severe symptoms and damages brain tissue. It is known that inflammation within the brain, spinal cord and nerves causes loss of function, such as blindness, paralysis and numbness, but we do not know the mechanisms responsible, and so we cannot effectively develop new 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)?**

REDACTED We expect our current research to add new strategies for neuroprotection in MS. REDACTED We also aim to identify therapies to prevent amputations in patients with diabetes.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use rats and mice only, and usually less than 400 in a year. We use genetically altered mice in some experiments, e.g. to reveal mitochondrial function, but the genetic markers are not harmful. Individual experiments are usually completed within a month.

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

Most experiments are directed at understanding MS, and the lesions are induced by immunisation so they do not involve surgery. Where surgery is involved, it is performed under general anaesthesia, and post-operative analgesia is administered

to avoid pain. We are mainly interested in the very early consequences of inflammation in the nervous system, and at this stage the animals either do not show any symptoms, or only very mild symptoms. We aim to protect the nervous system from damage, so the experiments tend to be mild in severity. Painful procedures are performed under local or, more usually, general anaesthetic. Demyelinating lesions, as in MS, are not usually painful in patients, and animals do not appear to be in pain either. Animals are more likely to lose sensation, resulting in numbness of parts of their hind limbs and tail, rather than experience pain. Animals can experience weakness in their hind limbs and tail, but these deficits typically last only up to a few days, and the purpose of our experiments is to reduce or eliminate these deficits, so they are typically not very severe. At the end of the experiments the animals are killed under general anaesthesia.

## Application of the 3Rs

### Replacement

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

#### Replacement

The study of inflammation within the brain requires an intact brain with an intact blood supply and an intact immune system, and it is not currently possible to reproduce these systems outside of the body. In particular our experiments indicate that a key factor in causing symptoms is an inadequate supply of oxygen to the inflamed tissue, and this can only be studied in the brain with a functioning blood system. Our aim is also to test novel therapies, and to do so we need a functioning blood supply to deliver the therapies, just as they would be delivered in patients. We also aim to improve function in the animals and patients, and to assess this we need to be able to watch how well the animals can walk along, before and after the medicine being tested has been given. It is therefore unfortunately necessary to use animals in the proposed research. (It is, of course, not possible to use non-protected animal species, such as worms, if the goal is to test medicines to help patients walk better.)

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We have significant experience in experimental neurology and we will employ this experience to ensure that minimal numbers will be used. Where possible, precautions are taken to employ statistical tests to predict the minimal number of animals to be used to achieve significant findings. We are also usually able to use the same sorts of examinations to monitor function and structure as are used in

patients (e.g. retinal imaging and MRI), namely examinations that do not cause pain or damage, and so can be repeated on different occasions (as in patients). We also employ small animal MRI using the institute's 9.4T scanner, which allows the same lesions to be monitored over time, rather than in different animals. These precautions reduce the numbers of animals used substantially. We also examine the nervous system of the same animals microscopically after death, using the same sorts of examination as are used in patient brain banks, and this again reduces the number of animals used.

## **Refinement**

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

## **Refinement**

We will employ mice and rats in the proposed research, and there is substantial evidence that findings made in these species can be meaningfully translated to the care of patients in the clinic. Indeed, as mentioned above, our own research has already been translated from therapeutic observations in rats and mice to the introduction of effective therapy for patients.

In experiments where we induce inflammation in a specific region of the brain and spinal cord, we choose “clinically silent” locations that do not cause loss of function.

We aim to prevent loss of function and so the animals typically have only mild, if any, symptoms. Most experiments do not involve surgery, but if surgery is employed any associated pain is controlled with opiate analgesics. The animals do not display evidence of suffering, and similar lesions are typically not painful in patients.

General anaesthesia is used for all procedures that may be painful, such as surgery, and electrophysiological examination (although such examinations are conducted without anaesthesia in patients). We employ state-of-the-art methods for our investigations to ensure the optimal data, just as are employed in the clinic. Notably, we employ small animal MRI to examine lesions over the course of disease, which not only reduces animal numbers but it allows lesions to be examined using the same technology as is used in patients, providing relevant clinical data.

Where possible we employ effectively non-invasive methods, as mentioned above, which help not only to ensure the least suffering to animals, but they also mean that the results are often directly applicable to findings in patients examined in the same way.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 60. The Development of the Microbiome of the Gastro-intestinal Tract of Neonatal Ruminants.</b>
<b>Key Words</b>	lamb, microbiome
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

**Overall Aim:** To describe the structure, development and sources of the neonatal lamb microbiome in health and disease.

**Objective 1:** *Describe the temporal development of the healthy gut microbiome of neonatal lambs.*

**Objective 2:** *Describe the sources of microbes of the healthy gut microbiome of neonatal lambs.*

**Objective 3:** *Describe the differences in structure of the gut microbiome between healthy and WM affected lambs.*

**What 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 a study of the common and serious lamb disease of "Watery Mouth". The cause of the "Watery Mouth" is not well understood. However, it is believed to occur as a result of changes in the gut population of bacterial of the neonatal lamb. This project aims to study this aspect of "Watery Mouth Disease" by comparing gut bacterial populations in healthy and diseased lambs. The study should provide the following benefits:- 1.The first description of the development of the healthy neonatal lamb gut microbiome in terms of its structure, changes over time, and key sources.

2. A description of the differences in the structure of the neonatal lamb gut microbiome in healthy and “watery mouth” diseased animals. 3. This data could identify significant disease pathogens and protective micro-organisms in common neonatal lamb disease. This could lead onto to novel therapeutic strategies (long term benefit) which have the potential to benefit animal welfare and productivity (long term), and improve human health through reduction in antimicrobial use. 4. The outputs will be written up as scientific publications which will be used by our research group to support further research grant applications; will be of interest to other researchers in the rapidly evolving area of microbiome research (human and animal); and will be of interest to the veterinary and pharmaceutical industries.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will study new born lambs and their mothers. Some animals will only be sampled on one occasion, some will be sampled at intervals over a period of about 6 months after birth. We will use approximately 20 ewes and 40 lambs in the study

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

The animals should only experience very mild and short term discomfort when a swab of faecal material is taken very quickly from their rectum. A subset of lambs will have a single blood sample collected. This again is a mild procedure and should only cause short term discomfort. Animals will remain under the care of their farms of origin throughout and at the end of the study.

## Application of the 3Rs

### Replacement

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

**Replacement**

We wish to study the microbial community of neonatal lambs in health and disease and therefore it is necessary to study lambs.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

**Reduction**

There have been no previous studies of lamb microbiome on which to base our number calculations, therefore we have based our estimates on studies on calves and humans, and hope our research will help inform future work into this

area. Statistical methods used in the data analysis will ensure significant effects are detected using the minimum number of animals.

### **Refinement**

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

### **Refinement**

The sampling that we will do is a mild procedure. Our sampling approach will use the minimum amount of lamb faeces necessary, lambs will only be handled and sampled by experienced staff (including veterinary surgeons). The lambs will be monitored after sampling by a vet and a farmer to ensure they do not suffer and adverse effects.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 61. Cellular senescence, plasticity and cancer: new frontiers and novel tools for diagnosis and therapy</b>
<b>Key Words</b>	Damage, Premalignant tumours, Cancer, Diagnostic tools, Therapeutic tools
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Cancer is a leading cause of morbidity and mortality worldwide. We are interested in the processes and molecular mechanisms that can contribute to the origin of cancer. When cells are afflicted by different types of damage (such as lung cells exposed to smoke, or skin cells exposed to ultraviolet radiation from the sun) they undergo a defence mechanism that results in a permanent stop of the division cycle. These damaged cells are usually eliminated by the immune system. However, when damage is permanent (as in chronic smokers), the system is deregulated, and damaged cells accumulate forming premalignant lesions or tumours that the immune system is not able to eliminate. These aberrant cells cannot execute their specific functions properly. Instead, their functions are altered, and they secrete a complex cocktail of inflammatory and tumour-producing factors in the surrounding tissue, which transforms nearby cells ultimately promoting cancer. We have strong evidence (*in vitro* and *in vivo*) of a causal role between these damaged or aberrant cells and the origin of cancer.

Importantly, we are developing tools capable of targeting these premalignant cells, which include novel probes and drugs. We aim to detect precancerous lesions and eradicate them in order to limit their progression to malignant tumours.

In conclusion, the main goals of our project are:

- i. to gain insight into mechanisms and processes that lie at the origin of cancer, thereby increasing our fundamental knowledge.
- ii. to develop novel diagnostic and therapeutic interventions, thereby meeting an existing clinical 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 overall purpose is a better understanding of the mechanisms that contribute to cancer initiation, which remains a formidable challenge in oncology. Such knowledge is crucial to develop novel diagnostic and treatment methods. To do so, it is important to isolate precancerous cells in order to identify the cellular type of origin and to characterise them. We also aim to validate novel probes and drugs to detect early-phase cancers and to implement novel therapeutic interventions. Our final goal is to expand our new therapies from preclinical studies to early-phase clinical trials. High-risk groups for cancer (for instance patients with a smoking history) may benefit from our technologies. In addition, the mouse models generated during our experimental approaches will be valuable to other scientists interested in cancer early detection and development of novel anti-cancer therapies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice have been widely used in cancer research and provide unique opportunities to manipulate their genes, 85% of which are shared with humans. Our work focuses exclusively on mice as animal models of human diseases. We anticipate the use of up to 10000 mice over 5 years, 7000 genetically altered mice obtained from our protocol 5, and 3000 obtained from projects with authority to distribute them.

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

In most cases, damage or tumour induction will have not a significant impact on the animals' general well-being. We will mainly focus our experiments on initial and intermediate stages of cancer progression. Short-term discomfort may result directly at the administration site, but the animals are expected to recover quickly. In case the mice show any signs of discomfort or pain, they will receive analgesics in order to reduce the administration-related effects. In rare occasions tumour induction may result in sudden death without preceding signs. Damage and tumour initiation will be a cause of genetic manipulation, the direct use of carcinogens or tumour cell implantation. Different substances, to either induce tumours or to be used as therapies once the tumours are established, will be administered directly through the blood stream, in the abdominal cavity or given orally. After tumour induction, the animals will be monitored closely for any evidence of tumour growth by imaging techniques (internal tumours) or by direct observation (subcutaneous tumours). Discomfort resulting in clinical signs such as hunched posture in combination with inactivity or respiratory distress will result in individual animals being culled. For other procedures such as treatment modalities with novel probes and drugs, most animals will show no more than mild clinical signs. In case of mice showing hunched posture in combination with inactivity or loss of body weight, mice will be culled. At the end of experiments, all animals will be sacrificed.

## Application of the 3Rs

### Replacement

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

#### Replacement

Identification of the cell of origin of cancer and the processes and mechanisms promoting malignant transformation of cells remains a challenge. Cancer initiation is a complex multi-step phenomenon that is modulated by different causal incidents (damage, inflammation, etc.), the properties of precancerous cells, and the surrounding tissue. Tissue environment may involve numerous cellular types and states (e.g. immune cells, damaged cells, proliferative cells, etc.). At present, it is essential to use animal models able to reproduce realistically the mechanisms, processes, tissue environment and complexity of events that contribute to the initiation and progression of cancer in humans.

To replace animal models we refer to cell cultures and the use of human tissues, whenever possible.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

REDACTED Calculations will be made to ensure that what is deemed to be a significant effect can be detected with the number of animals assigned to every single experiment. This quantitative assessment ensures that minimal numbers of animals are used. Damaged tissues, premalignant lesions, and tumours will be collected to perform complementary laboratory studies. Animals will be regularly followed up by imaging techniques in order to reduce the number of mice used. Different tissues will be collected at the end of the experiment to do a wide range of studies, avoiding this way duplication of experiments. We will perform pilot experiments to test our initial hypotheses/ideas on small numbers of animals. We will pay special attention to controlling sources of variability related to the environment, animals, animal handlers and the experimental procedures. To control the sources of variability we will use randomised experimental designs and blinded operators.

### Refinement

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

## **Refinement**

Our proposed mouse models replicate human cancer. Biological mechanisms and functions differ substantially in non-mammals organisms, especially in invertebrates. Consequently, mice represent the best species able to generate data likely to be directly applicable to human disease.

Animal suffering is minimised by the use of appropriate anaesthetics and analgesia. Animals will be terminated before they show sustained signs of discomfort or pain.

We have considered best practice guidelines, and proper husbandry/care measures and environmental conditions to improve the animals' quality of life.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 62. Development of a novel tracking technology for fish</b>
<b>Key Words</b>	Fish tracking, Automated vehicles, Acoustic tags
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Aims:

To test the suitability of using an automated underwater vehicle to track fish in coastal waters.

Objectives:

1. The initial project stage will develop the software and navigational capabilities to guide an automated underwater vehicle which can track an acoustic tag trailed behind a small vessel.
2. To catch and tag a wild fish to be actively tracked by the AUV as they move through coastal waters.
3. To generate GPS data showing the tracks taken by the tagged fish that can be mapped.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

As our coastal waters become more heavily used for recreation, industry and now increasingly for power generation, the likelihood of fish coming into contact with artificial structures increases. Any negative impacts of these potential developments on marine and migratory species can be better understood and reduced if we have knowledge of how individuals and populations move around and use an area. Acoustic telemetry is an established technology which has been used to track fish for a number of decades. Using this method, individuals are tagged with a small device that emits a sound signal which can be picked up by a receiver and allows the individual animal and its position to be identified. In the sea, tracking of tagged individuals may be done using a number of fixed receivers or by following an individual with a small boat whilst detecting the signal emitted from its tag. Both methods have their limitations and more recently, automated underwater vehicles (AUV's) have been used in limited studies to track large individuals or to follow a set course in the hope of detecting a tagged individual. In this project, we hope to

develop and test an AUV which will track fish as they move around coastal waters with the aim of providing fine-scale real-time data on the movements of individuals. If successful, this method can be applied more widely to better understand areas of key importance to a range of species. For example, as a priority species, under the UK Biodiversity Action Plan, sea trout are protected from over exploitation and sites believed to be important for this species are targeted for conservation management. Sea trout are a migratory species, moving between freshwater and marine environments to complete their life-cycle. Understanding how populations use coastal areas during their migration is a key component in the protection of this species, but currently data is lacking on how sea trout use coastal waters during their migration.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Sea Bass *Dicentrarchus labrax* Sea Trout *Salmo trutta* Salmon *Salmo salar* Grey mullet *Chelon labrosus* Dogfish *Scyliorhinus canicula* up to 20 individuals per species in total over 2 years

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

The fish will be caught in the wild and tagged with acoustic tags under anaesthesia using an injectable tag. When recovered from anaesthesia, the fish will be released back into the wild in the same place as they were caught. The severity level of this work is expected to be mild, and stress to the fish will be limited at all stages. The tag will remain with the fish for life, but due to the small size of the tag, the burden to the fish will not affect the long-term fitness of the fish.

## Application of the 3Rs

### Replacement

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

### Replacement

In the developmental stages of this work, as the tracking capabilities of the AUV are tested, a tag will be towed behind a small vessel or kayak to avoid using fish at this stage. Only when the AUV can track reliably, will fish be tagged and released for tracking. If the tracking capabilities of the AUV are not tested on fish, its reliability in the field cannot be assessed.

Review will continue throughout the project and alternatives will continue to be considered at all stages.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The AUV can only track one individual at a time, for a number of days so numbers will inherently be limited.

External advice will be sought to ensure minimum numbers of fish are used.

### **Refinement**

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

### **Refinement**

The species listed are suitable for tracking as they are free swimming, highly mobile species that are not bottom or cave dwellers and do not swim into the surf zone as tracking in these areas would compromise the tracking ability of the vehicle.

We will minimise the tag burden on the individual by using the smallest suitable tag based on individual body size. This procedure will be undertaken by trained and experienced individuals who can quickly and effectively assess the condition of the fish upon capture and who can make the necessary assessments of welfare throughout the process until release.

Other experts and our NACWO will be consulted regularly to ensure best practice and to minimise welfare costs to the fish.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 63. Characterisation of vaccine candidates against viral diseases</b>
<b>Key Words</b>	vaccine, immune correlates, antibodies, cytotoxic T cells, virus
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of the project is to develop novel vaccine candidates for protection against viral diseases. Those we are immediately interested in working towards are Ebola, Lassa Fever, Marburg, pandemic flu as well as seasonal flu and noroviruses.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Vaccines have saved millions of lives around the world and continue to offer enormous benefits in lowering health care costs globally by reducing the morbidity and mortality associated with infectious disease. However, new and improved vaccines are needed against emerging diseases, as well as existing infectious diseases for which existing vaccines do not offer 100% protection or where the protection is only short lived. We expect to be able to identify 1-2 vaccine candidates against severe viral haemorrhagic fevers that will be taken into clinical trials in collaboration with pharmaceutical companies as well as 1-2 vaccine candidates against influenza.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Adult mice (2000 over 5 years) and guinea pigs (750 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 levels of severity? What will happen to the animals at the end?**

The animals are expected to show no adverse effects after vaccination (either by injection or delivery of naked DNA) and testing for immune responses by taking blood samples. However animals infected with disease causing agents may show

signs of disease. Vaccinated animals are predicted to be protected by their immune response to the vaccine (this is why they will have been taken infected) but this may prove to be incorrect. The control animals to which we compare vaccinated animals are more likely to show disease signs. Clinical signs may include raised fur and hunched posture, weakness, inactivity, light aversion, and weight loss. These will only be allowed for a maximum of 24 hours before euthanasia. Some animals will be transferred to another project licence for infection should the containment facilities for the disease causing agent used require this and transfers will be in their established groups using climate controlled vehicles. Eventually, all animals will be humanely killed at the end of experiments.

## Application of the 3Rs

### Replacement

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

#### Replacement

The induction of the immune responses is complex and has never been recapitulated fully in cells in a laboratory setting, thus animals are required to provide the complex interactions of a whole body system. Animal challenge models are required to establish proof of protection delivered by a potential vaccine before decisions can be made to advance vaccine candidates towards clinical trials in people.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Group sizes are designed to give statistically significant results. Groups are designed to enable comparison between immunised experimental animal groups as well as with 'mock' immunised control animals. The mock immunised control groups may be used to compare against more than one experimental group at a time and this 'sharing' of control groups means animal numbers can be reduced accordingly. Animals that show appropriate levels of immunity after immunisation can be taken into 'challenge' studies. We have linked vaccination studies to challenge studies, to reduce the need for a second set of animals for challenge experiments

For studies where large volumes of blood are needed to detect antibodies the guinea pig is used rather than the mouse. This is because far fewer animals will be used in consequence to obtain the volume of blood needed for further analysis.

### Refinement

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

### **Refinement**

Mice will be used in some immunisation experiments where we want to look at how immune responses to the vaccines are generated. This is because mice have many available reagents to interrogate responding immune cells. This includes both early responses and later adaptive immune responses that may produce antibody. Guinea pigs are infectable with several viruses causing disease and provide a larger animal model for testing possible vaccines in which larger volumes of blood can be sampled during immunization schedules allowing different tests to be run with the same sample to measure antibody responses throughout the course of induction of possible protective immunity. This reduces the number of animals used compared to mice.

Vaccine constructs will be tested in the lab before use in animals. Adverse effects will be minimised by only allowing experimental groups with detectable immune responses to be taken into challenge studies. These animals should therefore be protected from disease or show little/lesser effect from exposure to the disease causing agent. Mock immunised animals may show clinical disease such as raised fur, soft faeces, hunching and weight loss) but as stated above this will be monitored closely and animals humanely killed within 24 hours if the signs continue.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 64. ROLE OF HOST DEFENCE PEPTIDES IN DISEASE AND THEIR POTENTIAL AS THERAPEUTICS</b>
<b>Key Words</b>	Obesity, infection, host-defence peptide, Defensin
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Humans resist infection through the prompt action of their own body to produce “host defence peptides” (HDP) that are small molecules that can kill germs. The HDP are present throughout the animal world and may help combat the new “superbug” infections that are not killed by the current antibiotics that we have. Recent work has highlighted that these HDP do additional, unexpected things like influence the skin disease psoriasis or how overweight/obese people are. The aim of this work is to understand what these HDP do normally in health and what goes wrong in disease and whether we can use similar molecules as therapeutic drugs to make us better.

REDACTEDThe mice are not unwell but do become fat when they grow older. We have also found that if the mice over express (produce too much of) a human HDP the mice are lean. We now wish to see the weight gain in these strains of mice when given a high fat diet and whether the lean mice remain lean. The mice will be tested to see if they have any similarity to late onset diabetics and whether the normal bacterial populations in their gut have changed. The incidence of these diabetes type II because of obesity and infection from antimicrobial resistant bacteria are a major burden on the UK NHS budget and understanding how they occur is likely to lead to new therapies.

We will examine the type of obesity the mice get and whether this increases when fat a high fat diet and are these animals more likely to get diabetes.

We will test the animals to see if they are more likely to be sensitive or resistant to infection by looking at their immune system and giving them non-infectious mimics of infection as if they are then that says the gene that has been removed must be important in protecting against infections and so may be an important clue for new

drugs. We will do a pilot experiment in the first instance to decide how best to do infection subsequently so as to cause the mice the least amount of distress possible.

We will use this information to inform in the long-term on the development of new drugs or approaches to control the weight gain and other illness we see in the mice.

**What 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 likely help understand what causes infections or inflammatory diseases such as obesity and what normally protects us from them. This will lead to potential therapies for weight regulation or infections,

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We work with mice as a part of our research programme, combined with using human tissue, cell lines and available mouse and human bioinformatic data. Mice that have many genes similar to humans and we and others have mouse strains with different gene deletions that tells a lot about what these genes do. Many immune and metabolic processes have been worked out in the mouse using this approach and this have been very useful in understanding the same processes in humans. We will use no more than 3,530 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 levels of severity? What will happen to the animals at the end?**

The majority of the animals used will be for breeding and maintenance and will not cause the animals any discomfort or harm. The GA animals under normal conditions kept in normal mouse cages in groups up to five do not show any evidence of being any less content than normal mice. Mice will be closely monitored for any adverse effects throughout their life and every procedure carried out. Some animals will be given high fat diets and will get heavier and they will be monitored for what happens to them as a consequence. They will be weighed on scales and their body length measured with a tape measure. They will need a light inhalable anaesthetic for this to stop them moving so we can get accurate numbers. This will not cause distress or harm. They will have blood samples taken from a vein to see how the chemicals in the blood are responding to obesity or exposure to inflammatory agents. We will not remove enough blood to cause any distress in keeping with the HO guidance. We will expose the mice to procedures that see if the mice have any evidence of type 1 or type 2 diabetes. This will be similar to how diabetic people are tested for their response to sugar administration or lack of it. This will involve keeping them away from food for a period of time and then giving them sugar and taking blood samples. The mice will be fasted overnight and then exposed to sugar either by injection into the body cavity or by putting it into the mouth. Blood samples are then taken at

regular intervals over a two hour period (maximum of seven samples) from the same nick on their tail to induce bleeding (like when someone cuts themselves with a razor by accident) to see what happens to their blood sugar. The nick will then be allowed to heal. They may also be fasted for four hours and then given the hormone insulin by injection to see what happens to their blood sugars again by taking blood samples. Only rarely will the same mouse have both these tests carried out. Some mice may suffer from low blood sugar and if this is obvious they will be killed using a humane method. The mice will be imaged to see where how much fat they have but this last only a few minutes and does not need an anaesthetic. It is similar to people having an MRI scan in hospital but takes much less time. We will need to inject the animals with various substances but this will not involve substances that would be irritant at the site of injection and the procedure does not cause animals distress. We will pick the animals up by cupping them in our hands rather than using their tails which causes the animal more stress. We will have to singly cage female mice for two periods of up to five days and male mice for 20 weeks. We will take steps wherever possible to decrease the adverse effect of social isolation by including cage toys including cardboard tubes or similar so the mice don't get bored. groups. Some animals will be exposed to inflammatory agents (eg. dead bacteria). Inflammatory agents such as these have a short time of effect in normal mice as there is no infection. These agents will be delivered in the body cavity or into the airways using light inhalable anaesthesia and a tube that goes down the airway. This is like when people have a bronchoscope. We may take a blood sample during the exposure experiment to monitor the response of chemicals produced from cells involved in the immune response. If the results show that the GA mice are more or less responsive to the inflammatory agents we will carry out a pilot study to determine with an infectious agent (bacteria of relevance to human infection) the minimum dose required to induce an effect in normal mice. Then we can see if the GA mice have an altered response. In the pilot experiments with bacteria we will use small numbers of mice (3) and address infection dose to reduce clinical signs and make sure that the animals are humanely killed before they become severely affected. We have a scoring system and the animals will be regularly monitored for adverse signs (lack of moving about; ruffled fur etc) using this system. In particular they will not be allowed to show evidence of difficulty in breathing for more than hour. We will see what happened to the animals in the pilot after their death by counting the number of bacteria in the animal's organs. We will see whether the HDP can rescue obesity or inflammatory responses. We will inject substances that increase the amount of HDP in the animal or breed to mice expressing human HDP or inject the HDP or a modified, shorter length HDP directly into the mice. Injection of these are unlikely to cause the animals any distress and are likely to make the obesity or inflammatory response less. We will use pilot studies with small numbers of animals to check the optimal dose and confirm a lack of mouse distress. This work will provide preliminary evidence that these molecules in the long-term will be useful therapeutics for infection/inflammation or obesity. Mice will be killed in a humane way

at the end of the protocols. Except the breeding and maintenance protocol which is used to provide mice for the obesity and inflammation/infection studies.

## Application of the 3Rs

### Replacement

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

#### Replacement

The responses of individuals to food, infection or inflammation are all complex and involve interactions between many different cells in the body. Pubmed literature searching shows that the genomic structures of defensins in non-mammalian species are too dissimilar to human to be of value when considering this rapidly evolving gene family. Zebrafish for example have only three defensin genes where mice and humans have 40-50. We can and do use cell based systems as a first step but to see what happens in real life we must use animals.

I have read the literature, searching using pubmed for non-animal alternatives for obesity modelling or airway inflammation/infection; searched in the NC3R 3Rs Resources catalogue (<https://www.nc3rs.org.uk/3rs-resources>) and (<https://norecopa.no/>) and cannot find appropriate *in vitro* methods to replace the animals (mice) that I propose to use.

We will use primary human cells from peripheral blood to test peptides *in vitro* for pathogen killing ability and signalling responses and we can induce mouse ES cells grown as cells in culture in the presence of a natural chemical to become macrophages and we will use this cell source where possible to monitor the effect of HDP on cell signalling. We will use *ex vivo* isolation of human airway samples post-surgery to determine pathogen killing in physiological tissue (<https://www.jove.com/video/56284/optical-screening-novel-bacteria-specific-probes-on-ex-vivo-human>) and mouse lines to validate the strategy before the final proof of efficacy in the whole animal. The immune system is key in all this work it is difficult to find an alternative to a whole animal system as the immune cells will have to come from compartments that would not be maintained in cell culture. We will constantly monitor the literature and speak to colleagues expert in the area, to be aware of novel replacement protocols and will consider anything relevant for this work.

Weight control ultimately requires brain interaction with signalling from the gut and so again the whole animal is required to monitor this but we will work out pathways as far as is possible in cell culture.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Efficient colony management ensures that only colonies that are actively being used are mated and produce animals. Those that are no longer required are cryopreserved and closed at the earliest opportunity. We now archive sperm as the main method of cryopreservation which reduces the number of animals required to secure a line. We will use good practice in experimental planning, including statistics such as power and resource equations and breeding calculations using current breeding figures to predict the number of mating required for experimental cohorts.

The number of animals used in experiments is determined by how many are needed to produce a mathematically meaningful answer. We are always aware of this and so we have done calculations to decide the least number of animals we will need. We have a chartered statistician on the project to help us with this. We use animals from the same family background so the background genetic variability is less. We also combine experiments so that controls do not need repeating. In addition we will use non-invasive imaging where possible to determine what is going on over the course of an experiment rather than using an additional animal group to determine long-term effects.

## Refinement

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

## Refinement

We are using mice as these are the least sentient being with a genome sufficiently similar to that of humans to do these experiments and because the toolbox of genetic techniques and reagents for study of this species is highly developed and readily available.

We will minimise severity in all our experiments to increase the welfare of these animals. To ensure this we will use observational methods and will refine and adjust these methods in the light of experience gained during the course of this work and from previous work in this experienced lab.

We have determined the best methods to use from our own experience, and the literature. Lab Animal and web sites (eg. [www.frame.org.uk](http://www.frame.org.uk)) will be used to check that where appropriate the techniques are refined and where possible animal use will be replaced. We will consult with the on-site vets and colleagues with experience in the models with which we are unfamiliar, ensure any training required is led by experienced trainer and added to the training record of my staff. We will use online sites such as journal of visual experiments (<https://www.jove.com/>) and procedures with care ([www.procedureswithcare.org.uk](http://www.procedureswithcare.org.uk)) to refine our protocol delivery. The vets

that oversee our work are always aware of everything that we do and the Government website NC3R has many new ideas for reducing animal experimentation and refinement of experiments. This includes the use of pain relief, animal handling and using the least invasive methods for drug delivery. We will use the NC3R Experimental Design Application to assist with experimental design. The recent Norway's National Consensus Platform for the advancement of the 3R's (Norecopa) Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) will be used to plan experiments as will the Fund for the Replacement of Animals in Medical Experiments (FRAME) planning poster to ensure we are using the most appropriate way to minimise animal suffering. We will ensure that where possible animal handling is done using tubes or cupping rather than securing the animal by the tail and with reference to publications such as *Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/labon.1217*

We will expose animals to the lowest dose of inflammatory agonist to achieve the response we require to study and will use raised cytokine levels after short exposure as our primary endpoint and only rarely use longer term exposure to determine the possibility of resolution. We will observe the guidelines on clinical assessment of pain and distress in rodents, by taking the FLAIRE learning module (online recognition and prevention of pain, suffering and distress in lab animals); assess the best practices in the NC3R resource section; follow the recommended safe volume for single bleed (ml) <https://www.nc3rs.org.uk/blood-sample-volumes> and the recommended volumes and sites of administration of substances to lab animals *Am Assoc Lab Anim Sci. 2011 Sep; 50(5): 600–613.*

Where new lines will need to be made e.g. where we wish to delete a specific gene we aim to use the most refined technology available (for example CRISPR/Cas9a). This should bring benefit both to the reduction of the numbers of animals used and refinement in the ability to create genetically altered mice of higher quality.

Where possible work will use tissue from genetically modified mice that have been subjected to killing by a schedule 1 method rather than experiment being done on live animals.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 65. Experimental therapy in cancer xenografts</b>
<b>Key Words</b>	
<b>Expected duration of the project</b>	2 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Prostate cancer is the most frequent malignant tumour among UK men. There are different sub-types of prostate cancer, which can be treated with different therapies (surgery, radio-therapy, hormonal therapy). The most aggressive form of this disease is called neuroendocrine prostate cancer (NEPC). Unlike other prostate tumours, NEPCs cannot be cured and usually lead to patient's death within a few months. The molecular mechanisms leading to the development of NEPC are still obscure. Therefore, there is a dire need for research projects aimed at identifying new therapeutic options for this incurable disease.

Here we propose to study a vast and largely unexplored area of the human genome: the long non-coding RNAs (lncRNAs) and T-type calcium channels (TTCC). Our goal is to identify new therapeutic targets for NEPC. To this aim, we will need to employ patient-derived prostate cancer xenografts (PDXs), i.e. cancer cells extracted from prostate cancer patients and implanted in immune-deficient mice. We have previously shown that PDXs are superior to other pre-clinical cancer models, since they accurately predict the efficacy of cancer therapies in human subjects, and since they enable us to predict the drug's stability and safety in human subjects (this preliminary work has been conducted at the BC Cancer Agency, Canada).

Consequently, the objectives of this study are:

1A) To analyse pre-existing PDX samples, in order to identify the lncRNAs that are specifically expressed in NEPCs.

1B) To study the function of selected lncRNAs and TTCC and to identify a method to "turn these genes off". These experiments will be performed in human prostate cancer cell lines (no animal model involved).

2) To select 1-2 therapeutic targets and test their efficacy on a neuroendocrine prostate cancer PDX models. Animals will be divided in two groups and treated with the experimental drug or with a control (non-effective drug). Tumour volume will be

measured at selected time-points for two months. We expect to see that our experimental treatment reduces the growth of NEPCs.

**What 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 contribute to understanding the pathogenesis of incurable prostate cancer and will provide a unique list of lncRNAs involved in this disease. If our pre-clinical tests are successful, these studies will also pave the way for the development of new treatments for human cancers.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will employ immunocompromised mice for 7-8 weeks (approximately 120 for the 2 studies: TTCC and lncRNAs).

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

The animals will be employed to measure the anti-tumour activity of selected lead compounds. Tumour bearing animals will be randomised to receive intra-peritoneal injections of the experimental drug or control substance. Likely adverse effects will be mainly due to the tumour (excessive weight loss, ulceration), while the treatment is expected to be only mildly toxic. Animals will have surgery to implant tumours just under their skin. During this procedure, the animals will be unconscious (under anaesthesia) and the recovery will be aided by giving animals analgesics and by keeping them warm. Hence, the overall severity limit will be moderate. Animals will be euthanized at the end of the experiment, or when adverse events occur and the pain, discomfort or suffering are not controllable.

## Application of the 3Rs

### Replacement

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

### Replacement

We have carefully considered non-animal alternatives, including the use of cell lines and human tissues. Some of these methods will be employed in the first 2 years of the project, to identify the most promising therapeutic targets, which will be then tested in animals. Animal tests on patient-derived xenografts (PDXs are an essential step towards clinical application of these therapeutic targets. Indeed, our PDXs closely mirror patients' characteristics and more importantly response to treatment.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We have conducted accurate statistical analyses to ensure that we use the minimum number of animals possible, while also obtaining meaningful results. We will also conduct a pilot study on a limited number of animals to ensure that our experimental drug “turns off” its target gene, before proceeding with a larger study.

### **Refinement**

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

### **Refinement**

We will use immunocompromised mice because they have been shown to allow the growth of patient-derived tumours that closely recapitulate the patient’s neoplasm. For this particular project, will ensure that distress and pain are minimized, via appropriate pain-reduction procedures approved and controlled by our REDACTED REDACTED . Welfare costs will be minimised by using appropriate enrichment methods and group housing, on the advise of the REDACTED. Appropriate aseptic and analgesic techniques will be employed during the surgical procedures.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 66. Pig models of poisoning &amp; drug toxicity</b>
<b>Key Words</b>	poisoning, antidotes, treatment, mechanisms
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Poisoning is a major global health problem causing hundreds of thousands of deaths each year. Self-poisoning with medicines ('attempted suicide') is responsible for 10% of all medical presentations to hospital in the UK. Medicines such as diltiazem and paracetamol are responsible for several hundred deaths each year in the UK.

Self-poisoning is an even greater problem in rural Asia. Here pesticide self-poisoning is a major public health problem and one of the three most important means of suicide worldwide, killing more than 150,000 people each year. Many of these suicides occur from organophosphorus (OP) insecticide poisoning, but other types such as paraquat and aluminium phosphide can be devastating.

The study of poisoning in humans (clinical toxicology) is a neglected area of medicine, with little active research. Few animal models exist with which to study what happens after poisons enter the body - information that is essential to find novel treatments. Few effective and affordable antidotes exist for severe poisoning.

This project will use pigs to identify effective antidotes for poisoning and to better understand what poisoning does to the body. This will be done by giving poisons to anaesthetised animals and studying the effect of poison and treatment. Lessons learnt from these animal models will be rapidly considered for studies and trials 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)?**

This project will increase our understanding of how poisons affect the body, in particular how OP insecticides cause our muscles and nerves to stop working and the lungs to become damaged. It may also find new treatments (or antidotes) for cyanide poisoning that are better at saving lives than our current treatment options

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use up to 150 pigs over 5 years. Previous work shows that detailed studies in a small number of pigs are able to provide scientifically powerful data that will guide human treatment

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

All animals will be anaesthetised at the beginning of the study so that they are unaware of any study procedures. They will then have minor surgical procedures to place monitoring and blood sampling tubes into an artery and veins so that blood samples can be taken for tests and the condition of the heart carefully watched. The wounds will be stitched up after insertion of the tubes. Poisons and/or treatments will also be administered via these tubes or by a tube placed into the stomach. All animals will be cared for by veterinarians who will closely monitor for adverse effects. They will be watched for the effects of the poison and how this is controlled (or not) by the antidote. At the end of the study, the animals will be killed by a humane method and tissues taken for analysis after death. There are no severe protocols on the license and no animal suffering except that associated with routine administration of sedative or other drugs before anaesthesia.

## Application of the 3Rs

### Replacement

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

### Replacement

It is not possible to set up models of poisoning in humans or to test new antidotes that have not previously been tested in animals. Studies done in test tubes or on computers are unable to determine the efficacy of antidotes or therapeutic interventions for poisoning in living humans because they cannot reproduce the complex multiorgan effects of the poisons against which the antidotes must work. Animal studies are therefore required.

Human patients presenting to hospital with self-poisoning are very variable. They have ingested differing amounts of different poisons, at different times, and have received different treatments before coming to hospital. Furthermore, the dose ingested is rarely known and the actual compound ingested may well not be known for several days, if at all. This marked variation between human patients makes clinical research difficult.

Large controlled studies in hospitals allow the variation to be balanced out but such trials are expensive, difficult, and only to be attempted when there is good evidence from both animal studies and early human studies that there is a reasonable likelihood of effectiveness.

Animal studies can be more controlled, with a specific dose of a particular poison administered at a specific time point, thus allowing much smaller numbers of participants.

We have shown that pig models of poisoning provide a large amount of relevant information on what poisons do in the body and whether treatments work - all information that can be rapidly translated into human studies

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We work with experienced statisticians to ensure that the minimum number of animals are used for each study, while maintaining scientific quality

### **Refinement**

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

#### **Refinement**

We have chosen pigs (either the Gottingen minipig which has been bred to be small or outbred farm pigs) for our studies because pigs are much closer to humans than rodents. Due to similarities with humans in how pigs handle and break down medicines, they have become an increasingly important model species for understanding the benefits and harms of new drugs.

The large size of the species has several further advantages including: a longer, and more clinically relevant, time course of study for most diseases; ability to repeatedly sample blood and tissues; and the use of readily available hospital equipment for humans to record changes and to image the animals.

Unfortunately, previous animal models of poisoning using rodents have not been closely related to the human situation and their data could not be extrapolated to clinical practice. For example, most studies of OP insecticide poisoning and its antidotes have involved measuring how many animals survive to 24hrs with or without certain treatments. However, these studies do not mirror what happens in people. The OP pesticide has been given in the wrong form and by the wrong route.

The treatment has been started: at the wrong time; with treatment doses that differ from doses used for humans; without the typical intensive care support available to humans; and without the intention of giving the animal comprehensive treatment. Our pig models address all these limitations.

All studies on this license will involve anaesthesia before poisoning. There are no severe protocols on the license and no animal suffering except that associated with administration of sedative or other drugs before anaesthesia.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 67. Mechanisms and outcomes of systemic inflammation and cancer in relation to cellular metabolism</b>
<b>Key Words</b>	Acute pancreatitis, treatment, kynurenine, metabolism
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We think that breakdown products of essential components of the diet influence the development of serious diseases such as acute pancreatitis, inflammatory bowel disease, sepsis and cancer, and also in the natural energy balance involved in cell aging. We aim to understand specifically how metabolism of a part of the diet called tryptophan and kynurenines is involved in those processes, to allow us to continue to understand and develop 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)?**

Increased understanding of kynurenine metabolism in serious diseases such as acute pancreatitis, inflammatory bowel disease, sepsis and cancer, and in cell aging, will help us to develop better ways of treating these conditions in humans and animals, for example by making new medicines, that act by altering that metabolism.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use rats – approximately 1700, mice – approximately 7300, over a period of 5 years of which breeding and maintenance accounts for: rats – approximately 200, mice – approximately 3000 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 levels of severity? What will happen to the animals at the end?**

A large proportion of the animals (approx. 60% of the total) will be used for breeding and maintenance of the colonies, and for generating new genetically-altered strains. The expected severity for these animals is mild. The majority of the animals to be

used (35% of the total) will have a moderate level of adverse effects, for example, being anaesthetised in a scanner, and having repeated injections, or having a moderate level of colitis. Because the diseases we are studying are very serious in patients – for example in severe acute pancreatitis, the risk of death to people who have it is around 1 in 5 – in order for our research to be meaningful, some of the animal models we use have to reflect that level of severity. Therefore, in some animals (fewer than 5% of the total), the level of severity is expected to be severe, with a risk of death and complications approximating that seen in human disease. At the end of each experiment, the animals used in each experiment will be humanely killed by experienced staff and blood samples and tissues taken for analysis, and analysed to help answer our research questions.

## Application of the 3Rs

### Replacement

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

#### Replacement

The diseases we are studying are complex, and although we can investigate lots of aspects using computer models, and cells in dishes in the lab, there are some complex interactions, for example between body tissues and the immune system, that can't be modelled in a petri dish. In addition, some of the technologies that we will use – for example genetic alterations – are at the moment only practically possible using mice and rats

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We always plan our experiments carefully, and work closely with statistics experts to come up with the experimental design that uses the fewest animals to get the right answer

### Refinement

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

#### Refinement

Our patients in the clinic are humans and therefore mammals, and we try to use the species that has similar general features – for example a cell-based and non-cell

based immune system working together – to understand the disease processes that we study. Therefore we use mammals, and rats and mice are the most appropriate species for this work. There are certain technical factors, for example the size of a rat being much more suitable for experiments that require drip lines into a vein for repeated injections, which makes rats more suitable for some experiments, and other factors for example the way we breed and develop genetically-altered mice that makes mice the most appropriate for other experiments. We always try to avoid using animals where we can get an equivalent or satisfactory answer without using animals, but when we do use animals, we minimise distress by careful and experienced handling, use of anaesthetics wherever appropriate or necessary, use of pain relief wherever pain might be expected, and careful monitoring by a team of experienced staff. In particular for some of our experiments, we will use implantable telemetry devices that give a constant readout of temperature and activity and can alert us early to any animals that are getting unexpectedly sick.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 68. Pregnancy complications: targeted interventions</b>
<b>Key Words</b>	Pregnancy, mouse, placenta, therapeutics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Complications of pregnancy affect around 1 in 6 pregnancies in the UK and cause enormous social and financial burden. Some of the most common pregnancy complications include fetal growth restriction, preeclampsia and gestational diabetes associated with fetal overgrowth. Fetal growth restriction (FGR), relates to the inability of a baby to achieve its genetic growth potential. FGR significantly increases the risk of stillbirth and also leads to a greater risk of adulthood diseases such as heart disease. Preeclampsia (PE) is associated with increased maternal blood pressure and the presence of proteins in the urine, indicating sub-optimal kidney function. In addition, PE is associated with an increased risk of having an FGR baby making PE a high-risk pregnancy for mother and baby. Gestational diabetes mellitus (GDM) is characterised by excess glucose in the blood which arises during pregnancy and puts the baby at greater risk of being overgrown. An overgrown baby increases the risks of complications during delivery and, in addition, a baby that is overgrown at birth is at greater risk of obesity and diabetes in adulthood. Thus, complications of pregnancy have implications that can last a lifetime. Despite these devastating consequences, there are no treatments for FGR/PE other than early delivery of the baby, akin to PTL, which is itself associated with poor outcome. One of the reasons for this lack of therapeutics is that we do not fully understand the mechanisms underpinning these complications of pregnancy. It does appear that abnormal placental function is key to the onset of these complications but many of the exact mechanisms remain elusive. As such, it remains imperative that we continue to assess the changes in placental function that accompany these complications and to target therapeutics based on this evidence. As such, we have already demonstrated, in mouse models of FGR, that sildenafil citrate (Viagra) is one drug that may have therapeutic value in the treatment of FGR. We have shown that Viagra improves placental blood flow (which is often impaired in FGR) and increases fetal growth as a result. Following these data, a human clinical trial has been funded emphasising the potential for mouse models to provide a good pre-clinical testing ground.

This project has 3 major objectives:

1. To identify whether signals derived from the fetus are important in the control of fetal growth and whether these signals are different/absent in cases of poor fetal growth
2. To identify new potential therapies for FGR/PE/fetal overgrowth by focussing on both dietary modifications and drugs already approved in the clinic for other diseases that share similarities to pregnancy complications (e.g. those designed to increase blood flow)
3. To target these therapies specifically to the placenta, both to minimise possible side effects of therapies and also to maximise the chances of success of these therapies. Targeting involves attaching a protein 'tag' to these drugs which allows them to bind only to the placenta, maximising action of these drugs at the required site. This should minimise the risk of possible side-effects caused by drugs being delivered to multiple 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)?**

Following this project, there will be a greater understanding of the placental mechanisms that underpin FGR, PE and the fetal overgrowth associated with GDM. As part of this insight, we will have a greater idea as to whether signals from the fetus to the placenta are important in fetal growth and whether these signals are altered/absent in complications of pregnancy. This project also has the potential to identify further candidate therapeutics, in addition to Viagra. These candidate therapeutics may include dietary modifications such as beetroot juice, which contains ingredients (nitrate compounds) shown to improve blood flow, already deemed safe for use in pregnancy. In addition, we will have evidence as to whether drugs targeted to the placenta only, give additional benefit in terms of safety for mum and baby, and in terms of achieving greater therapeutic value compared with the same drugs given systemically, i.e. not targeted. Overall this work will increase the likelihood of human clinical trials of drugs to treat pregnancy complications.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

All experiments will be conducted in mice and rats. We expect to use approximately 4000 mice and 1000 rats across 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 levels of severity? What will happen to the animals at the end?**

In our model of pre-term labour, we specifically induce PTL by targeting pathways known to be important in the onset of labour. This will allow us to test candidate therapies which we predict will delay this early onset of labour. The animal models

used in this proposal have mild pregnancy phenotypes including high blood pressure (PE), diabetes (GDM) and reduced fetal growth (FGR). In terms of the administration of potential treatments, most of these will be administered via the diet (water or food) thus minimising adverse effects. For substances that are unable to be administered in this manner, this will be via an injection, either under the skin or into the abdomen (which may be of moderate severity). This will cause a mild and transient pain but injections may need to be repeated on several days. Our previous experience suggests that animals tolerate this well and do not show long-lasting effects. For all the drugs/therapies that we propose, we do not expect any adverse effects but animals will be monitored for signs of pain/distress should unexpected outcomes occur. For experiments when surgery will be required (e.g. insertion of blood pressure probes), the animals will be kept at a surgical plane of anaesthesia. Following this anaesthesia, animals will be brought around but pain levels controlled by the use of painkillers as required. Whilst we make every effort to use sterile techniques to minimise the risk of infection following surgery, this minimal risk remains. If this occurs, and the animal found to be in pain/distress, we will humanely euthanase the animal. Following all end procedures, animals will not be re-used for any other procedure and will be euthanased humanely.

## Application of the 3Rs

### Replacement

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

#### Replacement

One of the reasons why so little progress has been made in developing drugs for pregnancy diseases is that clinical trials testing treatments in pregnant women are very difficult and ethically challenging. Thus, in order to assess the effectiveness of potential therapeutics, the use of animals is the only possible starting point.

We always run experiments using human placenta in the laboratory alongside animal experiments as a first step in determining effectiveness in women but such experiments cannot inform us of any general beneficial or harmful effects to mother and fetus or their function when a blood supply is intact. Computer modelling of the pregnant woman is just not possible with our present state of knowledge.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will keep the number of animals to a minimum by making as many observations/measurements as possible on individual mice (also aided by the fact

that each litter comprises multiple pups) and by removing as many tissues as appropriate for later analyses. This ensures that from one pregnant mouse, we can obtain multiple datasets.

As we have several years experience of similar experiments on mice we can be confident of the minimum numbers we will need to achieve statistical significance. Experiments will be designed so that the primary statistical test will be to assess 2 variables, treatment (treated versus untreated) and genotype (genetically altered versus wild-type controls).

## Refinement

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

### **Refinement**

As we require knowledge on placental function a mammalian species is essential for our work. Mice and rats have a uterus and placenta similar to that in women and also allows us to study genetically modified strains that have disease symptoms similar to those found in humans. Such accurate disease models are not available in any other species. The rat models of PE-like disease and FGR associated with inflammation described within this licence are established animal models in which to test candidate therapeutics; we choose to use these in preference to attempting to establish similar models in mice which would likely require significant usage of animals in defining and optimising new models.

We will minimise suffering by using anaesthetic for any potentially painful procedures and by careful monitoring of the animals to ensure they are not in discomfort. For recovery surgery procedures, analgesics will be used as necessary to minimise pain. Additionally, for administration of therapeutics, this will occur primarily via drinking water or in the food. Only if this is not possible, will injections or insertion of minipumps underneath the skin be employed.

Whilst we do not expect any adverse reactions from our candidate therapeutics, the use of targeted treatments will further limit any off-target effects.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 69. Cell and circuitry replacement in Parkinson's disease</b>
<b>Key Words</b>	Parkinson's disease, cell transplantation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The human central nervous system (CNS - the brain and spinal cord) commonly suffers diseases that destroy cells ("neurons"). The overall aim of the project here is to identify cells that can replace the neurons destroyed by the neurodegenerative disease Parkinson's disease. Parkinson's disease is a disorder where the neurons that help us start a movement degenerate. Once these cells are destroyed by the disease, the patient has little ability to move, and they become very slow or completely immobile. The objective of the research plan is to: (1) identify cells that can mimic the actions of neurons destroyed by Parkinson's disease; and (2) test the potential for these cells to recover the motor capabilities of an animal model of Parkinson'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)?**

We aim to determine if cells we have studied extensively in culture should progress toward clinical use. More specifically, the benefits of the experiments proposed here are that they will determine how effective the cells are at improving the functioning of an area of the brain affected by Parkinson's disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The studies proposed in the research plan here will make use of rats. To make statistical comparisons between animals that receive cell transplants and those that do not, the studies will use approximately 250 animals in total over the 5-year course of the project. This is the minimum number possible to get a meaningful assessment of the cells' ability to recover the fine motor functioning of a living animal.

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

The studies conducted here will involve surgery on animals, and this has innate risks. Traditional risks of surgery are mainly from anaesthetics and infection, and these will be limited by the vast experience of the scientist, and careful monitoring during the operative and post-operative period. Any signs of infection (i.e., poor wound recovery) will be treated with appropriate antibiotics, and the animal closely monitor until recovered. The animals are given appropriate analgesic both pre- and post- operatively, and should have no discomfort beyond that caused by the incision made in the skin during surgery. Any animals which display discomfort beyond 1 week post- operatively, will be reviewed (for infections or adverse events) and given additional analgesics as needed. The lesion model does not greatly affect the animal's ability to move, forage, feed or think. Any animals which do not feed, forage or move suitably after surgery will be fully assessed by trained staff (including a veterinarian, if necessary), and appropriate action taken.

## Application of the 3Rs

### Replacement

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

#### Replacement

The experiments are designed to be a final stage of testing to determine whether cells we have extensively screened *in vitro* have the potential for clinical use. Animal numbers are reduced to a minimum by the extensive use of *in vitro* testing to assess cell viability, potential for tumorigenicity, their ability to differentiate and grow in brain tissue. These will be performed before any *in vivo* work is conducted. The significant *in vitro* testing of the cells, however, cannot replicate the many biological systems (e.g., immunological, local tissue responses, protein - protein interactions) that can alter the effectiveness of cells transplants, and none can reveal the overall impact the cells have on improving movement in a living animal. Hence, the final stage of testing necessitates the use of living animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

When ultimate (*in vivo*) testing is warranted (i.e., when cells have shown promise in *in vitro* analyses), the size of animal groups for testing is determined through power calculations to identify the minimum number required to compare control and experimental animals. This is done through the advice of an on-site statistician that remains involved in the data analysis of such projects.

### Refinement

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

### **Refinement**

Rodents (instead of other animals, such as flies) are used for these studies as they are the lowest form of animal that we can use to obtain human-relevant (motor) behavioural measures. Human relatable motor skills cannot be meaningfully assessed in lower vertebrates, and there are no suitable alternatives that have significantly similar motor circuitry to humans. The animals (rodents) used, and the models produced, have been modified for more than 40 years to allow for a meaningful model of Parkinson's disease while having the least cost to the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 70. Dairy Precision Farming and Nutrition</b>
<b>Key Words</b>	Precision farming, production diseases, metabolic disorders
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

To generate data and information that will lead to developing a state-of-the art early-detection system for metabolic and infectious disease in dairy cattle, addressing some of the key challenges facing the UK dairy sector specifically diseases such as lameness, ketosis, and acidosis.

**What 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 potential performance of the modern dairy cow is hindered by the high incidence and prevalence of production, metabolic and also infectious disease. The proposed study will investigate non-invasive solutions that can identify appropriate indicators of disease and generate an early warning that can help farmers identify at-risk cows and hence allow for timely interventions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 250 adult cows 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 levels of severity? What will happen to the animals at the end?**

In order to develop non-invasive techniques, there is need to correlate the new non-invasive parameters such as cow behaviour (measured by (neck collars), cow gait, breath and in-line real-time milk sensors with the invasive traditional measures such as rumen pH at temperature and blood metabolites. At the end of the experiment, animal will either be returned to a normal production herd or be rehoused to a slaughter house.

## Application of the 3Rs

### Replacement

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

#### Replacement

It is not possible to achieve the objectives of the project without using dairy cattle because the technologies that will be developed through this research will only be applied in dairy cows and hence need to be tested on dairy cows. There are no mathematical models that might help us (or indeed that we might develop) that can be used to replace this study. Since the sensors being developed and tested are novel, there are no previous veterinary records on the farm that can be studied historically to address the aims of the study.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

To ensure that the minimum number of animals we will be used a power calculation using the appropriate statistical method will be undertaken. Power calculation will use known information from the traits of interest such as standard deviation for milk yield.

To increase discrimination between diseased and healthy cows based on different health indicators that will be measured such as cow activity we will account for additional sources of variation such as cow age, body condition score, parity and disease history.

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### Refinement

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

#### Refinement

The procedures which will be used are the most refined way to collect the relevant data. For example, the use of rumen boluses is less invasive than repeated naso-gastric rumen sampling to acquire information on the pH, temperature and other parameters required.

Where appropriate blood samples will be shared with the farm for routine management to reduce number of sampling from the same animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 71. Regulation of the progenitor cell niche in ageing</b>
<b>Key Words</b>	Regeneration, Nerves, Stem cell, Niche, Ageing
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The world's population is ageing: it is predicted that, by 2030, 20% of the population will be aged 65 and over. As we grow older our organs do not function as efficiently and in the event of injury or disease are less able to repair themselves. This leads to many of the health problems we associate with ageing and adversely affects quality of life. Nerves surround all organs in our bodies; however, during the ageing process nerves become damaged. Research has shown that nerves interact with stem cells, which are the cells that can contribute to organ repair/regeneration within each organ, and without nerve input stem cells diminish in number. To date, the way in which the stem cells and nerves interact in ageing organs has not been fully studied. Therefore, this study will test the hypothesis that nerve damage/loss around ageing organs leads to reduced numbers of stem cells, this in turn causes a loss of function and a reduced ability to repair and regenerate within such organs. The project will primarily use the salivary gland as a model, which is adversely affected by age and show symptoms of dry mouth. Understanding the relative contribution the nerves make to normal cell replacement and/or regeneration and the cells and signalling pathways involved will allow us to better understand how to regenerate ageing 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)?**

The intended benefits of this project are to: 1) Characterise the cellular associated with ageing in the salivary gland 2) Determine how the supporting environment (including peripheral nerves) influences progenitor cells during ageing 3) Test the efficacy of neuronal factors (such as the acetylcholine mimetic, Carbachol) to promote regeneration of aged salivary gland. It is predicted that, if nerve function is

improved, then the ageing organ will work better and be better able to repair and regenerate itself. Expanding this theory by applying it to all organs affected by ageing could play a key role in identifying therapeutic ways of improving organ function with age and following disease/injury without the need for life-long medication or complex surgery. Overall this would have a major effect on improving the quality of life of an increasingly ageing human population and reduce healthcare costs associated with age-related functional decline.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project is expected to use ~4500 mice (inbred and outbred strains and genetically modified strains) over the duration of the project (5 years).

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

Mice will be used for breeding and colony expansion. No adverse or harmful effects are expected from the strains being bred together and the techniques used for general husbandry. Mice will sometimes be injected with drugs. This will result in minimal and transient discomfort and we do not expect any adverse effects. Some mice will also undergo salivary flow measurements as a measurement of function. This will result in minimal and transient discomfort and we do not expect any adverse side effects. In order to study regeneration following injury some mice will undergo irradiation of the head and neck region, which will mimic radiation treatment that patients have for head and neck cancer. The rest of the body will be lead shielded as protection. Other mice will undergo surgical injury of the salivary gland or cell transplants into the gland. Dry mouth/dry eye may occur following glandular damage and will be alleviated with wet food/eye drops. Mild irritation and/or inflammation of the surgical wound site may occur. Mice will be monitored for ill health and will be humanely killed should the severity reach moderate levels. In order to study the role of nerves in regeneration some mice will undergo surgical or chemical impairment of the nerve supply to the salivary glands. Dry mouth may occur following salivary gland damage and will be alleviated with wet food. As stated above, mild irritation and/or inflammation of any surgical wound site may occur. Mice will be monitored for ill health and will be humanely killed should the severity reach moderate levels. In order to study the influence of age on tissue regeneration some mice will be aged up to 2 years. No adverse effects are expected from ageing alone. However, as age increases the likelihood of diseases or effects associated with old age, such as cancer, arthritis, poor skin condition and weight loss increases. Mice will be closely monitored and humanely killed should the animal develop aged-related health problems. Animals will be humanely killed at the end of the experiment or if a humane endpoint is reached.

## Application of the 3Rs

## Replacement

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

### Replacement

To understand how nerves and stem cells are influenced by age and the genes that are involved in these processes requires the use of genetically modified mice. Since chronological ageing and organ damage both involve the interaction between a multitude of different cell types (nerves, epithelial tissue, blood vessels, inflammatory cells) recapitulating this *in vitro* does not provide a faithful representation of the effects. Thus, alternatives to *in vivo* experiments are not possible.

*Ex vivo* experiments are used in place of some *in vivo* experiments. I have developed a novel *ex vivo* culture assay where tissue explants are irradiated and manipulated in culture, and I have demonstrated how closely such assays reflect the *in vivo* situation. Tissue for *ex vivo* experiments is collected from mice sacrificed using a humane killing method having not previously undergone any regulated procedure. No established *in vitro* cell culture assays exist that faithfully recapitulate epithelial repair/regeneration in primary tissue obtained from mice.

Human tissue (collected from surgical procedures and surplus, to be discarded) replaces some mouse *ex vivo* experiments.

The models used in the project will be reviewed at regular intervals and the possibility of incorporating an alternative will be addressed.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

This project aims to optimise colony management and experimental design to reduce the number of animals required for the project. Power analyses will be used to determine appropriate sample size. We will archive tissues from our animals, in turn reducing the need for multiple experiments for other tissues of interest. The project will also make use of collaborative ageing mouse colonies to share tissue as a resource. The project will use both male and female mice and powerful statistical analysis methods in order to maximise the amount and quality of information obtained from each animal.

## Refinement

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

## Refinement

In order to study nerve-epithelial tissue interactions during ageing and/or injury mammalian models must be used. Mice are the most appropriate species to be used due to their developmental/regenerative similarity to humans and the ability to use genetically modified mice. Lower animals (e.g. zebrafish) are not appropriate for use in such studies as the cellular interactions do not reflect those in humans.

No adverse or harmful effects are expected in the genetically modified mice. The use of tissue-specific (i.e. salivary gland) deletions will avoid whole-body genetic alterations which may have harmful effects (for example, on the brain or heart).

In radiation experiments the rest of the body will be lead-shielded to prevent whole-body effects.

Following surgical procedures mice will be given appropriate painkillers/anti-inflammatory medication and wet food will be provided following irradiation and surgery to alleviate symptoms of dry mouth and salivary dysfunction. Mice will be checked routinely post-operatively and throughout the study for symptoms of pain/discomfort. Mice will be humanely killed if any health-related issues arise that cannot be immediately treated.

All mice will be provided with appropriate housing that allows expression of normal behaviour, including but not limited to, places to hide and climb and nesting material. Mice will be housed together unless circumstances such as aggressive behaviour and fighting do not allow it.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 72. Mechanisms and targets for chronic pain</b>
<b>Key Words</b>	pain, nerves, non-nerve cells, pain killers
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Pain accompanies a variety of clinical conditions and the management of pain remains a difficult task. Chronic pain from nerve damage (neuropathic) can result from a number of factors such as trauma, infections, as well as being associated with various types of treatment such as the side effects of chemotherapy. Currently such pain is poorly treated by opiates and is resistant to alleviation from the use of non-steroidal anti-inflammatory drugs (NSAIDs). Anticonvulsant or antidepressant drugs are more effective in such pain control but the side-effects tend to be unacceptable. This lack of appropriate and effective treatments is due in large part to the incomplete understanding of basic neurology. Further, pain is also associated with inflammatory arthritis and at present inflammatory pain is mainly treated with NSAIDs, but they do have side effects.

Pain is the most debilitating and persistent symptom for people with RA, but it remains difficult to manage.

Modern treatments for RA are aimed at reducing joint inflammation. However, treating the joint swelling in isolation is often not effective in eliminating pain.

Glucocorticosteroids and disease modifying drugs reduce pain in RA, although the analgesic effects of glucocorticosteroids may not be maintained beyond 3 months. Non-steroidal anti-inflammatory drugs are effective in RA pain, but severe adverse effects limit their use especially long term.

Thus persistent pain is a large problem, as the primary aim of most RA treatment regimens is to reduce joint swelling and inflammation, and success in this can sometimes be misinterpreted as optimal management of the condition. This pattern of dissociation between levels of pain and swelling implies that other mechanisms are also making an important contribution to the person's pain

More work needs to be undertaken to better understand the 'mechanisms' of RA pain, so that we can develop new treatments that directly target the pain itself.

Given this shortage of suitable therapies for both neuropathic and inflammatory pain our research aims to identify new mechanisms underlying chronic pain in order to find new targets for analgesic therapies (pain relief). The project licence contains established animal models of chronic pain, a number of which are clinically relevant, namely: neuropathic pain, inflammatory pain and bone cancer pain. The objectives of this licence are as follows:

1. To elucidate new mechanisms and mediators involved in chronic pain.
2. To determine the effectiveness of compounds as analgesic or direct and indirect neuromodulatory agents.

The use of animal models is crucial to our understanding of pain pathophysiology and the development of novel analgesics. The translational animal models which are going to be used in this project provide unique systems responding to drugs used in the clinic.

The severity of the models will be limited as far as possible by limiting the time for which animals are kept following induction of the model. Rodents will be employed in these studies as they are the lowest vertebrate group on which these types of experiment can be conducted and their extensive use in biological research has already provided much information on pain 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 project will identify new mechanisms which are responsible for chronic pain in animals and humans. The identification of key players in such mechanisms will provide new therapeutic targets for the relief of chronic pain in diseases like arthritis.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We have estimated that will use mice and rats not exceeding 25,000/ 5 years and 15,000/5 years, respectively.

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

The use of animal models is crucial to our understanding of pain pathophysiology and the development of novel analgesics. We expect that our animals will walk less and lose some of their explorative behaviour. At the end of the experiments the animals will be killed humanely and tissue may be collected for analysis.

## Application of the 3Rs

### Replacement

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

### **Replacement**

The use of animal models is crucial to our understanding of pain pathophysiology and the development of novel pain killers. The translational animal models which are going to be used in this project provide unique systems responding to drugs used in the clinic. We are principally interested in pain mechanisms. Some studies of sensory phenomena are possible in humans. However, some mechanistic questions require more invasive techniques that are not possible or feasible at present in humans. In vitro techniques are also not sufficiently advanced so they can model the integrated actions of the nervous system. Thus, we will undertake some of our work in animals. Mostly we are interested in animal models of human disease or pathology, and so some of our experiments will make use of such models. Some of these models are short onset and short duration (hours) and can therefore be studied acutely in animals. For example we inject of a chemical agent in the hindpaw and measure the amount of pain-related behaviour induced. The most robust stimulus we will use is the formalin test. In man, this produces a brief but strong period of pain, which subsides over a few minutes. It is followed by a second phase of gentle throbbing pain, with some of the features of a toothache. In animals one sees a brief but intense period of licking the treated paw, which also subsides before giving way to a second period, lasting about 1 hour, of more gentle licking and favouring of the paw.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We routinely seek to reduce the number of animals studied by careful experimental design, the adoption of sensitive outcome measures with small variation and the study of only the most relevant time points. Where possible each animal is used as its own control. Where this is not possible groups of animals will be utilised. In these cases the numbers in each group will be the minimum required to allow valid statistical analysis.

We have several years of experience and most techniques are well established in our laboratory. Therefore, we often can reduce the number of control animals to the very minimum and use historical data. For examples, to reduce numbers of animals may be retested with more than one compounds and we always make sure that retesting won't cause more harm.

### **Refinement**

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

### **Refinement**

Rodents will be employed in these studies as they are the lowest vertebrate group on which these types of experiment can be conducted and their extensive use in biological research has already provided much information on pain processes.

The severity of the models will be limited as far as possible by limiting the time for which animals are kept following induction of the pain model. Also all animals will receive post-operative intensive care to ensure high standards of welfare are maintained. This will include cages remaining on heated mats, administration of saline, provision of soft, easily digestible food.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 73. Bioelectronic Medicines</b>
<b>Key Words</b>	Electrophysiology, Implantable Devices
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The purpose of this project is to understand how implantable devices and electrical signals can be used to regulate the nervous system to treat disease and organ dysfunction.

To do this we must first gain a better understanding of the anatomy and function of the nervous system, and how it exerts control of organ function. Secondly we must ascertain whether electrical regulation of the nervous system can be accomplished safely and 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)?**

Currently medicines treat a wide range of ailments in billions of people. However, there are a multitude of side effects and treatment resistant populations. Although in general successful, current treatments are expensive, socially limiting, and in most cases only a treatment and not cure. The potential for Bioelectronic medicine is broad, as all organs are controlled by the nervous system. Through implantation of devices that regulate the nervous system, and in turn organs, one can potentially reverse organ dysfunction and disease states completely.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Pig (650 over 5 years) and Sheep (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 levels of severity? What will happen to the animals at the end?**

The project has 3 stages. The first two phases will involve anaesthetised animals that are euthanised at the end of the study before they recover from the anaesthetic. Beyond the induction of anaesthesia, these animals will not experience any pain or suffering. In addition, these animals will give us the information we need to more

effectively and safely move to the next step of investigating treatment in animals with disease. There are no expected adverse effects with implantation and treatment as this is a terminal procedure. The final phase will investigate the safety and efficacy of Bioelectronic medicines and therapies in conscious and freely moving animals. Animals will undergo surgical implantation of the devices. The stability/reliability of the device in a conscious animal can then be investigated. This will be achieved using imaging technology e.g. MRI or CT scanning, to see how the resting body responds to the device, then the biological response following activation of the device will be studied. Looking both at the normal resting response and when the body is exposed to a minor inflammatory insult. At the end of the study, all animals will be killed by a schedule 1 method or will be perfused to allow tissue to be analysed.

## Application of the 3Rs

### Replacement

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

#### Replacement

A limited amount of testing has been done without using animals to give confidence nerve stimulation may treat disease. The science cannot be advanced further without using animals. Only a whole body system biology approach will give conclusive evidence and understanding that manipulation of the nervous system can be an effective treatment of disease.

A computer model does not yet exist to test nerve stimulation as a treatment of disease.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Pilot studies in small numbers of animals will be used to develop optimal methods, assess feasibility and outcome measures, and will define go/no go criteria for further studies. Statistical advice will be sought for study design to ensure adequate animal numbers are used. The number of animals used in the studies will not exceed the study size required by statistics to ensure reliable significance and result confidence.

In many cases chronic (recovery) animals will provide their own internal controls (e.g. stim on versus stim off), and multiple repeated doses (of mediators and stimulations) reduces group sizes further.

### Refinement

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

### **Refinement**

Pigs and sheep will be used for all experiments because they are the most appropriate species to determine efficacy and safety with respect to the devices tested. Their neuroanatomy and physiology is very similar to that in humans.

We will work with manufacturers and academic experts to ensure a continued refinement approach is adopted for all implantable devices, electrodes and leads. We will work toward fully implantable devices as advancement to external wires and head caps.

The systemic inflammation model is well-characterised and used experimentally in clinical and non-clinical studies, to determine the efficacy of medical treatments. Many diseases have an inflammatory component to them. Developing a chronic low-level inflammatory model allows investigation of a range of diseases and the potential benefits of Bioelectronic medicines and therapies in these diseases.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 74. Development of tolerogenic vectors for gene expression in skeletal muscles</b>
<b>Key Words</b>	Duchenne muscular dystrophy, gene therapy, immune tolerance, immune reponse
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Duchenne muscular dystrophy (DMD) is the most common and lethal inherited muscle disorder affecting 1 in 3500-5000 males. Affected boys demonstrate first symptoms in their early childhood; develop progressive muscle weakness and wasting leading to a severe disability (in early teens) and death at the age of 20-30. No cure or treatments are available, in part due to the immune rejection of cells corrected by gene therapy. We propose a novel approach combining the existing and well characterised gene delivery methods with factors triggering specific immunological unresponsiveness. Our ultimate objective is to improve the effectiveness of gene therapy to treat Duchenne muscular dystrophy, the most common and lethal inherited muscle disorder. For this we will use the dystrophic mouse as the most widely accepted pre-clinical model.

**What 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 successful completion of this project will lead to an improved treatment for otherwise highly debilitating and ultimately lethal disease and can find application in all other gene therapy approaches where immune responses are found to be a problem. More-over, results of these studies may help us understand some of the mechanisms causing cancer cells escaping the immune system surveillance.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The mouse models of DMD are considered the most appropriate for pre-clinical testing and their use is required for comparison of the efficacy of our method to other approaches. The number of animals needed for the results to be conclusive is 500

over the 5 year period. For breeding of genetically modified strains the total of 5200 mice across five disease models will be used over the 5 year period.

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

Transient discomfort from the handling and the administration procedure. Bruising at the site of injection. All these are very rare and animals will be observed daily. The overall severity level is mild. Animals will be killed at the end of the experiment.

## Application of the 3Rs

### Replacement

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

### Replacement

Animals are essential for this project as dystrophic pathology results from a complex interplay between muscle degeneration and regeneration and immune and inflammatory responses, which contribute to both muscle damage and repair. Therefore, after completing all the possible *in vitro* analyses of the gene targeting vectors, we cannot continue analysing these complex interactions and the tolerogenic efficacy in an *in vitro* system.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will keep the numbers of mice used to the minimum consistent with the aims and, wherever feasible, material from individual animals will be used for multiple analyses e.g. serum for creatine kinase assays, diaphragms for organ bath analyses and leg muscles for other analyses. Much of the work will involve histological sections. These will allow for a number of different analyses to be made in each individual animal (e.g. muscle morphometry and immunological infiltrations analyses).

### Refinement

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

### Refinement

There are several mammalian models of DMD: mdx mice strains, two dog, one pig and one cat models. The characteristics of each phenotype and pathology have been analysed for pathological similarities to the human disease and costs of the maintenance. There is a consensus amongst experts that the most appropriate model to test efficacy for DMD are the mdx mouse and the golden retriever muscular dystrophy (GRMD) dog model. Mouse model has been chosen as it allows comparing the results of our studies to other therapeutic modalities already described using the mouse model.

The tests to be used in this study are based on specific guidelines provided by TREAT-NMD, designed specifically to standardize experimental protocols that are used as efficacy readouts to allow comparisons of parallel efforts. Following an extensive consultation process TREAT-NMD identified a limited number of experimental protocols, which are appropriate for use in preclinical work and accelerate the development of new therapeutic modalities.

Importantly, in this study pain or distress are not a necessary concomitant to the validity of the experimental outcome. Therefore, animals will be observed and scored according to the checklist established in the initial experiment. Following administration, any animals showing signs of unwanted effects other than those resulting from injection itself would be killed by Schedule 1 method and if multiple animals display such symptoms the experiment with the particular drug will be terminated.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 75. Neural bases of action</b>
<b>Key Words</b>	Motor circuits, networks, neurons, movements
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

With our work we expect to gain new insights into how ensembles (networks) of neurons work together to control movements. Understanding the nature of these circuits, the genetics of them, how they are assembled and how they function is fundamental for the understanding of how we produce purposeful movements and why we fail to do so in a number of neurodegenerative 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)?**

We believe that the approach of measuring the activity of neurons within the living animal we will allow us to understand novel aspects of how the brain controls movements and identify neuronal elements (or brain regions) that are crucial for the production of movements. There is an ever-increasing incidence of neurodegenerative disorders that affect motor function to various degrees. These have an enormous impact on the life of millions of patients, with motor defects ranging from dyskinesia to complete loss of voluntary movements. We believe that our findings will be useful to identify and target more precisely those neuronal populations whose impairment leads to these severe motor defects.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use 3000 mice for the tracing and electrophysiological recordings and to breed a total of 17000 transgenic mice over 5 years to maintain the stocks and provide animals for the experimental procedures.

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

The advantage of using mice for our project is that we can selectively mutate genes of interest. To this aim we generate mutant animals by injections, for example, of genetic material in eggs followed by in vitro fertilization. To make sure that the

animals born from these procedures do indeed carry the mutant gene, we take a very tiny piece of tissue from the outer ear and test the expression of the genes of interest. This causes minimal distress or pain to the mice. Animals undergo surgical procedures and for this reason we expect them to show clinical signs of a moderate severity as a result of electrodes, fibers or cannulae implantation. Surgeries last about 1-3 hours, during which we will make a very small window in the skull to gain access to the brain. After which, we implant tiny screws and a probe no more than 5 mm long. We finally seal everything with dental cement. Very rarely the severity of these signs may be such that the humane end points may be reached. Animals are expected to reach moderate level of severity exclusively during surgery and during the period immediately following the surgery, which represents <2% of the time spent by the animal in this protocol. They are expected to recover very quickly from the surgery, typically they are already walking around the recovery cage 15-30 minutes after the surgery. They will be given painkillers and post-operative care just like people recovering in hospital. One day after surgery animals normally show no signs of discernable discomfort for the presence of implanted devices or as result of injected tracers during the recording sessions and/or the behavioural routines. Therefore, apart from the surgery period, animals are expected to reach only mild level of severity for the rest and longest part of this protocol. To study the visual system, we also perform injection in the eye, the capillary we use for the injection is very small, about 2-3 times the size of a hair. Mice recover quickly and normally show no signs of sight loss. Unless otherwise specified, the administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. To assess the behaviour of the animal, mice are kept on a diet so to increase their propensity to perform specific tasks in order to obtain food reward. These diets only take down the weight of the animal of about one tenth of their initial weight. At the end of the experiments mice will be killed using a large dose of anaesthetic followed by cardiac perfusion, which allows preserving the tissue for the successive analysis. At no point during the procedure the animal is conscious or feels any pain.

## Application of the 3Rs

### Replacement

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

### Replacement

The role of neural networks activity in motor performance can only be studied in the intact, freely moving animal. Implantation of chronic indwelling electrodes in humans is only permissible in a very small number of clinical situations and thus is impractical for research purposes. REDACTEDbut the models are extraordinarily simple in

comparison to the complexity of the brain, and cannot substitute for experiments themselves.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We intend to use the minimum number of animals consistent with achieving our experimental aims. The animals are often tested for long periods and thus considerable information is obtained from each animal, minimising the total number used. With an appropriate use of statistical methods and the use of inbred strains we keep the use of the animal at a minimal required level. Whenever possible we make use ex vivo recordings. This will reduce the instances in which we have to perform in vivo acute or chronic recordings which greatly decreases the number of animals used under these protocols.

### **Refinement**

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

#### **Refinement**

Mice are the experimental species of choice because it is possible to generate and acquire genetically modified strains, which allow the visualization and manipulation of selected neuronal populations. In order to trace neuronal circuits and record neuronal activity we implant microelectrodes chronically or acutely. The implantation of electrodes in defined brain regions might seem intrusive at first but the presence of the implants is completely painless. The surgical approaches used are the least severe available, involving the smallest amount of tissue damage. Animals are given extensive post-operative care including antibiotics and analgesics. Animals are closely monitored throughout the experiments and any signs of problems with implants or other aspects of surgery are immediately dealt with, or, if this is not possible, the animal will be killed. Similarly, animals are closely observed and monitored during the recording experiments and during interactions with other animals.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 76. Understanding mechanisms of bacterial pathogenesis for drug development</b>
<b>Key Words</b>	bacteria, infections, antimicrobial, pathogenesis
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Bacterial infections, particularly infections caused by antibiotic resistant bacteria, such as the hospital associated bacteria MRSA and Clostridium difficile are a major clinical challenge. There is a need for better and more effective drugs. The aims of this project are to identify pathways by which bacteria cause infection and to discover new antimicrobial agents which can effectively block infection.

**What 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 a need for novel antimicrobials against highly antibiotic resistant bacterial pathogens. This project will lead to a better understanding of how pathogenic bacteria establish chronic infections. It will identify the proteins that are key to bacterial survival. Additionally it will inform us about how the host produces defensive responses to bacterial pathogens. Along with shedding light on the biology of host-bacterial interactions, data obtained from this work will be valuable for the effective design of new drugs against bacterial infections that are hard to treat.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use ~3000 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 levels of severity? What will happen to the animals at the end?**

As the work will investigate bacterial infections it requires the production of adverse responses in mice. Following infection with bacteria all animals will be checked at frequent intervals for any symptoms of disease. The progress of bacterial infection in

mice is well understood and can be monitored frequently for external signs which include poor coat condition, reduced activity and increased rates of breathing. A combination of these external signs of disease reflects the potential outcome of the infection and can be used to identify, in advance, any animals likely to succumb to the infection. It is possible to monitor weight loss in conjunction with the external signs of disease and this additional measurement will be carried out on a regular basis. Any animals observed to display signs of distress or becoming very sick in the days following infection will be killed immediately using a schedule 1 method to prevent further suffering. Weight of individual animals will be recorded when they begin to display signs of sickness. If any mice show a weight loss exceeding 20% of the original weight they will be immediately killed using a schedule 1 method. While every effort will be made to cull mice in the late stages of disease it may not be possible to completely eliminate death, although we expect any deaths to be rare. At the end of each experiment all animals will be killed using a schedule 1 method.

## Application of the 3Rs

### Replacement

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

#### Replacement

The project seeks to investigate mechanisms of bacterial disease and to identify potential antimicrobial agents. It is necessary to use a mouse model of infection to understand the process of how bacteria establish an infection. Assessment of the onset and progression of disease and effectiveness of potential drugs is only possible in whole animals which have a functional host defence system and that can display the full extent of the pathogenic process. Although we will use a number of laboratory surrogates including 3D human infection models to study pathogens, it is unlikely that such models will completely replace a mouse model of infection, at least during the course of this programme. However we will also continue to review relevant literature and attend scientific meetings of relevance to determine whether there are alternatives to animal experiments in this context.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will conduct a number of experiments in the laboratory using different types of in vitro human 2-D and 3-D cellular models of infections, thus minimising the number of experiments we need to conduct in mice, The minimum number of mice necessary to provide statistically robust data will be used. This will eliminate the need for repetition of experiments, each of which would require the use of control mice.

NC3R's research design tool will be used wherever possible to optimise the experimental design of each experiment as it is being undertaken to ensure that the ARRIVE guidelines are being met in terms of reporting of experiments so that we can publish in journals that support the ARRIVE guidelines

### **Refinement**

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

### **Refinement**

Mice are the most accessible small animal model system for bacterial disease. A considerable body of literature has been generated using mouse infection with bacteria and this will provide substantial dataset with which to compare the results of the project.

All experimental animals including uninfected controls will be monitored as frequently as necessary to avoid infected mice from suffering severe disease. They will be monitored for signs of infection and the severity of the disease scored using a standard protocol. Mice showing the most severe symptoms will be culled using a schedule 1 method. Additionally, mice will be weighed and if the weight falls below 20% of the weight at the beginning of the experiment, they will be euthanized. Non-invasive imaging methods will be used where possible. If possible, specialised instrumentation which enable monitoring freely behaving animals will be used. We will also critically appraise what we do on an ongoing basis to seek out ways to improve our models to reduce harm to animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 77. Neuromodulatory regulation of hippocampal function</b>
<b>Key Words</b>	caffeine, hippocampus, memory, adenosine, plasticity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Over the past 15 years, there has been a dramatic increase in the availability and consumption of highly-caffeinated energy drinks. These beverages are marketed and sold primarily to a younger demographic with teenagers being the highest consumers. Recent trends indicate that a high proportion of adolescents are consuming energy drinks on a regular basis in quantities that exceed recommended daily allowances. It is currently unknown, though, whether habitual consumption of such highly-caffeinated beverages during adolescent development is associated with lasting changes in brain function. Caffeine enhances cognition by improving attention and augmenting memory, and caffeine's primary mechanism of action is thought to involve the block of a receptor protein in the brain known as the A1 receptor (or A1R). Indeed, recent evidence suggests that blocking the activity of the A1R in a brain region known as area CA2 of the hippocampus contributes significantly to the cognitive enhancing effects of caffeine. Interestingly, the A1R changes dramatically in area CA2 during adolescence and becomes much more pronounced with time (e.g., increased expression). As such, the aim of this proposal is to determine whether habitual use of caffeine during this period of A1R maturation in CA2 is sufficient to disrupt hippocampal physiology and cognition later in adulthood. To achieve this, human adolescent energy drink consumption patterns will be modelled experimentally in rats and caffeine-mediated changes in CA2 function will be assessed at the end of the dosing regimen.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Chronic inhibition of A1Rs in area CA2 induced by habitual caffeine use during adolescence may trigger long-lasting changes in hippocampal function. Changes in synaptic function and neuronal physiology in area CA2 following manipulations to

disrupt A1R function during adolescent development have yet to be explored in animal models. Indeed, experimental work described here is designed to mimic recent human caffeine consumption patterns closely. Evidence in support of a critical developmental window during which adolescents are susceptible to lasting neurological dysfunction resulting from habitual caffeine consumption may provide the necessary scientific justification required to amend policy to regulate the sale of highly caffeinated products to vulnerable populations.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats will be used as they are the species with the lowest degree of neurophysiological sensitivity that are able to perform the memory tasks upon which some of the work depends. It is estimated that approximately 473 animals will be used for this work. To ensure that the minimum number of animals are required, where possible, a within-subjects experimental design whereby each animal is its own control will be used. In addition, animals that undergo behavioural testing will also provide the tissue required some of the neurophysiological, morphological and molecular experiments in vitro.

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

For some experiments, it will be necessary to dose animals with compounds (i.e., caffeine) orally so as to mimic human patterns of consumption. Compounds will be consumed orally (by mixing with a palatable food source) and animals will be thoroughly habituated to the procedure before the start of any experiment. Animals will be monitored closely for a period of time following each dosing. If there are signs of distress resulting from the procedure then dosing will stop immediately and an alternative route of administration will be considered. The behavioural tasks utilised for this work will take full advantage of rodents natural propensity to explore novelty. In this regard, the level of severity is expected to be extremely low. All animals, regardless of experimental protocol, will be humanely killed either by terminal anaesthesia or a Schedule 1 method, decapitated and the brains used for experiments conducted in vitro.

## Application of the 3Rs

### Replacement

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

### Replacement

An understanding of the cellular processes that underpin learning and memory can only be achieved by studying the intact brain. Hence, the key experiments cannot be done with cultured neurons or by computer simulations.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

Each of the experiments proposed have been designed to achieve the required objectives whilst minimising the numbers of animals required to attain statistical power. Whenever possible, a within-subjects design will be used. In addition, a power analysis will be used to estimate the appropriate number of animals needed for each experimental condition.

### **Refinement**

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

#### **Refinement**

In order to make comparisons with human memory and with other published work on the substrates of mammalian memory it is essential to also use similar mammalian species for experiments covered by this project license. Rats are ideally suited to allow for a combination of behavioural, electrophysiological and molecular techniques with direct pharmacological manipulations. In using conscious animals, adverse effects of oral drug administration will be monitored closely for any signs of distress. All behavioural models to be used rely on measurements of the animal's spontaneous behaviour and thus avoid using any aversive motivators.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 78. Patient derived tumour models</b>
<b>Key Words</b>	patient derived tumour
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project aims to generate patient derived models of cancer to study tumour development in response to clinically relevant therapies, therefore providing a platform for a personalized approach to drug discovery

There is currently a high efficacy failure rate of novel compounds developed to treat cancer patients. The generation of models that reproduce the disease in humans is a high priority. .

We will generate patient derived tumour xenografts, which will be expanded and stored. The established tumours will be subjected to therapy regimes that will mimic those occurring in the clinic. Throughout these experiments, molecular and histological analysis will be performed using state of the art technology including next generation sequencing. In this way we will be able follow the development of individual tumours that are specific to cancer patients being subjected to therapy. Our data will be used to inform patient treatment.

Animal models are required to fully reproduce the properties of the three dimensional tumour tissues growing within specific organs in cancer patients. These properties cannot be adequately reproduced *in vitro*. Similarly, the effects of drugs need to be tested *in vivo* so that the effects of the tumour microenvironment, drug access and target specificity can be assessed. Mice are the most effective choice of species for these experiments and the availability of immune deficient strains allow for the grafting of human derived tissue with minimal rejection. *In vitro* cell culture and three dimensional tissue models are being developed and compared to the *in vivo* models in an effort to establish animal replacements. Non invasive imaging and molecular profiling are used to follow the development of the tumour and minimise the numbers of animals required at different time points during the course of experimental regimes. These assays not only reflect what is experienced by cancer patients but

provide a high level of information that will lead to a reduction in experimental repeats.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The aim of this programme of work is to develop clinically relevant models of cancer that will impact patient treatment. There is currently a high failure rate of new agents in oncology treatment, therefore the development of relevant models is a high priority. Patient tumour derived models have been shown to reliably predict clinical activity of compounds. REDACTED

**What types and approximate numbers of animals do you expect to use and over what period of time?**

7750 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 levels of severity? What will happen to the animals at the end?**

All procedures are designated as moderate. Most adverse effects will be related to surgical procedures for the implantation of tumour tissue and the growth of tumours superficially and within internal organs. In addition adverse effects may be related to the effect of therapeutic agents and anaesthetics for surgery or procedures such as imaging. All animals receiving procedures will be monitored for adverse effects and treated accordingly, e.g. pain due to surgery will be alleviated with analgesia. At the end of procedures animals will be killed humanely.

## Application of the 3Rs

### Replacement

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

### Replacement

Animal models are required to fully reproduce properties of the three dimensional tumour tissues growing within specific organs in cancer patients. These properties cannot be adequately reproduced *in vitro*. Similarly, the effects of drugs need to be tested *in vivo* so that the effects of the tumour microenvironment, drug access and target specificity can be assessed.

*In vitro* cell culture and three dimensional tissue models are being developed and compared to the *in vivo* models in an effort to establish animal replacements.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Non invasive imaging and molecular profiling are used to follow the development of the tumour and minimise the numbers of animals required at different time points during the course of experimental regimes.

These techniques not only reflect what is experienced by cancer patients but provide a high level of information that will lead to a reduction in experimental repeats.

Mice are the most effective choice of species for these experiments and the availability of mice with reduced immunity allow for the grafting of human derived tissue with minimal rejection, thus reducing the overall numbers of mice required.

### **Refinement**

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

### **Refinement**

Because the mice have a reduced immunity they are susceptible to mouse diseases.

The housing of these mice is thus refined with speciised caging and sterile bedding/diet , this protects them from catching infectious diseases.

The use of analgesia, pain relief , is given to the mice in surgical cases.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 79. Intestinal Immune responses</b>
<b>Key Words</b>	Intestine, infection, colorectal cancer, unconventional lymphocytes, immunity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our bodies are home to trillions of bacteria and most of them reside in our guts. Recent research has suggested a strong link between intestinal bacteria and inflammatory diseases including cardiovascular complaints, obesity, and more directly, inflammatory bowel diseases. Inflammation of the gut is also linked with an increased risk of colorectal cancers. A single layer of epithelial cells lines our gut and is the largest interface and first line of defence between microbes and our bodies. This layer of cells is interspersed with specialized immune cells that aid in its protection. In this project, we will explore the function of these special cells, named intraepithelial lymphocytes (IEL), and investigate how they identify an epithelial cell that has been infected with a disease-causing microbe, or is in the process of becoming cancerous. The aims of this project are (a) to explore the nature of the IEL response, (b) determine how IEL kill infected or stressed epithelial cells, and (c) identify molecules that IEL and epithelial cells use to talk to 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)?**

A clearer understanding of the early intestinal immune reactions that result from pathogenic infections, and the mechanisms used, will help us understand how the immune system mounts an appropriate response to clear the disease. This work will identify key factors that must be induced for efficient vaccine responses against gut pathogens. By aiding effective vaccine design, this could help us fight infectious diarrhoea, one of the leading causes of global morbidity. Moreover, a better understanding of how the local immune system recognises and reacts to the formation of intestinal epithelial cancers, will help us to formulate strategies to

prevent and treat colorectal cancer, one of the leading causes of cancer-related deaths worldwide.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Wild type (WT) and genetically altered (GA) mice will be utilised in these studies. Small pilot studies with groups of 4-6 WT mice will be used to establish each model with regard to infectious agent or carcinogen doses and time point, starting low and increasing dose if the scientific aim has not been met in order to minimise adverse welfare. Eventually, the numbers of mice per group will be optimised for each individual experiment, based on the endpoint and scientific aims. A total of approximately 3000 mice will be used in 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 levels of severity? What will happen to the animals at the end?**

GA mice will be bred on this license, but this is not expected to have any adverse effects. Some mouse lines may develop tumours, and will be monitored closely. If tumour formation begins to cause visible signs of distress, mice will be killed humanely. Mice may also be subjected to whole body irradiation, in order to perform bone marrow transplants. These mice may show signs of ill-health for about a week, but after that are expected to return to apparently normal welfare. Procedures to be carried out on the mice include tube feeding of infectious microbes, injections of immune-modulating or immune-boosting agents, transfer of immune cells by injection, and optical imaging, all of which are not intrinsically expected to cause harm. All of the infection models utilised will be mild to moderate severity, with some diarrhoea and will run for a maximum of 5-7 days, after which the mice will be killed humanely. The mice will be monitored very carefully throughout the duration of the study, as they may show signs of weight loss, ruffled fur, and listlessness, and significant weight loss or deviation from normal behaviour will result in the mouse being immediately and humanely killed. The cancer models will run for up to 12 months, and mice will be monitored regularly for the development of early tumours. As the project will document early immune responses to cancer, mice will be euthanized before development of more serious disease, thus keeping the severity within the moderate category.

## Application of the 3Rs

### Replacement

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

### Replacement

The proposal involves studying the responses of a number of different cell types and microorganisms working in concert to define intestinal immune responses. No tissue culture system is yet available that can integrate all the different cell types and allow measurement of tissue responses. Therefore, *in vivo* studies are the most appropriate here. However, where possible, *in vitro* co-culture systems involving intestinal epithelial cells and lymphocytes will be used.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

Efficient breeding practices will be utilised to reduce and refine animal numbers. The minimum number of mice required to show scientifically and statistically significant data will be used. Mouse numbers for all experiments have been estimated by power calculations based on published data of similar mouse models, as well as our own data on pathogen clearance. Where no pilot data exists, pragmatic numbers will be decided formally based on group experience and statistician advice. These estimates will be refined as the project progresses and more relevant in-house experimental data are available. As experiments may be started over a period of days, a randomised block design will be implemented, whereby animals are randomised to treatment type and order of dosing on any given day. This will reduce experimental variability, and thus animal numbers. Longitudinal studies on the same mouse will be performed using non-invasive imaging studies to follow infection or tumourigenesis in the animal, reducing numbers.

### **Refinement**

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

#### **Refinement**

For these studies, mouse models are most appropriate because their immune systems have similar complexity as the human immune system and it is very well characterized. The immune responses of mice to several oral pathogenic infections and tumour models have already been extensively characterized, allowing me to make predictions based on the information already available. My project requires good databases for proteomics studies, which are available for mice but not many other species. I will also need a number of antibodies against different cell-surface proteins, which are mainly available for mice and humans, but not another species. Furthermore, I will require a number of GA strains to characterize the molecular

determinants involved in intestinal immune responses, many of which are already established in mice.

For each experiment, a specific study plan will be drawn up detailing the rationale for the experiment and detailing the monitoring and specific end points to be used. These will be agreed in conjunction with the named vet (NVS) and will use the minimum severity possible in order to address the scientific hypothesis to be tested. The choice of dose and route of infection will be made to mimic the natural progression of infection, but with the lowest degree of clinical symptoms possible. Pilot studies will be performed wherever possible to ensure that severity limits are not exceeded in GA mice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 80. Mechanisms of blood cell development and leukemogenesis</b>
<b>Key Words</b>	blood cancer, drug treatments, drug resistance, leukaemia microenvironment, mechanisms of initiation and progression
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>No</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

While significant advances have been made to identify the molecular processes that regulate cancer initiation/progression, a great deal remains unknown. This project will gain a deeper knowledge of the mechanisms that mediate the development of stem cells into white blood cells, and to discover mechanisms that regulate the initiation, maintenance and progression of common blood cancers (leukaemias): chronic lymphocytic leukaemia (CLL) and chronic myeloid leukaemia (CML). We will use mouse models to understand the biology of white blood cell development and leukaemia initiation/maintenance, and as pre-clinical models to test established and novel drugs alone or in combination that have the potential to be future therapies. Specifically we will:

- A.** Identify the molecular events that regulate stem cell lineage commitment/maturation, and leukaemia development;
- B.** Assess how leukaemia development is impacted by changes such as cellular aging;
- C.** Use mouse models to test established and novel drugs for their ability to arrest/reverse leukaemia development;
- D.** Define the suitability of promising drugs for clinical trial, developing novel tests to demonstrate drug efficacy.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Greater knowledge of disease biology will aid in the discovery of novel biomarkers to assist clinicians in the stratification of patient groups, identifying those that will respond to specific therapies either alone or in combination in CLL and CML, and potentially other cancer types. This in turn will enable us to identify new drug targets and/or novel drug combinations with existing therapies, which will permit the delivery of rationally-designed clinical trials. Opportunities will be explored to ensure the findings are disseminated to ultimately benefit and better-inform the patient population, by approaching key opinion-leaders/physicians/policy-makers to advance research and clinical developments in this area. This approach will focus targeted therapies towards appropriate patient populations, establishing a personalised care pathway, and deliver quality adjusted life years for patients, through the reduction of disease burden.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice only, a maximum of 26,000 mice 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 levels of severity? What will happen to the animals at the end?**

Mice will be kept in ventilated cages to reduce the risk of infection. To follow the development of stem cells into normal lineages or leukaemia, populations of cells will be injected into mice that have had their own stem cell populations removed by irradiation: this enhances uptake of donor cells. Irradiation can cause toxicity, particularly in the gut, but we have made refinements to the protocol such as giving the mice two shorter doses as opposed to one long dose to reduce the toxicity. In addition, irradiation leaves the mice prone to infections so the mice may be placed on antibiotics for two weeks after irradiation. In our experience 90-95% of mice survive high doses of irradiation. In most cases mice will be injected intravenously, which only generates momentary discomfort (mild severity). In a minority of cases where we inject a small number of cells due to their rarity (<5% of transplanted mice), we will transplant cells directly into the thigh bone (femur) of the mice. The mouse will undergo a general anaesthetic and surgery, injecting the cells into the bone. Surgery typically takes 10 minutes and 95% of mice make a full recovery after experiencing a moderate amount of discomfort. Analgesia will be applied to alleviate the pain. To monitor cell growth/disease development, small volumes of blood will be taken (from a vein) generally once every two weeks causing mild discomfort to the mouse. If the transplanted cells are fluorescent/ luminescent, they can be imaged, enabling us to determine the distribution of the cells in specific organs. Mice will be immobilised with a general anaesthetic during the imaging process (99% recovery). In a minority of cases, cells will be removed from the femur to determine tumour load in the bone marrow, in a similar manner to the intra-femoral injection. A femur intervention will only take place a maximum of twice in the lifetime of the mouse (one in each leg) to protect the bone integrity. When treatment is intended, drugs will be

administered by an appropriate route (orally, by injection into the peritoneal cavity or subcutaneously), at a frequency based on the drug lifetime inside a mouse, for a specified duration. Blood sampling will be carried out to determine the ability of the drug to reduce disease load. At the end of drug treatment, mice will either be monitored to determine if the drug extended survival of the mouse compared to mice receiving control solutions, or the mice will be humanely killed and the relative levels of tumour in the organs will be determined.

## Application of the 3Rs

### Replacement

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

#### Replacement

While laboratory-based studies inform cellular behaviour in a physiological environment (i.e. a mouse), they cannot fully replace them due to the complexity of biological systems, which exists in a mouse. Importantly, substantial work will always be carried out in cell differentiation/microenvironmental models established in our laboratory, which will enable us to rationalise which experiments to carry forward into mice.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

As active researchers we are routinely exposed to new experimental approaches and will incorporate them into our future research program if they prove to be as reliable and robust as the proposed animal experiments. In this way we will endeavour to reduce the number of mice that are proposed for use in this project licence. The protocols described will gain the maximal amount of scientific information from an appropriate number of mice, by applying statistical approaches to our data (power calculations), and the procedures are chosen to be the least invasive, thus minimising the suffering of the mice.

### Refinement

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

#### Refinement

We have introduced a method of recording the health status of animals undergoing procedures. Each mouse is routinely checked and the data recorded using a health scoring chart to ensure robust analysis of the mice across different cohorts. This will not only identify scientific results due to the experiments being carried out, but also objectively enable us to identify the level of harm that specific mice are undergoing and ensure that this is kept to a minimal limit.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 81. Neuroprotection and neurorepair strategies in traumatic brain injury</b>
<b>Key Words</b>	Traumatic brain injury, Neuroprotection, Neurorepair
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to identify new neuroprotective compounds to support the management and potential treatment of traumatic brain injury, and also to support the long term tissue repair in patients. Traumatic brain injury (TBI) is a medical area where there is a strong need for better treatments, to improve the clinical care after the immediate and long term effects of brain injury in patients. TBI has a significant long- term impact on the quality of life of patients and is associated with significant medical costs. There are no treatments available at present and ultimately many patients have to live with very distressing disability. Furthermore, our knowledge of the TBI disease is limited, so studying the mechanisms will enhance our knowledge, and may be translated into a clinical setting to benefit the diagnosis and prognosis of TBI patients.

Cells in the brain are easily damaged irreversibly after acute injury. Most of the irreversible damage takes place in the immediate period after injury, i.e. minutes to hours. Intervention within this period with neuroprotective compounds could significantly minimise the disability and permanent deficits in patients. There is at present no satisfactory neuroprotective treatment after a traumatic injury to the brain. This remains one of the most significant unmet needs in neurology and trauma medicine, and our project addresses this need. The main emphasis of the project will be on therapeutic compounds, to administer after the injury, to protect and also support neurological repair, but some compounds could also be considered for protection against injury before the traumatic event. This would be particularly relevant in populations at high risk of trauma, such as military personnel.

**What 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 generated will be of value to other scientists working on neuroprotection and neurorepair. It will be published and presented at scientific meetings, to improve current knowledge of TBI as a complex disease. It will also be a significant step towards the discovery of new and better treatments. Ultimately, the work in rodents, with the ability to undertake advanced clinically relevant assessments (like imaging, genomics, histopathology) will facilitate a more refined and targeted subsequent assessment of the efficacy of treatments in higher species, before ultimately, the beginning of testing in humans in Phase 1 trials. In regards to biological markers (biomarkers), the information obtained will be of important value to clinicians, since currently there are limited clinical tests for evaluating severity, prognosis and therapeutic efficacy of treatment in TBI patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 years of the project, we expect to use a maximum of 2,200 mice and 2,200 rats, to achieve the aims of our studies on the acute and chronic consequences of injury. For each animal used, we will maximise the information obtained using complementary analyses and tests, so that each animal will be carefully individualised by an injury response profile, in the same manner that there is progress now in the clinic towards understanding of the individual variability in response to neurotrauma.

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

Under a general anaesthesia, a small part of the skull will be removed and a small area of brain will be injured using equipment that produces the minimum damage necessary for the study. Whenever possible experiments will be conducted without the animal recovering from anaesthesia, but some animals must be allowed to recover so that we can study the progression and treatment of the injury. These animals will be given analgesic during the recovery period. All surgery will be conducted using aseptic technique. The animals are not expected to have serious symptoms due to the injury – we aim to keep the injury to a minimum, which means that the effects are limited to changes in memory or movement that can only be detected using special tests. Any animals showing more severe symptoms will be humanely killed as soon as such symptoms are detected. Some animals will undergo an injury carried out without disrupting the skull, and also we will carry out in some cases repeated mild injuries, which are very relevant in the context of sports-related brain injury. The latter can significantly affect adolescents and young adults, hinder their normal brain maturation and increase the risk of neurodegenerative disease later in life.

## Application of the 3Rs

### Replacement

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

#### Replacement

Trauma in the central nervous system triggers a complex cascade of events, both in the affected organ and in other organs around the body. There is no alternative that would entirely replace the use of living animals for studies of the complex response of the injured nervous system. Clinical studies are challenging due to patient heterogeneity and the limited randomised controlled trials that can be carried out. In vitro modelling also fails to mimic the systemic injury response. Therefore, animal studies remain a necessary adjustment to improve our understanding of traumatic injury and to support the development of new treatments. However, our group works closely with the clinicians to fully integrate any clinical data in our studies and implement experimental outcomes like MRI imaging or blood sample analysis that are clinically relevant. We also have developed an ex vivo brain slice culture system that will allow us to undertake a lot of early discovery and compound screening testing, to minimise and replace the initial use of animals. We will also be working with various human and animal cell lines to undertake early testing studies, prior to translating any potential treatments into animal studies. Our REDACTED also has various collaborations with other national and international teams, with the objective to promote sharing of resources, including clinical and animal data /tissues, to maximise the impact and efficiency of our studies and enforce the replacement of early discovery phase animal studies.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

All experiments will be carried out following good laboratory practice and will be designed after careful examination of all the relevant literature, which will inform us as to the appropriate group sizes. All the animal studies will be designed so they are based on solid mechanistic evidence which is already available from in vitro experiments, and they address clinically relevant questions, based on clinical observations, thus having immediate utility for translation. Sample size calculations will be done before each experiment, so that studies are adequately powered statistically. We will maximize the amount of information obtained from every animal, by correlating the effects of treatments on the functional outcome with the effects seen in terms of protection of the brain tissue. This will be achieved by carrying out the tissue analysis at the end of the behavioural tests, thus obtaining tissue information in the same animals where we have the behavioural data. Furthermore,

in some instances we will carry out studies using approaches such as imaging, which will allow us to follow the same animal for a period of time, therefore reducing the number of animals used in chronic assessments.

### **Refinement**

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

### **Refinement**

We will use mice and rats, as these are the major species used in the research carried out so far by specialist groups working on brain injury worldwide. Most literature in neurotrauma research has been produced in models of injury in rats and mice, as they have a nervous system which is similar to the human nervous system. Both rodent species reproduce many of the relevant deficits seen in the traumatic brain injury affecting human patients. Furthermore, mice can be modified genetically. This could help establish which genes could confer increased resistance to injury, and would also help understand why certain compounds are neuroprotective – and why they may have different efficacy in different individuals.

Any animal which shows adverse clinical signs which deviate from the effects expected will be killed humanely and immediately. The neurological abnormalities associated with our models of brain injury will be moderate and will not be allowed to progress beyond the minimum required to achieve the scientific objectives of the project. Our group has developed good expertise on working with TBI models in laboratory rodents, and have improved the clinical and behavioural assessment in these models, particularly using non-harmful behavioural assessments using 24/7 constant monitoring and video recording in the housing cages. This not only minimises any confounding effects on behaviour assessment but also improves our care for the animals throughout the whole day and night. All the animals will receive appropriate analgesia after the procedures and be appropriately nursed with food/drink support and recovery housing (quiet, warm environment and back to their originally herd). REDACTED Critical neurological impairments are not expected in our studies, and are well-described in our humane endpoints to avoid any suffering in our animals. We will also be implementing the use of imaging and biomarker analysis to assess the disease progression in living animals, with minimally invasive approaches.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 82. NEURAL TISSUE ENGINEERING FOR NEUROREGENERATION</b>
<b>Key Words</b>	Neural injury, regeneration,, tissue engineering, transplantation, nanotechnology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We aim to study the usefulness of two new sets of technology tools (a) small particles called nanoparticles and (b) jelly like substances called hydrogels, to increase repair in injuries of the nervous system.

Through the following aims, our overall goal is to identify safe, medically relevant substances that can enhance repair in the injured brain and spinal cord, and to understand how these cells interact with the materials:

(AIM 1): Establish the safety and repair enhancing effects of nanoparticles in the injured nervous system.

(AIM 2): Establish the survival and repair promoting effects of nanoparticle-engineered transplanted cells in the injured nervous system.

(AIM 3): Establish the safety, protective ability and repair promoting effects of hydrogels carrying transplant cells in the injured nervous 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)?**

(i) Our work will provide information on the safety of nanoparticle systems for use in the nervous system and could provide a medically useful way of genetically engineering cells for medical applications; (ii) Our work will provide information on the use of nanoparticles for engineering transplant cells, and can lead to the development of multifunctional nanoparticles as a new class of medical imaging agent; (iii) We will generate new information on the manner in which cells of the nervous system interact with nanoparticles; (iv) We will investigate the use of hydrogel materials as protective cell delivery substances for transplantation into the injured nervous system; (v) We will generate new information on integration and

breakdown of hydrogel materials in the brain and spinal cord, of high value in the development and testing of such biomaterials for neurological applications.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats and Mice, 1650 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 levels of severity? What will happen to the animals at the end?**

The project is classed as being of MODERATE SEVERITY. The experiments will consist of making small areas of injury in the brain or spinal cord of immature or adult rodents. This will be followed by the therapeutic process such as injection of nanoparticles or stem cells in a hydrogel material into the area of injury, to try and improve repair. (1) All surgical procedures cause some discomfort associated with the surgery and the placement of sutures. This will be controlled by administration of pain relieving drugs. (2) The small risk of wound infection will be reduced by using good surgical techniques. (3) Mothers of neonatal animals can sometimes abandon their young that have undergone surgical procedures- this is a common problem. To reduce this several approaches may be adopted including 'smell conditioning' and gentle handling of the pups for a few minutes daily prior to removal of the pups and rolling of pups in used bedding before returning pups to the mother. (4) Introduction of nanoparticles and cells into the circulatory system may block small blood vessels. We will be able to establish the chance of such effects in our trial studies and adjust the experiments to safe levels accordingly. All animals will be humanely euthanised at the end of the experiments and tissue removed for the experimental assays.

## Application of the 3Rs

### Replacement

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

### Replacement

We aim to establish the usefulness of new technology platforms to enhance repair in brain and spinal cord injury. Several factors underpin the success of such methods. These include the intactness of the protective sheath called the 'blood brain barrier' surrounding nervous system cells, and the interactions of blood vessels with nervous system tissue. This complex situation cannot be fully mimicked accurately in a dish. Second, the intact, 3-dimensional structure of the nervous system tissue as well as the relationship between host tissue, introduced substances and cells, and the immune system will be important factors that will affect the success of these experiments. It is impossible to model the full complexity of these processes in a dish and undertaking purely non-animal studies could lead to unwarranted and harmful

speculation on the potential usefulness of these new therapies.

REDACTED

Wherever possible, our new models will be fully exploited to screen new therapies such as cells and materials before proceeding to animal work.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

1. In all cases we will conduct small trial experiments to establish if a particular intervention is safe before proceeding to testing in larger groups.
2. We have used a widely recognised statistical approach to calculate the minimum numbers of animals which will be used per animal that will yield meaningful results.

### **Refinement**

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

#### **Refinement**

Rats and mice are the chosen species for these experiments and we consider these to be the most refined models as:

- (1) These are the lowest mammalian species where nervous system injury trauma models have been established, including in newborn animals
- (2) Numerous nanoparticle, hydrogel and neural cell transplantation studies have been conducted in rats and mice making it easier to design our experiments
- (3) Our own early studies on new technology platforms have been conducted using rat and mouse cells
- (4) By conducting experiments in adult rodents, we can study repair promoting strategies within a biological system that naturally repairs poorly
- (6) Numerous genetic variant models for mice have been established, allowing applications from this work to be extended to transgenic mice in the future
- (7) Several tried and tested models of nervous system injury have been developed in rats, and have been in widespread use for many years.

In terms of reducing welfare related concerns, we have sought extensive advice from veterinary surgeons, a statistician and our animal care and welfare officers in the planning of this project:

- (1) We have chosen the least sentient species feasible for the experiments
- (2) Pre and postoperative pain relief will be administered in all cases
- (3) A series of trial studies will be conducted for all cells and materials to identify and eliminate those with potential harmful effects
- (4) Wherever possible, we will use our dish models for screening new materials, to

identify best candidates to take forward to animal testing.

(5) We have clearly defined end points at which the experiments will be terminated using a humane procedure

(6) Animals will be housed in a well-equipped facility and provided environmental enrichment.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 83. Vascular cytoprotection during inflammation</b>
<b>Key Words</b>	Blood vessels, Inflammation, Healing, Heart disease
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Each day in the UK 435 people die from cardiovascular disease, 530 suffer a heart attack of whom 190 die. Although often considered a 'lifestyle' problem related to poor diet, obesity, lack of exercise, Type 2 diabetes and smoking, many other factors are important in cardiovascular disease. These include genetic causes and our focus, the influence of inflammatory diseases. For example rheumatoid arthritis (RA), lupus and vasculitis (inflammation affecting arteries) damage the endothelium, the layer of cells that lines the inside of our blood vessels. The endothelium is important for maintaining blood flow and preventing the development of blood clots. Inflammatory diseases result in a poorly functioning endothelium, which is one of the earliest features in the development of atherosclerosis, the disease that slowly but steadily blocks arteries and causes heart attacks and strokes. Consequently the risk of heart attack is increased 3-fold in RA and 5-fold in lupus and often affects those 40-50 years of age. We believe that the discovery of new drugs, aimed at improving endothelial function in patients most at risk, may slow down or prevent the development of atherosclerosis.

However, our understanding of the way in which the endothelium protects itself against injury remains relatively poor. REDACTED Our aims over the next five years are to improve our understanding of how these proteins work. We will use predominantly studies in human cells with targeted animal experiments when indicated. The ultimate objective is to identify new targets against which drugs can be developed. The new drugs will be designed to switch on protective genes and switch off deleterious responses in the endothelium. In this way we anticipate they protect against atherosclerosis and the development of heart attacks and stroke.

**What 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 believe that therapies are required that act to reduce the incidence of atherosclerosis-related disease in those patients known to be at high risk. For example those with diabetes or inflammatory diseases. Our focus is endothelial

dysfunction and how we might reverse that. We believe that we have identified pathways that are important for endothelial protection. The current project will seek safe and effective ways to switch the protective pathways on and so enhance endothelial function. Ultimately if drugs can be developed to mimic these responses then patients shown to have endothelial dysfunction might benefit from these therapies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The project will make use of wild-type and genetically modified mice. Based on the numbers used in our projects over the last five years we anticipate using up to 4000-5000 mice which would be a reduction from approximately 5500 over the five years of the current licence.

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

All protocols are graded mild or moderate. The most common potential adverse effects include an unexpected reaction to a short acting general anaesthetic, biological reagent or drug. In these cases mice will be humanely killed. In the event of reaction to general anaesthetic, the animal would be culled without recovery from anaesthetic. Special monitoring is in place for certain strains e.g RIP140 transgenic mice. In consultation with the local vets and the animal care staff, we have introduced a monitoring programme for these mice which has largely eliminated problems, with any sign of respiratory distress leading to culling. For all procedures listed, at the end of the experiment the animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

Increasingly and wherever possible, our laboratory performs experiments using cultured human endothelial cells to replace animal studies. REDACTED This allows more extensive study of human rather than animal cells. We have also generated immortalised cell lines from mice which can be stored and used over many years to minimise animal usage. However, chronic inflammation, blood vessel injury in disease and the development of new blood vessels cannot be modelled fully using isolated cells or blood vessel samples. Whole animal studies are ultimately required to definitively confirm that beneficial actions of new treatment approaches discovered using human endothelial cells in the laboratory, actually occur in live functioning blood vessels. We therefore propose to use established protocols in the mouse

experiments and only when extensive experiments have yielded new discoveries in human cells. In this way we will minimise the number of animals required and any associated suffering. We constantly look for replacement non-animal models in the published scientific literature, we attend relevant scientific conferences to learn about these and we discuss alternative options with our scientific collaborators. In addition we have searched PubMed and EURL-ECVAM databases.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

In this application we have removed 3 of the most invasive protocols from our current licence to reduce the impact on the animals and the number of animals used by up to 25%. We have also received statistical advice to ensure the validity of our data and to restrict the number of animals used. The advice helps considerably in understanding and so planning the minimum and maximum number of animals required to reach statistical significance for each experiment, so minimising animal use.

Further aspects of experimental design include: Introduction of scanning strategies e.g. ultrasound, CT or MRI scans. This approach allows measurement over time in a single animal, in place of a more harmful technique that can be only used once. Scanning techniques will help reduce the numbers of animals required by up to 50%. Careful harvesting and storage of post-mortem tissues allows subsequent use in different experiments/ projects, so optimising data output and reducing the total number of animals required.

We have refined the sub-cutaneous air pouch model. This model involves the injection of air under the skin of the mouse to form a small pouch. Substances that cause inflammation are injected into the pouch and then white blood cells are collected from the pouch a few hours later. In addition, at the end we can now remove the pouch and analyse it to increase the amount of data obtained from each experiment and increase its relevance. We use freezing of embryos to avoid unnecessary breeding of genetically-modified mice.

## **Refinement**

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

### **Refinement**

Mice have proved invaluable for our studies, largely due to the availability of genetically-engineered strains to model human disease. For example mice deficient in the low-density lipoprotein receptor have proved to be a good model for human atherosclerosis with minimal animal suffering. Transgenic mice allow the activity of the signalling molecules we are studying eg. Protein kinase C $\epsilon$  and Erg to be specifically increased or decreased and the effect on the protection of vascular endothelium determined. The alternative to this would be to resort to inhibitory compounds against protein kinase C $\epsilon$  and Erg. However, agents like these are typically less specific and often toxic to the animals. The genetically-modified animals generated to date and effecting protein kinase C $\epsilon$  and Erg have a mild phenotype only.

Our protocols have been reduced in number, removing those with highest impact on the mice. The remaining protocols have been refined so that they are all mild or moderate and typically mild in practice. Examples include the subcutaneous air pouch of inflammation and Matrigel model of angiogenesis (a liquid is injected under the skin and this activates local growth of new blood vessels). We have not seen any significant complications with these models. In all experiments our routine is to maintain animals in a warmed cabinet with drinking water available where appropriate during the experimental procedure. Where indicated analgesia is administered as standard following guidance from the local vet.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 84. Gene Function and Regulation in Sexual Development and Cancer</b>
<b>Key Words</b>	Cancer, Development
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall goal of our studies is to understand the mechanisms that drive the development of organs and how these mechanisms are deregulated in pathological conditions such as cancer. Our focus is on the sexual development of the embryo, a fundamental biological process and on cancers of the prostate, gonad and adrenal. Our specific objectives are:

1. To identify and study the function of specific genes in the formation of the gonad, prostate and adrenal gland and how they can drive tumour development in these organs.
2. To study the interaction of different cell types that are important in organogenesis and in tumour development.
3. To generate genetic preclinical models of cancer to study tumour formation and its response to clinically relevant 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)?**

Insight into the process of organ development will provide information on how programs of development are organised during early embryo development and why they sometimes fail and give rise to malformations or are inappropriately activated and induce tumour development. Our work on organ development has important implications for basic understanding of fetal development and has clinical relevance with respect to the causes of birth malformations in man such as Disorders of Sexual Development. REDACTED our studies on prostate, gonad and adrenal development and cancer will inform the search for novel biomarkers of disease, understanding the

role of genetic changes in tumour progression and the identification of possible targets for drug development as well as providing mouse models for preclinical studies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

10500 mice in 5 years

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

With the exception of the breeding protocol, which is designated mild, all procedures are classified as moderate. Some genetically modified animals will show clinical signs relating to the gonad, prostate or adrenal, including the formation of tumours. Tumours and drugs used to treat the tumours may result in some animals losing body weight, becoming lethargic and developing skin rash and abdominal bruising. The bladder may be blocked. However, in most cases these will be expected to occur and so we will monitor for them and the effects on health. Tumour formation will be monitored to ensure that they do not impair animal health and are within size limits. Other adverse effects are related to surgical procedures and effects will be minimised through the use of analgesia. At the end of procedures animals will be killed humanely.

## Application of the 3Rs

### Replacement

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

### Replacement

Although we strive to develop *in vitro* systems, the study of the complex interactions of tissues that are required for organ development during embryogenesis and during the process of carcinogenesis can only satisfactorily be done using *in vivo* systems. Nevertheless, we are continually developing *in vitro* cell culture and three dimensional tissue assays and comparing them to the *in vivo* models in an effort to establish animal replacements.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

For all our experiments we use the minimum amount of animals possible. We consult colleagues with statistical expertise to ensure that the optimum and minimum number of animals are used to obtain significant data.

Areas where we have actively sought to refine our experiments to reduce the animals even further are:

1. We have set up in vitro organ culture and 3D systems for gonad, adrenal and the prostate. We have used these systems to study the cellular interactions important in organogenesis
2. We are continually developing methods to introduce genetic changes in vitro either in cells, grown in a dish or as 3D self organising structures called organoids, or in tissues that we then grow as grafts to minimize experiments which require complex genetic breeding and therefore reducing animal number.

### **Refinement**

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

### **Refinement**

We use the mouse as a model system because of the available techniques that allow genetic manipulation and the vast background knowledge we have of its embryology, cell biology and pathogenesis

The mice are held in specialised caging which protects their health status.

The use of analgesia, pain relief, is given to the mice in surgical cases.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 85. Development of novel therapies for for Autosomal Dominant Polycystic Kidney Disease</b>
<b>Key Words</b>	Kidney, polycystic, therapeutics, translation, disease
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Polycystic kidney disease (PKD) is a very common cause of kidney failure but currently remains without a cure. Our main purposes are to better understand polycystic kidney disease and to develop novel therapies to treat or cure the disease.

Our current research using cells grown in the laboratory has identified a number of potential drug targets which may benefit patients with polycystic kidney disease. The aim of this project is to confirm these findings in animal models of PKD to see if they are able to slow the growth of kidney cysts.

Mice and rats affected by PKD will be treated with the aim of identifying novel drugs which slow the progression of this disease. Drugs will be administered at regular intervals and animals monitored to determine their effectiveness in slowing the progression of PKD. to the hope is that we will then be able to translate the findings from the animal experiments into 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)?**

Our research and aims has two main purposes, firstly to control disease and find new therapeutic targets and secondly to better understand the progression of polycystic kidney disease. As part of this project we will also be developing new imaging methods to track the progression of PKD in our animals. This will have the benefit of reducing the number of animals required as the same animal can be imaged over a period of time to assess the ability of our drugs to slow the growth of

cysts. Both of these aims will result in better quality of life for a patient suffering with PKD in the future. Some of our results may then extend to other kidney disease such as kidney cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We are planning to only use mice and rats that present with elements of kidney disease that resemble that which effects our patients. We estimate to use a total of 2300 animals over the 5 years 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 levels of severity? What will happen to the animals at the end?**

REDACTED Late stages of PKD lead to kidney failure however, we will closely monitor our animals and not allow them to progress to failure. Mice or rats bred will be used to determine what treatments provide benefit and all animals will be sacrificed at the end for further analyses.

## Application of the 3Rs

### Replacement

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

### Replacement

REDACTEDexperiments in cells cannot mimic the whole body or organ systems and the disease that affects them. Thus, ultimately it is only possible to test our treatments using rodent models of human disease.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Before performing any experiment, we read all relevant published papers to avoid unnecessary repetition. We then design our experiment and talk to our statistician colleagues to get a statistical estimation of minimum numbers of mice required to achieve a meaningful result (statistical significance). We are also in constant contact with animal technicians and have optimised our breeding strategy to avoid unnecessary breeding of animals. The use of modern imaging similar to that available in a clinic will ultimately reduce the numbers of experimental animals needed.

### Refinement

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

### **Refinement**

REDACTED These animals develop polycystic kidney disease with accompanied decline in kidney function. Disease develops rapidly in these genetic models of PKD, and therefore most protocols will be completed before the age of 8-10 months. Animals will not be kept beyond the age of 12 months.

Animal welfare will be supported by housing animals in appropriate social groups and caging will provide sufficient bedding and shelter to encourage species specific behaviour. We will also use appropriate pain relief or anaesthesia to minimise any pain suffered by the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 86. Safety Testing of Medicinal Products Using Small Animal Species</b>
<b>Key Words</b>	Regulatory, Small Animal, Safety Assessment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The expansion of scientific and medical knowledge has led to the development of drugs which can treat or alleviate the symptoms of many illnesses. Whilst progress has been made there is still a need to develop medicinal products to diagnose and treat many human conditions such as Cancer, Ischaemic Heart Disease, Sepsis, Stroke and Alzheimer's disease. 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. This project licence authorises the conduct of studies in laboratory small animal species to evaluate the hazard profile of pharmaceuticals in terms of general toxicity and potential life time exposure.

The primary aims of this project are to support the development of these new 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 humans 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of tissues from surplus stock animals to support drug development and the validation of non-animal alternative methodology.

**What 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 eg metabolites, impurities and drug degradants. The principal benefit of this project is the generation of safety data to allow regulatory decisions regarding human exposure. 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 types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 year life of this Project Licence, it is estimated that 55,000 mice, 82,000 rats, 8,000 hamsters and 2,760 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 levels 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 the intended human exposure. As most therapies are taken 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 oesophagus. Most animals are treated daily in this way, occasionally studies may 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. 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 test material or its metabolites within an animal’s circulatory system. These may also be analysed to detect any effects on 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. 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. Many toxicological effects of the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. 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

### Replacement

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

#### Replacement

At present there are no scientific and legally acceptable evaluations of systemic toxicity which will satisfy regulatory requirements and provide sufficient safety data other than use of animals. Validated *in vitro* tests for specific organs are available and used to replace or refine procedures wherever possible. As new *in vitro* methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace *in vivo* procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

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.

Wherever practicable, and by looking across studies, the combination of endpoints eg general toxicity, reproduction and developmental toxicity, safety pharmacology, mutagenicity etc in studies is considered, to reduce overall animal usage.

As most studies involve the examination of tissues following treatment opportunities for re-use are very limited. Tissues are collected to support drug and in vivo developments from any surplus stock animals.

### **Refinement**

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

### **Refinement**

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 biologically appropriate species, and the species which most closely relates to man. Typically studies are performed on small animal species before testing progresses to larger animals such as dogs, minipigs and primates.

Generally the rat is the rodent species of choice in safety assessment. There is wide knowledge of the response of rats to various substances and a wealth of background literature. Rats are large enough to provide repeated blood samples, thus requiring significantly fewer rats than mice to achieve the same objective. Mice (or hamsters) may be used when considered a more appropriate species, for example, if they more readily absorb the test material, are more relevant biologically or improved tolerance depending upon objective of the study.

Rabbits may be used when considered a more appropriate species, for example non-pregnant range finding studies prior to conducting reproductive toxicology studies in pregnant rabbits; local tolerance or vaccine development studies as the actual intra muscular or subcutaneous human dose volume can be administered

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.

Socially compatible species are routinely group housed with environmental enrichment which encourages species specific behaviours without not adversely impacting study outcomes. Occasionally it may be necessary to single house animals for example to collect urine samples or for the administration of test substances. All such occurrences are conducted in accordance with project licence limitations and under the oversight of the local Animal Welfare and Ethical Review Body.

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, under guidance issued by the site's Animal Welfare and Ethical Review Body (AWERB).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 87. The pathogenesis of Guillain-Barre syndrome</b>
<b>Key Words</b>	Guillain-Barré syndrome, auto-immune, paralysis, mouse-model, therapeutics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Guillain-Barré syndrome (GBS) is a rare but serious disorder caused by an autoimmune attack on the peripheral nerves (those connecting the brain to the limbs and organs). Initially affecting the feet, hands and limbs, the paralysis in its most severe form can reach the diaphragm whereby artificial ventilation and intensive care therapy are required. It is triggered by a preceding infection, such as flu or food poisoning, and in most cases will require treatment in hospital. Normally, the body is programmed to protect itself against attack from its own immune system, however, when this 'self-tolerance' fails autoimmunity occurs and can affect many different organs and nerves. Current evidence indicates that autoimmunity (antibodies) directed against nerve glycans (diverse sugar structures in nerve membranes) is an important pathogenic factor in the induction of this disease. We aim to investigate what glycans the antibodies are targeting, and how these antibodies damage the peripheral nerves, leading to paralysis. No new therapies have been developed in decades and patients receive non-specific treatment, to which not all patients respond.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Developing a refined mouse model of GBS will allow us to identify novel therapeutic targets and test new therapeutics before they proceed to human trial. By first studying the mechanisms of nerve injury in our mouse models, new information on the nature of the key pathogenic pathways will allow us to design, test and report on novel interventions in autoimmune neuropathies in GBS. New treatments will benefit patients, especially those that respond poorly to current non-specific treatment and health professionals who will be required for a shorter period of care. Any

mechanisms of injury identified may benefit the wider scientific community as there can be involvement of similar pathways across a diverse range of nervous system diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project uses transgenic (genetically engineered) mice that have been adapted to help us to determine what components of the nerves are under autoimmune attack, information that will be used in the design of new treatments. Over the course of the 5-year licence we expect to breed 9300 mice, the majority of these mice will not contain the genetic alteration we need for our studies and will therefore not be used in any further study. This is an unfortunate effect of our breeding protocols, as many of our transgenic male mice are infertile. We have estimated that 1300 of the total number of mice will be used in our studies. Mice will be at least 4 weeks and no more than 6 months old when placed on experimental procedure.

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

To induce immune attack it is necessary to inject the mice with antibodies and other modifying agents once a day over several days. Mice will typically be injected into the abdominal cavity, subcutaneously and/or into a tail vein. Serum samples, to monitor the immune response, are collected from a small cut on the surface of the tail; a couple of drops of blood gives us a large enough sample for our tests. Immunisation may cause temporary discomfort or pain; generally, these procedures are tolerated well by the mice who quickly return to normal behaviour. Some of the mice will develop an injury to their nerves that interrupts the messages from the brain to the muscles in the chest and may have difficulty breathing. Animals are expected to reach a severity level of moderate to severe. Accordingly, animals will be monitored very closely and if their symptoms become severe (determined by non-invasive whole body respiratory functional tests) they will be killed to prevent further suffering. In our extended injury model, injection of antibody into a specific site in the mouse hind leg is facilitated by anaesthesia. In a fully conscious animal, it is not possible to appropriately restrain the mouse to perform this specialised injection. The mice undergoing this short anaesthetic (max. 10 min) recover quickly and resume normal behaviour. A further two models studying nerves in the neck or hind-leg require what is termed 'major surgery'; these procedures will be performed on a small number of mice. Under anaesthetic, an incision is made in the skin overlying the neck or of one hind leg to expose the muscle and nerves. The neck muscles will be imaged and defined solutions applied. Estimated time for the entire procedure, from induction of anaesthesia to recovery, is up to 5 hours. The nerves innervating the neck muscle or the sciatic nerve in the leg is 'crushed' by pinching with a pair of forceps. The sciatic nerve crush procedure is short, the wound is closed and the animal regains consciousness within 15 minutes of the start of surgery in the case of

the sciatic nerve. Animals undergoing anaesthesia are expected to make a rapid and unremarkable recovery; it is very unusual for an animal to fail to recover. The mouse will experience temporary paralysis of the affected limb but this does not deter them from eating, drinking or moving around the cage. They will be monitored closely for signs of infection or other adverse effects, although these are rare, and given pain relief, as required, following the vet's recommendation. A small number may nibble at their paralysed foot; to distract them from self-harm their cages will be environmentally enriched. At the end of our experimental procedures, all animals will be killed humanely, most commonly by carbon dioxide overdose. Some animals have an overdose of anaesthetic as a method of killing. A small number of our genetic mice have rare adverse effects, such as failure to thrive; these animals are sacrificed before their symptoms become severe.

## Application of the 3Rs

### Replacement

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

#### Replacement

Modelling a complex diseases like GBS, involving the interaction between the nervous and immune systems cannot be fully replicated without the use of live animals and cannot be fully replaced by an artificial substitute system. However, we have developed an artificial membrane system that will allow us to screen hundreds of patient samples to identify common immune targets. This information will guide animal work. We will continue to seek any new and appropriate alternatives that become available as the project progresses.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Within our experimental design, the principles of The National Centre for the replacement, refinement and reduction of animals in research are followed, particularly with regard to increasing the robustness of our research and minimising unnecessary animal studies. By using transgenically modified mice we are enhancing the model and its likely success. By screening patient samples on array, we can selectively determine the substances we inject into our mice to elicit a far more defined immune response that correlates more closely with the human condition. Based on past experience, we maximise sample collection from individual animals ensuring the maximum amount of experimental data is collected. Our new peri-sciatic injury model allows an internal control in the non-injured leg, removing the need for a control animal, and delivery to this specific site means that injury to

the whole body will not occur thus reducing suffering. To minimise the number of animals used in live animal experiments, we can also explore antibody binding in tissue removed from an animal and kept alive outside the body. This reduces suffering and allows us to take at least 2 nerve-muscle or nerve preparations from 1 sacrificed animal and perform several experiments on the tissue, reducing by 1 the number of animals needed in each experiment. We also prepare cell culture in vitro (cells growing in a petri dish) on which we can carry out many different techniques. However, these techniques cannot be a complete substitute for a whole animal model.

### **Refinement**

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

### **Refinement**

Mice have a well-characterised nervous and immune system and the transgenic models we need exist in that species. They are the lowest vertebrate species in which both of these properties exist and are essential for the success of our work. All animal work will be performed in well-resourced, fully equipped modern facilities. During and following all animal work the mice are carefully monitored for any adverse effects by experienced staff. We give analgesia, soft diet, and any support necessary to keep any harm to the absolute minimum. On the rare occasion when an animal is suffering, it will be killed humanely. Advice from the NVS and NACWO is readily available and will be taken in such circumstances. Our whole system model affecting the breathing of the mouse will be refined to the leg in our new model. Additionally this leg injection site is far less invasive than major surgery and site-specific injury will induce less suffering.

## NON-TECHNICAL SUMMARY (NTS)

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NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 88. Cell Death and Inflammation in Tissue Repair and Cancer</b>
<b>Key Words</b>	Cell Death, Inflammation, Cancer, Anti-cancer immunity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Anti-cancer chemo- and radiotherapy work by activating cellular processes whose purpose is to kill the cell. A key problem is, however, the acquired resistance of cancer cells to bypass the cell death programmes. Cell death acts as part of a quality-control and repair mechanism that eliminates potentially harmful cells, and failure to do so is linked to cancer. However, it is now recognised that killing cancer cells with chemotherapeutics, while important, is not sufficient to provide long-lasting protection from the tumour growing back.

One promising approach to improve cancer therapies is to stimulate the patient's own immune responses against breast tumour cells. This can be achieved by inducing tumour cells to undergo immunogenic cell death, meaning that the patient's dying cancer cells stimulate a specific anti-tumour immune response, which in turn can control, and sometimes eradicate, residual cancer cells.

Our projects are aimed at harnessing the complex relationship between cell death and immunity to elicit robust immunogenic cell death.

Therefore, understanding the mechanisms that regulate immunogenic cell death might allow us to kill cancer cells, and at the same time activate a specific anti-tumour immune response to generate a clinically durable anti- cancer response.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Our research will assist the design of future clinical trials and the development of novel anti-cancer treatment combinations. We will identify new and more effective treatment combinations to treat solid tumours in humans. Moreover, this work will benefit the basic research community by increasing our fundamental knowledge of how our body defends itself from pathogens and cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will require the use of various strains of mice genetically modified for proteins that regulate the body's defence against pathogens and cancer. We anticipate using around 24,300 mice in total for the duration of this 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 levels of severity? What will happen to the animals at the end?**

We will conduct research into how the body defends itself from cancer. Animals are not expected to show an overtly harmful phenotype, other than localised inflammation and tumour development. Tumours will be primarily subcutaneous tumours, tumours of the lung, mammary gland and intestine. In certain cases, animals may also develop distant tumour colonies in lung, liver and bone. Tumour burden will be limited to the minimum required. Occasionally, tumours may ulcerate and very rarely they will compromise locomotion. Depending on the tumour model, cancer cells may migrate to distant organs (metastasis). Metastasis may present as (e.g.) weight loss, palpable internal tumours or lymph nodes or compromised respiration. Animal suffering will be minimised by making every effort to keep the tumour models employed at the subclinical levels. Tumour burden will be assessed also with the help of imaging. Other adverse effects associated to the experimental manipulations described in this project include risk of infection and minor pain or discomfort that will be dealt with using aseptic techniques, antibiotics and analgesics. Toxicity may arise from the use of anticancer agents and radiation. This is not expected to be a regular occurrence as they are delivered at previously determined well-tolerated doses. To minimise any possible adverse effects, we will closely monitor animals undergoing experimental procedures and pay attention to any signs of suffering. Also for genetically altered mice that are not yet the subject of a specific experiment, common problems affecting each genetic background will be monitored. Animals will be humanely killed at the end of each procedure. We have also indicated several guidelines that regulate when animals should be humanely killed before the end of the procedure.

## **Application of the 3Rs**

### **Replacement**

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

### **Replacement**

The ability of tumours to grow depends on interactions with cells of the surrounding tissue. Therefore, it is essential to study tumour biology in animal models *in vivo*, as only limited information can be obtained from culturing cancer cells in incubators. Additionally, metastasis is a process that can only happen within the whole organism *in vivo* and no non-animal alternatives are available for that either. However, I plan to continuously monitor the research in an attempt to replace sections of *in vivo* work with *ex vivo* or *in vitro* alternatives wherever possible.

For example, we will be using heterotypic organoid cultures where we grow mouse-derived tumour organoids in the presence of immune cells. This will allow us to streamline our research and rapidly evaluate tumour-immune reactions *in vitro*.

We are also using the fruit fly as a model system to study certain aspects of tumour defence mechanisms. This will help us to focus our questions and refine our *in vivo* experiments in mice.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

In some experiments animals will only be used to generate primary tissue cell cultures that avoid invasive procedures and uses fewer mice. We will use well-characterised cancer models that minimises the requirement for pilot experiments to define *n* numbers. We use statistical power calculations to help determine the most appropriate number of mice to use to test an experimental hypothesis. The use of highly inbred, genetically altered animals will decrease the natural variation and improve signal to noise. Demand for genetically altered mice will be carefully assessed before breeding and crossing, and mouse numbers with unwanted genotypes will be kept to a minimum by optimising crossing designs. Moreover, we will make use of CRISPR/Cas9-mediated genetic engineering to generate one-step genetically modified cancer models. This will dramatically reduce the number of mice needed.

### **Refinement**

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

### **Refinement**

Cancer is analysed in the context of the host and, therefore, these studies need to be mainly performed in vivo. The reasons why mice are the best choice as cancer experimental models can be summarized as follow: (1) the physiology of cancer in mice is consistent with the human disease; (2) the need for working with genetic modification (knock out, transgenic models). In mice, many models are available, as are well-defined techniques for de novo production; (3) they are economic, easy to handle, produce multiple offspring and they have a very short gestation period as well as a functional survival time. (4) well-defined inbred mouse strains and mouse cancer models minimise variability in the responses between individuals, thus ensuring fewer animals are required. We will continuously refine our model such that we optimally power our experiments and use just the right numbers of animals to generate data that is reliable and robust, yet avoids the need to repeat experiments beyond statistical significance. We will commit to working within the guidelines on tumour growth in animal models, as outlined by the NC3R, and in the guidelines for the welfare and use of animals (British Journal of Cancer. 2010 May 25; 102(11) 1555). All animal work will be performed in close collaboration with skilled animal technicians and trained research staff. Moreover, we are collaborating with leaders in the field so that we can use the respective mouse cancer models with utmost efficiency.

For the adoptive transfer procedure, we will use alternative anesthetizing procedures should they become available.

## NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 89. Regulation of stem cells and tissue morphogenesis by Fibroblast growth factors (FGFs)</b>
<b>Key Words</b>	Adult neurogenesis, Fibroblast growth factor signaling, Hypothalamic tanycytes, Brain
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Human medicine is facing two major challenges relating to brain function: First, its inability to readily replace or repair cells that are lost to neurodegenerative and aging processes; Second, malfunctioning of brain circuits that normally regulate hunger and satiety in response to hormones and signals received from other organs, which ultimately leads to eating disorders such as obesity. Accumulating evidence from rodent studies suggests that many parts of the adult brain harbor dormant or slow dividing stem cells. It is hypothesized that manipulation of these stem cells to generate new brain cells of the desired type, could prove effective in replacing damaged or aged neurons, or to modulate circuits that control appetite and energy expenditure. However, we know very little about the biology of these stem cells. Our over-arching research goals are to: (i) find out how abundant are these stem cells in multiple brain regions; (ii) Identify the set of molecules and factors that maintain and/or regulate their division, with a particular focus on a family of cell growth factors, termed fibroblast growth factor (FGFs) (iii) Test whether the stem cells can actually be genetically manipulated to produce new cells of the desired type, and what is the consequence of this cell production for brain function; and (iv) discover how ageing affects the biology of these stem cells 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)?

In the immediate term, our results will further our understanding of novel population of stem cells in the mammalian brain, and this knowledge will be valuable to a broad range of scientists and clinicians. Identifying the key regulators of brain stem cells and how they function at molecular level will, in the long run, lead to development of compounds and intervention strategies, to experimentally modulate brain stem cell function in vivo. Collectively, these efforts could lead to activation of stem cells to generate new cells and repair brain, and/or to modulate brain circuits that regulate appetite and energy expenditure to counteract the urge for food uptake or increase energy expenditure to promote weight loss.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We anticipate using approximately 4000 mice during the 5 year life of the license. The majority of these will be transgenic mice, carrying one or several types of genetic modifications.

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

We propose to study and characterise novel population of stem cells in the brain, in their in vivo environment, in both young and aged animals. We also aim to understand the function of genes that regulate the biology of such stem cells and what impact would manipulation of these genes have on animal's metabolism and biology. To address our aims and objectives, we propose to use a set of genetically modified mice that by themselves present no phenotypes. However, treatment of these mice with certain chemicals, allows the activation or deletion of genes, specifically in stem cells of interest. Moreover, such chemicals simultaneously activate inert but traceable fluorescent markers that enable visualisation of the transduced stem cells and their progeny. Other substances of interest are chemicals that label dividing cells. These substances are delivered through one of several routes to maximise their effects, depending on the age of the animal or the desired length of the experiment. This includes application via injections into the abdomen, introduction into the stomach through inert tubes (oral gavage), or by inclusion in the drinking water or diet of the animal. Injections and oral gavaging yield transient discomfort. At the doses, routes and length of treatments considered, the chemicals themselves have no toxic effects on the animal. Following gene activation or deletions, animals are culled by humane methods and stem cells of interest and their daughters are visualised in sections prepared from their brain. We anticipate that our gene activation/ deletion studies will impact animal behaviour, in particular their level of appetite and how much energy they spend. To test this impact, another cohort of substance-induced animals will be placed on high fat content diet for a limited amount of time. Under this paradigm, the mice invariably gain weight in a manner and to a level that does not affect their normal behaviour or movement, allowing us to test whether our substance-induced mice eat more and/or show resistance to, or

become more prone to the High fat diet-induced weight gain. We aim to accurately measure the level of accumulated fat in these mice by scanning their bodies in a specialised (non-lethal) X-ray based scanner, whilst the animals are under general, but recoverable, anaesthesia. Great efforts will be made to ensure animals are fully anaesthetised and recover properly from this procedure. We also aim to test whether the effect of gene activation/ deletion impacts energy expenditure from the animal's energy stores (fat tissues etc.) and we propose to evaluate this using sensitive equipment that measure oxygen uptake and heat production by the animal, whilst they are singly housed for up to 5 days. We will allow the animals to acclimatise in the new experimental cages, before the onset of experiments. To monitor changes in blood hormones that are critical to energy expenditure, small blood samples will be collected from tail veins, using methods that cause only transient discomfort. In another set of studies, we propose to test the effect of ageing on the stem cells of interest and their biology. For this, we will compare the biology of stem cells in brains of young (3-6 months old) versus old mice (aged up to 2 years), after they have been humanely culled. Ageing of mice to 2 years may result in age-related diseases such as mild cancers of skin, lung or liver, or arthritis. Mice that manifest the former will be promptly and humanely culled and excluded from further study. Mice that may show arthritis will initially be treated with analgesic to alleviate pain, but all non-responders will also be humanely culled. All animals will be maintained as groups - up to 5 per cage, in individually ventilated cages (IVC) that provide air and temperature-controlled environments in addition to adequate bedding for animal comfort. To determine the genotype of animals, a small tissue biopsy from their ear cartilage, a procedure that produces transient discomfort, will be collected and the extracted DNA will be screened for genetic modification. Animals have ad libitum access to formulated food, as well as water, and are regularly monitored by trained staff. Bar the effects of potential over-anaesthesia, we do not expect any animal to die as a result of proposed procedures. At the end of experiments, all animals are humanely culled. However, animals that are fully recovered at the end of procedures may be kept alive at the establishment (with the agreement of a vet), with a view to their re-use on procedures if appropriate and licensed. Some animals may be re-homed if it is in the best interests of the animal and they have been through the establishment's re-homing scheme. Naturally, any animal - whether experimental or control – that shows any deviation from normal behaviour (e.g. lacking exploratory movement, reflexes etc.) or show signs of discomfort (e.g. arching, withdrawal etc.), will be promptly and humanely culled.

## Application of the 3Rs

### Replacement

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

## Replacement

In the proposed work, we aim to study brain stem cell biology and the impact that genetic manipulation of these stem cells may have on brain function and organs outside the brain. Study of other stem cell populations (outside the brain) show that stem cell biology is heavily influenced by the environment in which the stem cells reside and grow. Stem cells receive many instructive signals, some of which remain undefined and poorly characterized in the brain. In vitro or ex vivo models cannot wholly recapitulate this environment and so there is a need for work on stem cells in their natural environment, in live animals. Similarly, critical cross talk between multiple organs (e.g. between brain and the adipose tissue or pancreas during regulation of metabolism) cannot be modeled in culture.

Through literature searches and consultation of data bases as well as expert colleagues, we have sought other alternative methods to the proposed in vivo work in mice. A limited number of short term and specific questions can be answered using non-protected species, such as fruit flies (*Drosophila*) or worms (*C. elegans*), but the range of transgenic strains that are needed to manipulate gene function for the proposed work is not available in these species. Other alternatives include work with less sentient species such as zebrafish or using emerging technologies in which small organs, such as the brain, can be grown and analysed in vitro. However, marked differences between the biology of zebrafish and humans precludes their use, and mini-organs are still at the exploratory phase and, at present, not suitable for our proposed studies. Nonetheless, we will continue to monitor this technology and should it evolve to stage where it can be used for our work, we will incorporate it into our work plans.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

We will take the following steps to ensure animal usage is minimised:

Where possible, we will generate and maintain mice carrying multiple non-interacting transgenes (compound transgenic) to save on maintaining single transgenic mice. Moreover, where possible, we will maintain our transgenic mice as homozygotes to enable efficient transmission of an allele to their offspring, upon breeding.

We will review our mouse colonies regularly and promptly discard any spare mice that would be generated as a result of our selective breeding programme (e.g. wild types or mice of undesired genetic status), so that animals are not kept for longer than necessary.

We will carry out proper statistical analysis to ensure that only the required number of animals are generated and used for each experiment.

We will endeavour to share tissue from culled mice with other users and colleagues to minimize their animal usage.

We have already developed an ex vivo organotypic brain slice cultures model. We are currently optimizing this model so that it can be used to follow stem cells and their descendants over several hours/ days. This would eventually remove the need for sacrificing experimental mouse cohorts at close time-points to reach the same goal.

### **Refinement**

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

### **Refinement**

Mice are the correct species for this work because of their strong resemblance at genetic, anatomical and physiological level to humans. Therefore any finding is likely to have direct relevance to human biology and medicine. Moreover, for the types of genetic and experimental manipulations proposed, there is already a plethora of transgenic strains available, which would otherwise take many years to develop and model in other species.

For experimental manipulations, we will use optimized and well-established protocols that result in least discomfort to animals. For example, we have already taken the initiative and established that for long term administration of some chemicals, delivery of substances via drinking removes the need for uncomfortable daily intraperitoneal injections. We will continue to re-evaluate our existing protocols and consult other users to seek less harmful experimental approaches and protocols.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 90. Investigating the role of inhibitory interneurons to understand and halt neurodegenerative diseases</b>
<b>Key Words</b>	Alzheimer's disease, interneurons, hippocampus, GABA, pharmacology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;

(b) translational or applied research with one of the following aims:

Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Alzheimer's disease is a common form of dementia and an urgent global challenge affecting over 800,000 people in the UK alone. These staggering numbers are expected to continue to rise with our increasing aging society, leading to a future health crisis. There is no effective medication to treat dementia-related disorders and this clearly needs urgent directed attention.

The common signs of Alzheimer's disease are an accumulation of proteins called amyloid- $\beta$  ( $A\beta$ ), and clusters of microtubule-associated protein tau in the brain, as well as a loss of brain cells leading to brain dysfunction and cell destruction (known as neurodegeneration).

Although much research has focused on either molecular, genetics or biochemistry studies of Alzheimer's disease, which has helped us identify valuable markers of the disease process, ***much less is known*** about the changes in the 'wiring' of the brain in this condition. Addressing this knowledge gap will deepen our understanding of early brain activity dysfunction underlying Alzheimer's disease, allowing us to

investigate ways to modify and intervene to correct the intended function of brain cells.

In this project we will initially focus on investigating the underlying **causes** of Alzheimer's disease, **bridging the gap** in our understanding of pathological changes in **brain circuit** behaviour that leads to progressive neuronal death. We will then aim to manipulate specific brain cells to correct the imbalance with pharmacological agents to halt neurodegeneration.

**What 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 outcome of this novel, unexplored basic research project is underpinning mechanistic information of the brain that and the possible causes of Alzheimer's disease at a brain network level that will lay the foundation for wide applicability to future therapeutic settings aimed at improving human health. The important question is whether deciphering the changes in synapses involving specialised inhibitory interneurons bring us close to finding a cure for Alzheimer's disease? If the desire is to develop targeted therapies that will prevent or halt the disease from developing, then unequivocally, yes.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Experiments will be performed using control wild-type rats and mice, and genetically modified mice using the first amyloid protein precursor (App) knock-in mouse models, as well as other mouse variants. The Alzheimer's disease is expressed in these mouse models when the genes from both parents are expressed. We expect to use a total of 1200 animals over a 5 year period and this number is based on previous success rate in breeding.

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

Using an interdisciplinary approach, we aim to breed genetically modified mice that harbour 2-3 familial forms of human genes for Alzheimer's disease. These mice models not show sign of harmful clinical phenotypes except a decline in memory performance as they age, this will not impact on their well-being. We will perform cranial surgery under anaesthesia, however animals may experience pain after the surgery for which pain relief will be given to alleviate any suffering. Very rarely animals haemorrhage or seizure during these procedures. Some protocols involving behavioural memory related tasks will involve positive reinforcements to motivate animals to find food treats located in a maze. This is to investigate whether certain drug treatments improve or halt neuro-degeneration and memory loss associated with Alzheimer's disease. However, behavioural protocols may involve some

transient weight loss for short periods of time, if this occurs then food and water intake will be closely monitor. The final end point of the animals will involve surgery to obtain brain slices procedures, which is will be performed under general/terminal anaesthetic to ensure no pain is felt.

## Application of the 3Rs

### Replacement

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

#### Replacement

We aim to use human derived cell line, and also build artificial computational networks, but the results obtained from this approach would still require verification in naturally developing brain tissue from a mammalian species. No computer model is currently available that can replace the use of animal tissue for this objective, as there is insufficient information on the network connectivity and circuit activity involved. Nevertheless, computer models will be used to assist the interpretation of the data obtained in experiments from animal tissue.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will ensure that the minimum number of animals will be used by maximising the information obtained from each animal. For this, experimental design will be optimised to obtain answers to the questions addressed, and statistical power analysis will be employed ahead of commencement of experiments. However, physiological experiments are special in that the number of animals will largely depend on the success rate of recording.

### Refinement

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

#### Refinement

Mice are sufficiently close to humans to reveal principles of synaptic communication and are species that are much used in behavioural and cellular studies of the

synaptic circuitry and the inhibitory system, which enables us to build upon a large body of research already carried out, and to relate our findings to previous results.

Our primary model is stimulation and recording from a slice preparation in vitro. This is the most refined model that can be used for the study of synaptic communication of relevant architecture. We will employ state-of-the-art stimulation and recording techniques to maximise the information yield from each experiment.

## NON-TECHNICAL SUMMARY (NTS)

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NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 91. Practical Training Course in Microvascular Surgery</b>
<b>Key Words</b>	Microvascular, Surgery, Technique, Course
<b>Expected duration of the project</b>	5 year(s) 0 months

## Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>No</b>	(a) basic research;

(b) translational or applied research with one of the following aims:

No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
Yes	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This course has the sole aim and objective of providing suitably skilled surgeons with highest quality instruction and technical training to allow them to undertake (or support) microvascular surgery on patients in their clinical practice.

Microsurgery refers to surgery on vessels or structures (such as nerves) which is not possible with the naked eye, requiring and as such uses magnification to allow the surgeon to achieve a successful outcome.

**What 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 training surgeons will acquire microvascular skills that can then be transferred to standard surgery, improving outcomes and shortening training and operative time

**What types and approximate numbers of animals do you expect to use and over what period of time?**

65 rats will be used. They provide a standard size of vessel in a standard surgical exercise allowing reproducible, reliable training. As the course has been running for almost 25 years the techniques of anaesthesia are very reliable and as we restrict surgery to the femoral and epigastric region and adverse events are kept to a minimum.

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

The procedure is always conducted under terminal anaesthesia (animal does not recover from the anaesthetic). End-to-end and end-to-side anastomosis (suturing of blood vessels to allow normal blood flow) will be performed on the femoral artery and vein and on the epigastric artery and vein. Not all anastomoses will be technically successful, however, as all animals are terminally anaesthetized, failure of an anastomosis will not cause animal welfare concerns. Occasionally, due to operative error, haemorrhage will occur; under such circumstances if it is considered that blood loss is excessive the animal will be immediately euthanased. Under no circumstances do the rats ever experience pain or distress. The rats are killed humanely after completion of the training exercise.

## Application of the 3Rs

### Replacement

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

### Replacement

As the success of microvascular anastomosis is so important to the surgical patient there is a need for an animal model with flowing blood and natural technical complexities (akin to the human situation) to ensure the surgeons can practice in a situation that the success of the surgery can be fully assessed.

There is no adequate non-living substitute to living blood vessels for learning the critical aspects of microvascular surgery.

To ensure maximal capability of candidates at the commencement of the microvascular course, they must have previously developed and demonstrated exceptional surgical skills in keeping with higher surgical training in a relevant surgical specialty (Oral & maxillofacial surgery, Plastic surgery, Neurosurgery, and Otolaryngology/H&N surgery). Further they will demonstrate to the course faculty, capability to handle synthetic substitutes for animal tissue and the necessary

technical proficiency with microvascular instrumentation to ensure learning on the animal for microsurgical model is maximised. This evidence will be sought on the first morning of the course.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

The numbers of animals used is the minimum required for acquiring the desired surgical skills. Prior to commencement of procedures on animals, a model preparation is used.

A single animal is used per candidate per day ensuring complete utilisation of the resource prior to termination of the animal.

The procedures are carried out under non-recovery anaesthesia so that no animal will experience adverse effects.

### **Refinement**

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

#### **Refinement**

As detailed previously, these vessels provide an excellent correlate for the human microvasculature used in free tissue transfer (eg, human radial artery etc) and it is for this primary reason that the rat femoral system is the system/animal of choice. The rat femoral and epigastric vessels are the standard animal model used widely in the UK and beyond for this purpose

Minimising of animal stress is ensured by the technical expertise of the BSU staff, by way of constant/consistent monitoring of animals throughout the anaesthetic (induction to termination). Candidates and faculty are reminded of their responsibilities to the animal and its welfare and means to assess alteration in depth of anaesthesia.

The course is reviewed following provision of formal candidate and faculty feedback at the completion of each annual cycle, to ensure animal usage is optimised for the purpose of the licence. As already stated, there is also an annual AWERB review of the course in keeping with best practice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Function and Regulation of the Immune system in health and disease</b>
<b>Key Words</b>	T lymphocytes, signalling, autoimmunity, cancer
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We aim to understand how cells of the immune system and in particular a subset of cells called T cells are regulated. Specifically, our work is focussed on understanding how signalling molecules within the cells interact with each other in order to regulate the behaviour of the cells. These signalling pathways are involved in the development of the cells, their maintenance under normal steady state and in their function during an immune response. Currently we do not understand how these signalling pathways interact with each other to determine how a cell behaves and improving this understanding is fundamental to our proposal and the main objective we hope to achieve in the shorter term. Our long-term goal is to be able to manipulate these signalling molecules and, on the one hand, to improve immune responses to pathogens and cancer cells, and on the other hand, to down-modulate inappropriate immune responses, such as in the cases of autoimmunity.

For example, information obtained on genetic mutations that occur in people with autoimmune conditions, has pinpointed genes which, when mutated, increase the likelihood of contracting autoimmunity. Knowing that a gene can increase susceptibility to disease does not explain how that gene functions. Understanding how is important knowledge required to try and develop therapies that will be effective in the clinic.

**What 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 be able to understand important biochemical pathways that regulate immune responses. Such understanding will advance the field in the short term and ultimately may lead to the development of drugs that target these pathways in a very specific manner and so avoid many of the side effects seen with current therapies. In

addition we may be able to manipulate these pathways, for example, to improve immune responses against tumours.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Our studies use mice as a model organism and we expect to use approximately 30,000 mice over the course of 5 years. These animals have specific gene modifications and the majority (~80%) will be used in the breeding program and to supply postmortem tissues for analysis. Approx. 20% of the animals may be used to investigate the immune response to immunisation, infection, autoimmunity and cancer, processes that can only be interrogated fully in the intact animal.

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

The majority of animals are used in the breeding program or as donors of organs that can be used for the study of immune cell behaviour in the lab and have a "subthreshold" severity rating, which means they do not show any adverse effects as a consequence of their genetically manipulated status and are maintained under best practice conditions of husbandry. A proportion of animals will be subject to procedures that lead them to experience only very mild, if any, forms of discomfort. An example would be administration of a protein or compound which may give transient discomfort if injected, but has no detectable adverse effects on the animal's health. However we are trying to understand why the immune system fails and how to avoid this, and in order to do this some animals will develop signs of disease, for example swelling of joints in a model of arthritis, decrease in body weight in a model of colitis and evidence of tumour growth in studies of cancer. Such animals will be closely monitored and euthanased if they exhibit symptoms that are compromising to their wellbeing as determined in consultation with qualified veterinarians. At the end of all procedures under this license animals will be euthanased.

## Application of the 3Rs

### Replacement

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

### Replacement

Our studies rely on looking at the immune response to infection or other conditions such as autoimmunity or cancer in the context of the whole body. The immune response is a highly complex process involving multiple different cell types and molecules that work together. The function of these cells or molecules depends on where they are in the body, and often they move around the body. We cannot

replicate these processes outside the body. Whenever possible, we use cell culture systems to address specific questions.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

Our experiments are carefully designed such that the results will be clearly interpretable using the smallest number of mice. We consult statisticians whenever necessary to ensure this is the case. We also continuously look for ways to reduce the numbers of animals used and have adopted several practices that allow us to do so.

### **Refinement**

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

#### **Refinement**

We use mouse as our model organism as manipulation of genes of interest is well established in the mouse. The mouse immune system has been extensively studied and, in addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms; its immune system bears extensive similarities to the human, thus making the findings more relevant in terms of usefulness. Animals are closely monitored for any ill effects, typically by visual assessment and weighing. Weight loss can predict ill effects before they are seen visually. When performing experimental manipulations we will use the least invasive and distressing procedures available, and use the least number of manipulations, to reduce pain, suffering, distress, and lasting harm to animals. We will also use measures such as administration of analgesia where appropriate to minimise suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 92. Safety Testing of Medicinal Products Using Dogs and Minipigs</b>
<b>Key Words</b>	Regulatory, Safety Assessment, Dogs, Minipigs
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project licence authorises the conduct of studies in laboratory dogs and minipigs to evaluate the safety, quality and effectiveness of medicinal products for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening conditions in man, in terms of general toxicity and whole body system exposure.

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, Alzheimer'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 humans.
- 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of blood/tissues to support drug development and the validation of non-animal alternative methodology.

As a specially protected species, the dog is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species with relevance to man, and that there is no other acceptable candidate species of lower neurophysiological sensitivity/status.

**What 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 provision of robust safety data to facilitate sound decisions by national and international Regulatory Agencies regarding human exposure to medicinal products. Without these studies, progression of new medicines to early human studies and to patients could not occur safely or in the current regulatory framework. Validation and refinement of test methods may be completed for specific techniques and may be published to the wider scientific community.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 year life of this Project Licence, it is estimated that 4,800 dogs and 2,300 minipigs will be used. These numbers include a small proportion of re-use of the same animals.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels 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 are 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 slings 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 test material or its metabolites within an animal's circulatory system. These may also be analysed to detect any effects on 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. 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 the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. 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. Animals that do not need to be euthanased at the end of a study and have experienced no more than moderate effects and have met the requirements for keeping alive may be reused or rehomed under AWERB oversight, in line with ASPA/HO requirements. In the case of rehoming, animals will undergo a programme of training and socialisation to enable transition to a domestic environment. Potential adoptees will also be assessed for their suitability to provide for the animal's needs.

## Application of the 3Rs

### Replacement

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

### Replacement

There are currently no scientific and legally acceptable evaluations of systemic toxicity that will satisfy regulatory requirements and provide sufficient safety data other than use of animals, though validated *in vitro* tests for specific organs are used wherever possible. As new *in vitro* methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace *in vivo* procedures. Where available, review of scientific articles,

non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.

As a specially protected species, the dog is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species with relevance to man, and that there is no other acceptable non-specially protected candidate species.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

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.

In general, toxicity studies are initiated in rodents before progressing into larger animals. This approach, combined with background literature searches and looking across at other study types, can lead to earlier decisions on whether or not to continue development of a particular test material, refinement of study designs and reduced use of dogs and minipigs.

In recent years, the general availability and use of minipigs has also increased, with an associated reduction in the proportional use of dogs.

### **Refinement**

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

#### **Refinement**

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 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, whilst 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.

Socially compatible species are routinely group housed with environmental enrichment which encourages species specific behaviours without adversely impacting study outcomes.

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).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 93. Adoptive cell transfer for cancer immunotherapy</b>
<b>Key Words</b>	Adoptive cell therapy, Chimeric Antigen Receptors, Cancer, Immunotherapy, Immunology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall goal of this project is to develop novel, more effective treatments for cancer by harnessing the tumour-killing potential of patient's own immune system.

Certain types of immune cells are known to be capable of specifically recognising and killing tumour cells. Our team is performing research into the interplay between immune system and cancer trying to increase the potential of immune cells to recognise and kill tumour. We are using mouse models of cancer that better mirror human malignancies.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The main benefit of this study is to address an unmet medical need, in this case cancer, ultimately saving lives. Data generated in the course of this work will be used to develop the next generation of immunotherapeutic treatments for a broad range of cancer types. We will also investigate and refine the mechanisms underlying current immunotherapies and produce a package of pre-clinical data to support evaluation of the novel immunotherapeutics in cancer patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We plan to use approximately 12,500 mice and 600 rats 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 levels of severity? What will happen to the animals at the end?**

Overall, we expect to observe a moderate level of severity in mice or rats used in this study. The procedures proposed in this project to generate mouse cancer models have been previously established and refined to reduce adverse effects of tumour growth on animals to a minimum. Depending on the tumour type and site of transplantation, growing tumour may restrict locomotion of the animal or can metastasize affecting internal organs and eventually leading to animal's death. Thus, it is imperative to observe the mice for signs of moderate severity such as weight loss, distension of abdomen, body temperature changes and/or skin condition. Other procedures in this project such as administration of anti-cancer drugs or adoptive cell transfer may lead to weight loss, piloerection and/or hunched posture. Rigorous monitoring of adverse effects will be maintained, and any animal approaching the predetermined humane endpoints will be humanely killed via an approved Schedule 1 method, as will all animals that complete their assigned studies with no adverse effects.

## Application of the 3Rs

### Replacement

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

### Replacement

We will employ in vitro alternatives whenever possible. However, there are no composite in vitro techniques that sufficiently mimic the complexity of an in vivo system. Therefore, animal models are essential to understand the intricate interplay between tumour cells, the tumour microenvironment and the immune system.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will use a variety of techniques to reduce the number of animals and will keep our work in adherence to NC3R's ARRIVE guidelines. The sizes of experimental groups and the number of repeated experiments will be kept to a minimum while ensuring that reproducible results are obtained with clear biological significance. State of the art technology will be used to perform multi-parametric analysis in every tissue or cell sample. This will maximise the amount of data obtained from a single experiment and therefore reduce the amount of mice required.

### Refinement

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

### **Refinement**

We intend to use mice and rats in the proposed study. The reason for using mice is that they are the lowest vertebrate with the best characterised disease models for cancer efficacy studies and are the most commonly used species for antibody generation. Our work will also require rats as they respond to a broader spectrum of antigens than mice, thus providing an alternative source of antibodies.

The techniques used in this programme of work will be those that are accepted by drug development authorities and which follow the guidelines on animal welfare. The studies will be designed to use the simplest and least invasive methods possible to obtain the data required for completion. Duration of tumour growth will be kept to a minimum and use of non-invasive imaging techniques will be employed to monitor tumour progression and adverse effects.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 94. Development, function and regulation of the immune system</b>
<b>Key Words</b>	Lymphocytes, immunology, cancer, vaccines, signals
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The immune system must be precisely regulated so that it will fight off infections and tumour cells effectively, while not damaging the normal organism. T lymphocytes (“T cells”) are cells found throughout the body and which have key roles both in attacking “foreign” material but also regulating the activity of the immune system as a whole. Our research objective is to systematically map and identify the key signalling pathways and molecules that control lymphocyte function. One focus is how lipid and protein kinase pathways integrate information from antigens, cytokines and nutrients to control metabolism, inflammatory cytokine production and T cell migration/trafficking and hence determine cell fate choices in lymphocytes.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

When T cells fail to function correctly the immune system fails, often resulting in death. The present project will characterise molecules that act as messengers inside T cells to regulate their function. The control of T cells is critical for immune responses. Understanding the signalling pathways that control T cell activation is essential to identify targets relevant for the treatment of autoimmune and inflammatory diseases. The laboratory has an integrated research program to explore signalling pathways inside lymphocytes. These studies will generate new

information about mechanisms that control immune responses and identify new targets for therapeutic intervention in the immune system that can be used for vaccination, to fight bacteria and to treat cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This application is to support a group REDACTED scientists for a period of 5 years and proposes to use up to 20,000 mice including wild type and genetically modified mice.

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

Based on previous experience, more than 95% of the mice to be used in this programme will exhibit only mild outward signs or none at all. The great majority will be killed humanely for the collection of tissues and cells to be studied in detail in the laboratory. Some animals (up to 5%) will be challenged with microorganisms or tumour cells. Infections are likely to cause deviation from normal welfare in some of these mice, but all will be killed humanely at as early a scientific endpoint as possible. Similarly, tumour studies will be conducted (in up to 5% of animals) in a way that will ensure that only minimal changes in welfare are caused. Some animals (2-3%) will be irradiated, to permit the introduction of immune cells derived from other mice (akin to bone marrow transplants as carried out in humans). We will use a standard procedure for this and mice will receive welfare support to ensure that the effects of the irradiation are minimised and resolve completely.

## Application of the 3Rs

### Replacement

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

### Replacement

Lymphocytes isolated from peripheral blood from normal human donors can be used to study some aspects of lymphocyte behaviour but immune responses are very complicated and require that lymphocytes traffic between the blood lymphoid organs and peripheral tissue. To identify important modifiers of immune responses, it is necessary to carry out immune function tests in live animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We work in partnership with skilled animal experimentalists and use well validated mouse models. We ensure through constant discussions and consultation with statisticians that the number of mice we use for experiments is appropriate. We constantly seek to reduce our animal usage through improvement of in vitro models. The minimum number of mice required to show scientifically and statistically significant data are used.

### **Refinement**

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

### **Refinement**

This project will use mice because mice are an appropriate, well established model for studies of the mammalian immune system and are a good model for the human immune system. We will adopt early scientific endpoints for all experimental interventions.

## NON-TECHNICAL SUMMARY (NTS)

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NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 95. Leukocyte recruitment and retention in acute and chronic inflammation</b>
<b>Key Words</b>	Galectin, Atherosclerosis, Leukocytes, Inflammation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Atherosclerosis is a chronic inflammatory disease of the major arteries, characterised by progressive accumulation of immune cells and fatty deposits within developing plaques. The endothelial cells which line the arteries become leaky to blood-borne lipids, and develop an adhesive and attractant surface for white blood cells, principally monocytes. The monocytes migrate into the wall and ingest modified lipids, eventually becoming lipid-engorged 'foam cells' which drive further trafficking. Failure to resolve this inflammatory infiltrate leads eventually to plaque rupture, with formation of blood clots that causes heart attacks and strokes. Whilst lipid-lowering statin treatment has helped reduce disease incidence, atherosclerosis remains the primary cause of mortality worldwide, highlighting an unmet need for new treatments.

I have been studying a family of proteins called the galectins, which are made by endothelial cells and white blood cells, and have actions that influence inflammation. My research demonstrated that galectin-1 and galectin-9 regulated recruitment of white blood cells and clearance of inflammatory infiltrates in acute inflammation. However, there are currently no studies of these galectins in atherosclerosis or their actions on monocyte trafficking and foam cell formation. This application will redress this by investigating: how these galectins influence monocyte recruitment in laboratory models mimicking human blood vessels; their location in

arteries of humans with atherosclerosis and in mice developing a similar disease; how their manipulation influences development of murine atherosclerosis. These studies will pave the way for in-depth analysis of how galectins influence arterial disease and justify studies aimed at developing new 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)?**

I propose to look at a class of proteins which have been shown to modulate inflammatory responses in general but have been completely overlooked in the context of cardiovascular disease (CVD). Understanding the effects of Gal-1 and Gal-9 on monocyte recruitment and behaviour will provide important pre-clinical information for the use of novel galectin-based therapeutics in the treatment of CVD. Furthermore, increasing our understanding of galectin biology in CVD will also open the opportunity to explore galectin-glycoprotein interactions by state-of-the art glycomic approaches, which could provide further avenues for therapeutic intervention.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

All our studies will be performed with wild-type and genetically altered mice. We expect to use no more than 11,000 mice over a period of 5 years

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

The majority of the animals in this study will be used in breeding programmes to generate genetically altered animals to perform critical tests of how inflammation is regulated in vivo. We anticipate that ~80% of procedures will be at a mild level of severity with the rest being at a moderate level of severity. The anticipated adverse effects will be transient pain and brief discomfort following injection of substances into the bloodstream or into the peritoneum or airpouch. Any animals that display discomfort or abnormal sickness behaviours will be killed by a humane method. Animals will be humanely killed as soon as possible after we have obtained all the data outputs needed to complete the study.

## Application of the 3Rs

### Replacement

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

### Replacement

In line with the principles of the 3Rs we will aim to minimise the use mice in all my experiments through inventive in vitro studies but equally as important we need to demonstrate that the concepts we plan to test translate to animals before we proceed to human studies. Although no animal model can truly represent a complex disease process that occurs in humans the ApoE<sup>-/-</sup> diet-induced model of atherosclerosis represents a well validated model which shares clinical similarities to human atheroma. Furthermore, the pathology is reliant on monocytes for the development and persistence of the inflammatory response and as such represents the best murine model of atherosclerosis for our studies.

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>

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

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Where pilot data exist we will always perform statistical power calculations to ensure that we use the minimum number of animals needed to obtain statistically significant results. In designing our experiments we aim to ensure randomisation of animals to different treatment groups and we will try to ensure that people performing analyses are blinded to animal treatment groups. We will consult trained statisticians before using any new protocols and all staff performing animal experiments will attend appropriate training on key aspects of experimental design

We will aim to use the NC3R's experimental design tool to aid experimental design and we will also look to publish in journals that support the ARRIVE guidelines for reporting

## **Refinement**

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

## Refinement

The mouse is the most appropriate species to conduct these studies as it is the lowest vertebrate species to give an inflammatory response similar to that seen in humans. The type of inflammation we generate in mice shares many features in common with acute and chronic inflammation in man. We see a similar time course of white blood cell recruitment in mouse models of acute inflammation to that seen in man and in mouse models of chronic inflammation the same set of inflammatory mediators drive the disease process to those seen and targeted in human disease.

With our proposed programme of work we will aim to systematically 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 subsequent experiment

In our experiments we will use scoring schemes with clearly defined action points and humane end points to reduce animal suffering. Clinical scoring schemes for our animal models of inflammation have been developed to allow maximum collection of scientific data outputs whilst minimising animal suffering.

In addition before conducting each experiment, it is discussed with the NACWO and NVS to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to acquire the scientific endpoints. We will also 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 the next experiment

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 96. Role of Wt1 in development and tissue homeostasis</b>
<b>Key Words</b>	Wt1, development, adipose stem cell
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Tissue development and maintenance are tightly regulated by a variety of genes under a tight regulation. Our group has identified one key player in these processes which are significant in both health and disease (Wt1, Wilms Tumour protein gene). Wt1 is known to be involved in several important diseases including cancer and kidney diseases as well as crucial developmental steps including the formation of heart, kidneys, spleen, and diaphragm etc. However, it is not well understood how Wt1 carries out these functions. The project that I am proposing aims to better understanding of the role of Wt1 (and its interacting pathways and genes) in tissue development and homeostasis in general, with a focus on adipose (fat) tissue and tissue of the diaphragm between the chest and abdominal cavity.

**What 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 identify the role of the gene Wt1 and its interacting partners in regulating tissue development and how the body functions. We will find out how these genes work, how their expression is controlled and what happens when that regulation goes wrong, and which will improve our understanding of how and why diseases develop. We will share our knowledge with scientists at meetings and in journals and with patient groups and the general media.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice during this study. We expect that we will use a maximum of 8,700 mice over five years. Most of the mice will be used for breeding to produce animals with the appropriate combinations of genetic makeup (these animals will not undergo any experimental procedures). Up to 1,500 mice may be given an injection of substances that alter the way their genetic alteration behaves or may have their diet altered also to look at the impact of an alteration to how the gene functions.

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

To study the function of genes (with a focus on *Wt1*), we will breed mouse lines with small changes in these genes. For example, some mice will be genetically modified so that we can alter production of the gene of interest (e.g. by giving or withholding drugs from the mice), or by attaching fluorescent markers to genes of interest as a way to track cells/tissues that produce the gene. In tissues where the gene(s) of interest has a function, detectable effects ('phenotypes') are expected when the gene is disrupted (e.g. we might see kidney or obesity problems). The upper limit of severity in this project is moderate. To study the potential stem cell property/function(s) of the cells that express *Wt1* (or altered expression of *Wt1*), we will transplant these cells to mice to test if they form e.g. functional adipose tissue. Post-surgical infections may occur and primary tumours may arise in treated animals. The experimental animals (with the correct combinations of genetic modifications) will be used to study their phenotypes (while the phenotypes develop) or culled humanely using regulated procedures for tissues for histological or molecular analysis.

## Application of the 3Rs

### Replacement

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

### Replacement

The regulation of embryonic development and adult tissue homeostasis is complex and cannot be determined in isolated cells alone. Multiple tissues, cell types, as well as change-over-time information are required. Animals are needed to address these questions in a physiological and pathological context.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will aim to use statistical power calculations (based on previous studies and published research from others and ourselves) to determine the minimum number of mice needed to produce meaningful results in our experiments. We routinely cryogenically preserve sperm/eggs from the mouse lines to reduce numbers of mice needed to maintain these lines. My lab will also make cell lines from mice with the appropriate genotype. We can then use these cell lines to prepare material for e.g.

drug testing before taking them to animals, thus helping to reduce total mouse numbers needed.

### **Refinement**

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

### **Refinement**

We use mice because they are good models of human disease; for example the genes associated with obesity that we wish to study (WT1) are expressed in both humans and mice and appropriate mouse models are available. To minimise harm to the animals we only use models that provide informative, reproducible results without causing other unnecessary illness to the animal. Throughout our studies we strive to minimise suffering. For surgical procedures, appropriate anaesthesia, analgesia, postoperative care and aseptic techniques will be used. Mice will be checked routinely post-operatively and throughout the studies for symptoms of pain/discomfort. Mice will be humanely killed if any health-related issues arise that cannot be immediately treated.

We also strive to refine our protocols, so if a newer version is published by another laboratory, which clearly demonstrates refinement, then we will adopt this protocol.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 97. The role of inflammation in vascular disease</b>
<b>Key Words</b>	Inflammation, cardiovascular disease, atherosclerosis, therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

#### **Clinical Need :**

**Why we are studying cardiovascular disease:** In common with much of the developed world, and increasingly in common with the developing world, the majority of deaths in the UK are due to cardiovascular disease (CVD). In the main, these are heart attacks and strokes which are the acute clinical manifestations of the chronic inflammatory disease atherosclerosis<sup>1,2</sup>. Although in recent years risk factors for formation of atherosclerosis have been identified, and benefit for patients can be achieved through life style modification and pharmacological control of these (for example by lowering levels of circulating cholesterol using statins), there is no cure for CVD. Indeed, direct clinical intervention is still based on invasive surgery, for example to re-establish blood flow in blocked arteries using vein grafts or inserting stents. This comes at great cost. In 2009, in the UK, the direct healthcare cost of all CVD was £8.7 billion and the total economic cost (including healthcare cost, informal care and loss of productivity) was estimated at £18.9 billion. The average cost of a hospital admission for a CVD event is estimated to be ≈ £5,000. Thus, the ‘holy grail’ of CVD research is to develop new treatments which can cure the disease. This would benefit patients, the NHS and the wider economy.

**Our solution :** We wish to apply our knowledge of the molecular and cellular pathways that cause CVD to identify new ways of intervening in the disease process, and ultimately to allow the development of new anti-inflammatory drugs that will prevent the development of atherosclerosis, thereby dramatically reducing the risk of heart attack and stroke.

The overall purpose of our project is **to understand whether it is possible to target the processes of inflammation in order to treat CVD**. Many of our studies begin with identification of new inflammatory pathways in patients with CVD. These are then tested in the laboratory using cultured cells to reproduce the disease process. When we have a good understanding of the biology of these pathways, we move to mouse models of inflammation which allow us to verify that these inflammatory networks operate in the body. Finally we move to disease models of CVD to show they are relevant to pathology and that we can intervene in the initiation of and progression of disease. We also regularly review the scientific literature to ensure that we are using the most refined animal models and so that we can respond to new developments in model design, particularly where newly emerging *in vitro* techniques could replace animal 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)?**

This work will significantly enrich the knowledge base in our field of expertise as it is directly intended for testing novel molecular interactions with the potential to translate to clinical treatments using novel compounds or new targets for existing drugs. Our mechanistic knowledge will be important for the scientific, medical and pharmaceutical communities. We also hope to identify new treatments that we can use in patients with CVD. This is important for patients, because at the moment there is no 'cure' for CVD. Our pioneering studies have already identified new inflammatory pathways from which we are trying to develop new anti-inflammatory drugs. Our data is thus likely to be used by basic scientists and clinical scientists to inform the design and outputs of their own experiments. As required by our funding partners, data originating from these studies will be published in high impact scientific journals confirming with the ARRIVE guidelines provided by NC3Rs, and presented at national and international symposia and conferences. Thus benefits from our work include transfer of knowledge, training opportunities for future clinicians and academic scientists as well as improvements in treatment for UK patients and the healthcare industry.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice for our experiments and expect to use up to 6000 over the five year term 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 levels of severity? What will happen to the animals at the end?**

Our major model is a mouse that gets atherosclerosis when fed a high fat 'western diet'. Although these animals develop atherosclerosis they do not suffer from heart

attacks and strokes, and this form of disease modelling is thus relatively benign in terms of animal welfare during the progression of disease. Animals are ultimately culled at the end of the experiment so that we can measure the levels of disease prevalent in the cardiovascular system. The model requires prolonged feeding of a high fat diet (up to 40 weeks, but routinely 6-14 weeks). The animals relish the diet provided, but it does render them obese and they develop greasy coats. Some animals experience administration of agents or cells prior to, or during the feeding protocol. In some instances, for example delivery by osmotic minipump, this requires surgery under anaesthesia to implant the device. Some animals may also be subject to periodic instillation of pollution particles into the lung to model a known risk factor for CVD in man. This is a rapid and highly reproducible protocol which can be achieved without undue distress (other than scruffing and handling). The cumulative levels of severity on this license fall into the moderate category.

## Application of the 3Rs

### Replacement

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

#### Replacement

The complex disease pathways we are interested in involve the interaction of several cell types and regulatory signals derived from dietary and other environmental sources and these are hard to recreate *in vitro*. We also do not have access to samples from humans in all stages of disease indeed, cardiovascular disease does not display clinical signs until it is extremely advanced. This means that studies on the processes supporting disease establishment and development cannot be studied in humans, as these stages of disease development are complete by the time patients arrive in clinic. As mice share the main components of their Cardiovascular and immune systems with humans, the early stages of cardiovascular disease can be modelled in appropriate strains of mice susceptible to disease. A wide range of genetically manipulated strains and therapeutic reagents are available for mice and allow intervention in the disease process, thus they are the best model for us.

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.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

We have prior experience using the models proposed here, which will inform the design of experiments in this study. This experience allows us to be informed by inter-individual variation in responses and take this into account when we are designing and powering these new investigations. Similarly targets and approaches are always informed by evidence from our human in vitro studies and ex vivo studies in patients. All our experiments run serially with outcomes from initial animal groups informing the design of subsequent experiments. The scientific team includes scientists with proven experience working with murine models who meet regularly to discuss data, and we seek advice from local statisticians and colleagues. We have built in checks in our workflow to ensure that experiments do not progress if statistically significant results are not evident upon an intervention. Importantly our experimental design strategy is informed by use of the NC3R's experimental design assistant (EDA : <http://www.nc3rs.org.uk/experimental-design-assistant-eda>) and in conjunction with adherence to the ARRIVE guidelines, to ensure the minimal numbers of animals are utilised in order to gain valid experimental outputs.

Importantly although we are primarily interested in the cardiovascular system, many of the molecular pathways we investigate operate in more than one organ. This means that therapeutic targets may have broader applicability to other chronic inflammatory diseases. Similarly it is clear that cardiovascular disease has broad systemic complications, and indeed that injury to other organs such as the lungs or immune activation at remote sites can feed back to the cardiovascular and haemostatic system more widely. Thus we are proposing to study information in a variety of sites and to maximise the useful information we collect from each animal, we will collect blood, and solid organs. These samples can later be used to investigate the wider significance of our pathway or therapeutic intervention. Importantly we publish extensively in high impact journals and work closely with collaborators at other institutes to maximise exchange of information. We also share samples with our colleagues so that maximal use is gained of each individual animal.

## Refinement

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

## Refinement

We have chosen our models based upon the experience of ourselves and scientific peers to best recreate the inflammatory and systemic picture seen in humans AND meet the strict welfare conditions we adhere to in the UK. Indeed many of our models are dietary driven or simple and quick to perform, and all have been refined by our past experiments. Our endpoints are generated either using blood and tissue sampling post schedule 1 procedure or generated under non recovery anaesthesia to ensure handling and experience for individual animals is minimised.

In addition before conducting each experiment, it is discussed with the named veterinary surgeon to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to acquire the scientific endpoints. We will also 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 the next experiment.

With experience we have been able to refine our use of these models. For example, for some readouts in models of atherosclerosis it has been possible to reduce the duration of disease modelling from 14 weeks to 6 weeks, with concomitant benefits in animal welfare. Additionally, in animals rendered thrombocytopenic, we recognised a low level of mortality associated with excessive internal bleeding due to aggressive interactions between animals in the same cage. These animals are now housed separately for their own welfare, and to avoid escalation in experimental animal numbers.

In addition before conducting each experiment, it is discussed with the NACWO and NVS to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to acquire the scientific endpoints. We will also 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 the next experiment.

## NON-TECHNICAL SUMMARY (NTS)

NOTE:

The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 98. Mitochondrial diseases: pathogenesis and therapy</b>
<b>Key Words</b>	Mitochondrial disease, experimental therapy, gene therapy, neural stem cells, hypoxia
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Mitochondrial disorders are genetic diseases affecting the mitochondria - cellular organelles that provide most of the energy required by our cells. As a result the brain, the heart and the skeletal muscle, which are the organs with the highest energy requirements, are the most affected, although any organ may be involved. Mitochondrial disorders are thus highly complex and can range from relatively mild diseases (such as muscle weakness) to devastating early onset brain diseases. This project is aimed at (a) investigating the mechanisms causing mitochondrial diseases and (b) developing new therapies to treat these 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)?**

No therapy is currently available for these conditions, although the preclinical work carried in the last 5 years by us and other groups opened new hopes for the future. However, in spite of the major advances in our understanding of mitochondrial biology over the last few decades, the role and function of several genes and proteins that are commonly mutated in human mitochondrial disorders remain unclear, as do the mechanisms by which these mutations cause disease. This project will be aimed at filling these gaps in our knowledge, in order to develop new therapies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project involves the generation of mouse models bearing abnormal genes that are mutated in a number of severe human mitochondrial disorders; it is anticipated that approximately 20,000 mice will be used. However, the progresses already made

in our lab towards the application of new treatments in patients may drastically reduce this number. Animals are needed in our research because mitochondrial diseases affect multiple organs, and are often extremely severe disorders; therefore, their complexity cannot be reproduced by cellular models. In addition, to validate potential therapies that can eventually be translated to humans, it is essential to analyse their feasibility and safety in animal models. Anyway, cell cultures will be used to complement animal work, for instance for the initial testing of new potential therapeutic compounds. Experimental protocols for animal work will ensure that the minimum possible number of animals is used, and that they endure the least possible suffering. The procedures applied under this licence include administration of pharmacologically active chemical compounds to correct the bioenergetics defects, adeno-associated viral vectors (AAVs) to deliver therapeutic gene (e.g. re-introducing the missing/mutated gene), and stem cell transplantation to repair damaged tissues.

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

It is expected that at least 70% of animals will experience minimal stress/discomfort related to the handling and administration of chemicals or viruses. 30% will experience moderately stressful situations, in part related to the clinical signs they can develop (such as muscle weakness or minor movement disorders) and in part due to invasive procedures required for the experiments, including minor surgery requiring anaesthesia. The procedures the animals will undergo under this licence will be minimally invasive and include (i) metabolic manipulation by administration of special diets, including ketogenic and high fat diets, (ii) administration of chemical compounds and AAVs, and (iii) non-invasive behavioural tests. The only procedures requiring surgery are the implantation of stem cells into the brain, and the sampling of the cerebrospinal fluid, which surrounds the brain. Pain relief will be provided under the supervision of the named veterinary surgeon. At the end of each experiment, the animals will be sacrificed by approved schedule 1 methods (such as dislocation of the neck or administration of CO<sub>2</sub>). A limited number of animals (less than 5%) will be killed by introducing a tissue preservative directly into the heart whilst the animal is under terminal anaesthesia.

## Application of the 3Rs

### Replacement

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

### Replacement

1. Primary cell cultures generated from human patients often do not reproduce the defects present at the level of the tissues.
2. Mitochondrial disorders are rare diseases that affect mainly organs and tissues. This complexity cannot be reproduced by any cellular system.
3. Fibroblasts from patients become quickly old and the bioenergetic and metabolic properties changes between passages.
4. To validate potential therapies that can be eventually translated to humans, it is essential to analyse their efficacy and safety in living animals.

However, cellular models will be used whenever possible to replace and complement the experiments in living animals.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

The number of animals needed in each experiment will be calculated by appropriate statistical methods, eventually using pilot studies when no preliminary data are available.

The number of breeding pairs will be kept to a minimum by appropriate matings, keeping the clinical features into account.

Since mitochondrial diseases are often multi-systemic, and in order to maximise the information from a single animal, we will collect tissue samples from almost every organ and keep them stored for future analysis. These samples will eventually be provided to other scientists, that could help us in characterizing specific aspects without need to breed mice specifically for their experiments.

Cryopreservation will be used to preserve important lines and remove the necessity to hold stock for extended periods.

The personnel using animal models will be carefully trained and monitored to ensure good practice in animal work. We strictly adhere to the moderate endpoints specified in the PPL..

### **Refinement**

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

#### **Refinement**

The mouse is the animal of choice in studies on genetic diseases because of its genetic and biochemical similarity with humans. The protocols for the production and phenotypic characterization of recombinant mice are well established, so that little optimisation is required.

The phenotype of our recombinant mouse models is normally milder than that of the corresponding human disease, and animals do not develop clinical signs that would exceed a mild severity limit. Sometimes however the expected severity will be moderate, in order to mimic the clinical severity of the corresponding human disorder. Humans suffering from mitochondrial dysfunction due to mutations in genes that are ablated in our mouse lines present with extremely severe, in fact consistently fatal conditions, in most of the cases characterized by progressive brain, and/or muscle, and/or heart failure. There is therefore a need to develop mouse models which recapitulate these signs in order to (a) investigate the pathogenic mechanisms in target organs and whole organism, (b) evaluate the response of the whole organism to the genetic defect, (c) search for biomarkers to monitor disease progression, and (d) establish quantitative endpoints and outcome measures that can be exploited in experimental treatment. In any case, humane endpoints will be adopted to prevent unnecessary suffering; for instance, body weights and clinical conditions will be carefully monitored. Most of the procedures will be non-invasive, causing only temporary discomfort. When surgery is required, supervision by the NVS or other properly trained personnel will be required, until competence in the procedure has been acquired. Analgesia will be provided as required after surgery. Only fully recovered operated animals will be subjected to behavioural tests that can imply fatigue or exhaustion (e.g. treadmill test) and the acquisition of scientific information cannot be obtained by alternative tests (e.g. spontaneous activity cage).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 99. Nutritional means to improve the health and performance of cattle</b>
<b>Key Words</b>	Cattle, Rumen, Nutrition, Health, Sheep
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>Yes</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The objectives of this project are to advance knowledge and understanding of nutritional factors affecting digestion in cattle and sheep to improve their performance, metabolism, health and product quality, and to reduce their environmental impact.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The project will advance the knowledge and understanding of the nutrition of dairy cattle, beef cattle and sheep. More accurate feeding will help prevent disease or ill-health in cattle and sheep, and improve the production and quality of milk and meat. A greater knowledge of the digestion and metabolism in cattle and sheep will assist in improving the welfare of animals by for example, better supplementation of cows grazing grass, or reducing health problems around calving and lambing. This project will deliver greater knowledge on the quality, efficacy and safety of alternative forages and by product feeds (e.g. from bioethanol production) that are likely to become available as a consequence of climate change or energy production from crops. This project will also contribute towards protecting the environment through developing strategies to reduce the environmental impact of cattle and sheep through, for example, reducing methane production and excretion of nitrogen and minerals.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Cattle and sheep are the main species to be used, and the project will use approximately 400 dairy cows, 200 growing dairy or beef animals and up to 8 sheep.

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

Most of the studies that will be conducted will be under conditions similar to that found on well managed commercial farms and commensurate with the code of practice, and will use forages or supplements that are available to cattle and sheep farmers. To understand the effects of alternative forages and feeds, or dietary strategies on animal performance requires performance to be monitored. Milk yield will be recorded and samples taken for subsequent analysis in the same manner as on commercial dairy farms. Similarly, live weight and condition of cattle and sheep will be measured in the same manner as on many livestock farms. To understand the underlying mechanisms of different forages and feeds requires samples of blood to be taken to determine metabolic status, samples from the rumen (the first stomach in a cow and sheep) to determine microbial metabolism, the liver to determine aspects of metabolism, or faeces and urine to determine digestibility. Most of the procedures employed in this project are widely used in commercial practice by veterinary surgeons to monitor health in dairy, beef and sheep, except for the insertion of a permanent rumen fistula which is required to obtain rumen fluid samples from cattle and sheep to allow the microbial population to be monitored accurately, or to provide the starter culture for in vitro studies. To determine the effects of dietary treatments on aspects of fertility requires the reproductive cycle to be managed. With any of these sampling techniques there is a risk of infection following the procedure, or pain or distress during the procedure. These will be minimised by the used of appropriate pain killers and by using trained personnel. The advice of a veterinary surgeon will be sought whenever necessary. At the end of the procedure the animals will be inspected by a veterinary surgeon and either returned to a farm or killed by a humane method (Schedule 1).

## Application of the 3Rs

### Replacement

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

### Replacement

The purpose of this project is to improve the performance, health and welfare of cattle and to reduce their environmental impact. Because of the complex interactions between the diet and the cow, using cattle is the only scientifically viable option. This project does employ non-animal based alternatives (e.g. to measure the potential effects of different diets on methane output), and therefore reduces the use of animals. However, these techniques are limited, and to determine whether they have a real effect in cattle requires them to be fed to animals and animal performance, health and metabolism monitored.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Before a study commences the number of animals required is determined in consultation with a statistician. The number to be used is based on the literature and from similar studies in the subject area. Study design techniques such as change-over designs, where each animal receives each diet, or factorial designs are also employed to reduce the numbers and ensure that a significant difference can be detected.

## **Refinement**

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

### **Refinement**

The use of cattle is necessary as there are no non-animal based substitutes that accurately replicate the combined effects of diet on intake, digestion, rumen metabolism, liver metabolism, milk production and growth. Additionally, as some of the dietary strategies may alter behaviour, the use of cattle is required. The animals will be kept, whenever possible, under conditions similar to that encountered on well-managed commercial farms and commensurate with the Code of Practice. For example, some studies will require the cows to graze at pasture, and others to be housed in stalls or on straw over the winter. Where sampling is required, animals may need to be housed individually but will have sight and contact with others at all times. The use of appropriate pain killers will be used when required. If animals need to be restrained for example to measure urinary nitrogen output, they will have sight of other animals at all times and will be restrained for the minimum period to ensure that an accurate sample is obtained. The animals will be cared for at all times by trained and competent staff. All studies will be approved prior to commencing by a local animal ethics and welfare body. This body is made up of people with animal welfare, statistical design and animal health experience. The advice of a trained and experienced veterinary surgeon will be sought at all times during the planning, operation and at the end of the study.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 100. Ischaemia Reperfusion Injury and Organ Transplantation</b>
<b>Key Words</b>	Transplantation, Ischaemia-reperfusion Injury, Rejection, Treatment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Organ transplantation is the life-saving treatment for many diseases. Two related mechanisms of injury during organ transplantation include the damage caused by the lack of oxygen during transplantation and the immune response to the transplanted organ. The aim of this project is to study these mechanisms of injury and investigate the efficacy of targeted therapies to reduce injury and improve organ function after transplantation.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Approximately 1000 patients die or are removed from that transplant waiting list every year, although this is thought to represent only a fraction of the true health burden. The main contributor to these 'avoidable' deaths are shortage of organs suitable for transplantation, compounded by premature failure of organs after transplantation. The data generated by this study is essential for conducting human clinical trials on novel therapeutic agents in transplantation. It is anticipated that this programme of work will generate new insights into mechanisms of injury during transplantation and identify new targets for therapy. Therapeutic approaches developed as part of this program will directly inform the design of clinical trials to ameliorate injury during transplantation. Moreover, many of the mechanisms of injury in transplantation are expected to be common to other diseases such as heart attacks and strokes. It is expected, therefore, that the findings from this study will also be applicable to treatment of such other conditions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to 6125 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 levels of severity? What will happen to the animals at the end?**

The experiments in this project are designed to study the mechanisms and the treatment of organ injury in transplantation. Some animals will be used as organ or tissue donors. The removal of tissue will be under terminal anaesthesia and will therefore not cause distress to the animals. Some experiments will involve the transplantation of organs (heart or kidney) into other mice, or causing injury to organs (kidney or part of the liver) by interrupting their blood supply for short periods. These surgical techniques have been refined and optimised to ensure that, when performed successfully, the animals can make a full recovery from procedure. Animals with heart or kidney transplants are therefore not expected to display any clinical signs. Technical failure of these complex procedures will be noted at the time of the operation and the animals will be culled under anaesthesia. If technical failures lead to adverse effects soon after recovery of the animals, the animals will be culled as soon as adverse effects are noted. Late failure of heart transplants will also not result in clinical signs. Failure of transplanted kidneys can result in gradual and slow-progression of clinical signs such as weight loss. The animals will be culled if these adverse effects are displayed. The risks of wound infection or wound dehiscence after operation are generally very low (<5%). Animals will undergo a maximum of two invasive procedures under general anaesthesia followed by recovery. The majority (>80%) of the animals are expected to recover well from the procedures and are not expected to show signs of adverse effects that impact materially on their general well-being. No more than 20% of animals are expected to show clinical signs of a moderate severity as a result of the effects of surgery or treatment with drugs. Rarely the severity of these signs may be such that the humane end points may be reached. Mice will be killed if they show significant signs of ill health, such as weight loss, piloerection and hunched posture or inactivity. If animals display mild signs, animals may be killed if they do not improve after up to 24 hours of observation.

## Application of the 3Rs

### Replacement

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

### Replacement

The definitive study of mechanisms and therapies to reduce injury during organ transplantation requires examination in intact animals. However, as part of this project, we are also making extensive use of fresh live tissue and organs from deceased human organ donors, to enable many of the questions to be answered as

best as possible without the use of animals. Replacement of animal use with human tissues and organs are therefore a fundamental component of the proposed studies.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

Through the use of human tissues and organs, we will reduce the number of animals used to achieve the aims and objectives of this experimental programme. The experiments are design to reduce experimental bias and improve the validity of the data generated through randomisation and systematic blinding of the experimental groups. Only therapeutic agents that have shown efficacy in vitro will be examined in vivo to reduce the number of animal experiments.

We will use multiple tissues and organs from each animal to maximise the data generated and reduce the number of animals used in the project. Tissue from culled animals will also be shared proactively with other researchers to reduce animal use by other research groups.

### **Refinement**

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

#### **Refinement**

The models used are optimally suited to achieve the aims and objectives of the study. We have refined the protocols and procedures for the generation and maintenance of these mice to maximise the likelihood of the success of the experiments and to minimise stress and harm to animals. The vast majority of the experiments are designed such that the animals only experience minor discomfort, and serious ill health or death is never an expected end-point.

In the heart transplant procedure, the heart from a donor animal is transplanted into the abdomen of a recipient animal. In this model, subsequent failure of the transplanted heart does not result in any ill effect in the recipient animal. Similarly, in the kidney transplant procedure, one of the recipient animal's kidneys is left intact, and only removed later if the transplanted organ is functioning. This ensures that the recipient animal does not experience adverse clinical effects even if the transplanted organ fails soon after transplantation. In both models, the health of the recipient

animal is not dependent on the function of the transplanted organ. Similarly, in experiments in which the blood supply to the kidney or part of the liver is temporarily interrupted, the duration of the interruption is limited to ensure this does not lead to adverse clinical signs (but allows biochemical detection of changes in organ function). These refinements minimise adverse effects experienced by the animals, while allowing the generation of important data by the experiments.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 101. Imaging of Ageing and Ageing-related Diseases</b>
<b>Key Words</b>	Ageing, obesity, neurodegeneration, iron, inflammation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The project will advance knowledge of ageing, metabolic diseases e.g.. obesity, non-alcoholic fatty liver disease and neurodegenerative disease e.g., Alzheimer's disease, in which age is a major risk factor.

Specific objectives:

1. Generate non-invasive imaging assessments of organ health, e.g., rate of brain ageing and/or prediction of future brain ageing; degree of liver fat, and other features of liver disease such as inflammation and fibrosis. Such imaging assessments may be biomarkers, that can be used to assess accelerated ageing and disease in animals and humans.
2. Determine if inflammation, changes in metal content and metabolic derangements occur in ageing, metabolic and neurodegenerative diseases, and whether they act in concert to accelerate ageing and/or disease.
3. Develop new ways of imaging/seeing certain biological processes, molecules, cells and/or metals in the body without 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 already high costs of unhealthy ageing including metabolic diseases e.g. obesity, and neurodegenerative diseases, e.g. Alzheimer's Disease, are rapidly mounting due to increasing average age of the world's population, and escalating adult and childhood obesity, and the absence of cures for these diseases. By studying ageing, metabolic and neurodegenerative diseases together, common and possibly synergistic processes may be identified which may provide novel putative therapeutic targets. Medical imaging methods such as MRI provides a safe 'window'

into the body. The methods can also be translated to the clinic, aiding accurate diagnosis, disease and therapeutic monitoring for patients and drug development.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The project has been designed to minimise the numbers of animals used, with human studies, in vitro and ex vivo studies being performed whenever possible. We expect to use 2200 mice and 500 rats (normal, mutant and genetically altered) over the duration of the project (5 years). Mice and rats are known to show typical behaviour and learning strategies, and the effects of ageing, obesity and neurodegeneration can be replicated in these animals. Mainly mice will be used as the wealth of standardised inbred strains and genetically altered strains available allow confounding factors such as genetic variability, gender, etc, to be eliminated as variables. Therefore, they are very well suited to use in these studies.

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

We are using animals that are mutants/genetically altered or given substances to age faster, develop metabolic and/or neurodegenerative diseases. Those ageing quicker or with neurodegenerative diseases may have problems with locomotion or abnormal behaviour. Animals with type 2 diabetes and non-alcoholic fatty liver disease may show signs of discomfort. All animals will be monitored but even more closely if they display any symptoms, e.g., weight loss, subdued behaviour. Animals with 20% or greater weight loss will be killed humanely. Should the ability of the animals to live normally be impaired then the animals will also be killed humanely. Animals will undergo imaging (under general anaesthesia), given injections (may be under general anaesthesia if appropriate), behaviour testing and blood samples taken. Such procedures will be conducted in a manner that minimises suffering and closely monitored and if unexpected adverse effects observed, animals will be killed. After general anaesthesia, hydration and wet-mash food may be provided if appropriate. Local analgesics may be applied to wound sites if needed. At the end of the experiment, animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

Animals are used as in this licence on the basis that it is considered unethical to test theories or precipitate induction of ageing/unhealthy ageing for study purposes in

man. In addition with respect to ageing studies, the relatively much shorter lifespan of animals, allows ageing investigations throughout life, not possible to do in man.

Mice and rats are known to show typical behaviour and learning strategies, and the effects of ageing, specific aspects of metabolic disease and neurodegenerative disease can be replicated in these animals. Investigations in ageing, metabolic and neurodegenerative diseases, results from communications between organs throughout the body and needs to be performed in the context of a whole animal rather than in vitro. Further, we are particularly interested in interactions between the different organs and various biological systems dispersed throughout the body such as the immune system.

Mainly mice will be used as the wealth of standardised inbred strains and transgenic strains available allow confounding factors such as genetic variation, gender, diet and environment etc, to be eliminated as variables.

Analyses of biological fluids such as urine, blood, and post-mortem tissue analyses will be performed to provide additional knowledge or aid optimization of live animal use in studies.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

All experiments are designed to use the minimum number of animals while still producing scientifically and statistically significant results, and power calculations are performed whenever possible to support experimental design. Usually, information is provided from taking tissues, often after animals have been humanely killed. However the use of medical imaging allows the same animals to be studied over differing time points in life and thus increases data obtained whilst drastically decreasing animal numbers as without imaging animals would be killed at each time point to gain necessary data.

## **Refinement**

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

### **Refinement**

Rats and mice are used as they age similarly to man, and obesity, diabetes and non-alcoholic fatty liver disease (NAFLD) can be induced in them by high fat and/or sugar feeding, as in man. While diabetes can be induced by chemicals in animals, we have chosen to do so mainly by high fat and/or sugar feeding, which does not affect animals as adversely as chemically-induced diabetes. Similarly, we are choosing to use genetically altered mouse models of neurodegenerative diseases rather than inducing disease by chemicals, as the latter affects animals more adversely.

We are employing medical imaging, which is non-invasive, such that each animal can provide data at different time-points. Not only does this decrease the number of animals that needs to be used as cohorts do not need to be killed at each time point, it also improves the robustness of the data as each animal acts as its own control. Imaging quality is improved/refined by ensuring that animals are kept still during the imaging process by using inhalation anaesthesia. Inhalation anaesthesia is used because animals recover quickly and promptly return to normal behaviour, e.g., eating and drinking very soon after. Additionally, in this project we will determine if medical imaging methods can provide biomarkers of brain ageing or disease, such that it may be unnecessary to perform invasive brain sampling or kill the animal to study the brain. Furthermore, increasing liver fat deposition as observed in NAFLD, is asymptomatic but imaging is able to non-invasively detect liver fat, again, making invasive liver sampling with its associated mortality and morbidity unnecessary.

Learning and memory are impaired in ageing and neurodegenerative disease and this will be tested in animals. The methods chosen in this project to test for these deficits, do not require the application of painful stimuli to the animals, which is a more refined approach than could be the case if alternative tests were chosen.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 102. Manufacturing of influenza virus defective interfering virus: testing of protection from respiratory virus disease in mice and assessing dosing and administration regimens</b>
<b>Key Words</b>	Defective interfering virus, respiratory viruses, influenza virus, antiviral
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

It has previously been shown that a defective-interfering influenza virus has high activity and broad-spectrum activity against possibly all respiratory viruses.

This antiviral is being produced commercially on a large scale with the aim of testing this in people in a virus challenge clinical trial.

We are validating a large scale production process by testing that the commercially produced antiviral has the same activity as that produced earlier by a small scale process.

**What 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 hundreds of respiratory viruses which all cause a similar disease. At the present time the only vaccine available is against influenza and this gives at best about 60% protection. In some groups protection is negligible. There are antivirals against influenza but these are not very effective and are giving rise to resistant viruses. We expect that our new antiviral will be successful in protecting and/or treating people from a variety of respiratory virus infections. Because of the broad spectrum of efficacy it is not necessary to identify the virus causing the infection which reduces the time to administration and significantly improves the treatment outcome. This approach will have the added advantage that it will hugely reduce secondary bacterial infections and the use of antibiotics.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

It is anticipated that a maximum of 5500 mice and 2000 embryonated chickens' eggs will be used over the 5 year 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 levels of severity? What will happen to the animals at the end?**

Animals will be inoculated via the nose with infectious virus but most will remain free of any disease as a result of the antiviral treatment. Many mice will be in control groups which will receive harmless materials. However, some animals receive the virus challenge dose without antiviral (the virus control) and these will succumb to respiratory infection. All animals will be closely monitored and those showing severe disease will be humanely killed to reduce suffering. At the end of the study all animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

The project requires study of respiratory disease which is a multisystem event involving interaction of a virus with highly specialized respiratory tract cells, and the entire multicomponent defence system of the animal. This cannot be mimicked in any way. Similarly our antiviral treatment impacts on all these facets of the animal and this cannot be reproduced in any way either. The project will provide essential data that is required to satisfy regulatory authorities for approval for testing of 244 DI virus in humans. The current regulatory requirement is that the data is obtained using animal testing but we will stay alert to any new developments and if any non-animal alternatives become available we will adopt them.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We have developed a very good infection system over a number of years. Using this experience we will use experimental routes that contain the minimum number of animals that is compatible with statistical robustness, and avoid the need for repeat experiments. We have carried out power calculations to identify the minimum number of animals per group required to provide statistically robust datasets and will use this to inform experimental design. We have used this approach in the past for

generation of publishable data. We will use independent advice as appropriate to ensure that regulatory requirements are met.

### **Refinement**

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

### **Refinement**

Laboratory mice are the animals being used here, and we have considerable experience of this system. Mice respond to infection with respiratory viruses in a very uniform way, allowing the use of small groups of animals. In work under previous ASPA licences we have established a very reliable and reproducible scoring system to assess the progression of respiratory virus disease and to record the symptoms in the animals. Over recent years in conjunction with increased frequency of observations and incorporation of daily recordings of the weights of the test groups of mice as an objective measurement of general health the scoring system has been refined to more accurately predict animals likely to experience the most severe infection. By combining these elements we have refined the assessment of animals to reduce the number that succumb to infection. All experimental animals including uninfected controls will be monitored daily by trained and experienced staff for signs of infection and the severity of the disease scored using a standard protocol. If any animal becomes seriously ill they will be immediately humanely killed to reduce suffering. The number of animals that die as a result of infection will be constantly monitored to refine the assessments procedures wherever possible to reduce this number.

Wherever possible we combine experiments to allow use of fewer control groups than would be the case if experiments were conducted separately and this has led to a reduction in the total number of animals used. We will continue efforts to refine the model further and the numbers of animals that succumb to infection will be monitored to continuously reduce this proportion as far as reasonably possible.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 103. Pathophysiology of acute lung and organ injury</b>
<b>Key Words</b>	inflammation, intensive care, lung injury
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The systemic inflammatory response syndrome (SIRS) is a frequently fatal condition where the body mounts an overwhelming immune response to an insult, culminating in organ failure. This can be caused in many ways. Burn injuries are an important 'sterile' cause of SIRS, while SIRS caused by an infection is known as sepsis. Acute respiratory distress syndrome (ARDS) is a related condition in which the lung is the primary organ affected, for example during influenza/pneumonia. ARDS, SIRS and sepsis are frequently linked with each other, as sepsis/SIRS may lead to ARDS, and ARDS may lead to sepsis/SIRS. Together they form the major cause of deaths within the intensive care unit each year. Depending on severity, 30-70% of people who develop either of these syndromes will die, and the Sepsis Trust has estimated >40,000 deaths per year in the UK from sepsis. Unfortunately no therapeutic options exist to treat these conditions apart from antibiotics, and treatment consists mainly of keeping patients alive in intensive care until they either die or recover.

**What 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 understanding of the precise mechanisms behind these conditions is still lacking (hence the absence of treatment options). The current project aims to improve this knowledge using 'classical' methods to evaluate physiological and inflammatory processes alongside imaging, systems biology and next-generation sequencing technologies. Hopefully therefore these studies will drive the development of novel therapeutic strategies in patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Animals used within this study will be mice. It is expected that no more than ~6000 animals would be used over the 5 year course of the project.

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

As SIRS/ARDS/organ injury have such high mortality in patients, it is inevitable that our animal models must have the potential to induce some signs of distress. These would include various signs of illness including reduced mobility, loss of appetite, diarrhoea, weight loss and abnormal breathing. As far as possible we will design protocols in which such symptoms are minimised while still allowing our scientific goals to be reached. However, in some experiments, particularly those in which potential treatments are being evaluated, it is necessary that models incorporate such 'clinical symptoms' in order to translate the basic studies to patient treatment. The majority of protocols under this programme of work are categorised as moderate. There is however 1 small-scale pilot protocol (involving up to 100 mice) which is categorised as 'severe'. NACWO/NVS advice will be sought for assessment of severity symptoms, and scoring sheets used to minimise suffering. Ultimately the aim of this protocol is to enable development of moderate protocols for later use within the studies. All animals will be humanely killed at the end of studies.

## Application of the 3Rs

### Replacement

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

### Replacement

At present, it is still impossible to produce a complete non-animal replica of such complex biological processes as SIRS or ARDS. Complicated interactions occur between different organs and the immune system which cannot be modelled in vitro. Furthermore, an important aspect of the project is to explore the impact of 'pre-disposing factors'. Where possible we will ask questions in cell-culture based systems, but ultimately any findings from these need to be reconciled in vivo.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We have substantial experience with the majority of protocols within this project, which allows us to predict with some accuracy the number of animals required to detect statistical differences. To help limit variability and group sizes, we will use inbred animals, and as far as possible, male animals only. Whenever we can, we try to maximize the information from each animal, taking samples for multiple analyses from a single animal at endpoint, as long as this does not compromise

experiments. Experiments will be planned and reported in line with the NC3Rs ARRIVE guidelines.

### **Refinement**

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

### **Refinement**

All dosing and blood withdrawal volumes will follow LASA guidelines, while anaesthetic and pain-killing regimes will follow either our well-established protocols or be developed in discussion with NVS.

The mouse is genetically well characterised and numerous research tools (antibodies, reagents etc) are available. Moreover, the use of genetically modified mice is an invaluable tool to dissect out the roles of genes of interest. Thus, the mouse is the least sentient species in which we can address the questions raised in this Project.

For those models in which injury to the lungs or other organs needs to develop over days to weeks, we will take various steps to minimise discomfort. These will include: close monitoring; terminating experiments as early as is consistent with scientific goals; administration of pain-killing drugs; and maintaining animals within a warmed, oxygenated box, which allows organ injury to develop while the animal's wellbeing is less affected than it would be in the general environment.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 104. Therapeutic Antibodies in Haematological Disorders</b>
<b>Key Words</b>	Monoclonal Antibody, Immune therapy, Haematology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The main objective of this project licence is to understand how novel monoclonal antibodies, an antibody produced by a single clone of cells or cell line and consisting of identical antibody molecules, effect animal models of blood disorders. This understanding will underpin the development of these new treatments for these blood disorders. Present therapies for blood disorders suffer from side effects and patients developing resistance to the treatments or not responding initially. This leads to many patients unfortunately still suffering from the clinical symptoms of these debilitating conditions which reduce both the quality of life and can reduce lifespan.

With the data provided by this project licence we intend to develop new therapeutics for blood disorders (including anaemia and haemophilia), bringing the opportunity of long term relief to a larger number of patients than can currently benefit from present therapies. We aim to generate new treatments, based on a class of drug called monoclonal antibodies. During the course of discovering new monoclonal antibodies, we will also be addressing the questions of which patients will respond and whether our treatments can help those who do not respond to the currently available therapies. This licence will also provide essential supporting data for the antibody therapies being evaluated, enabling us to determine the way our molecules are working.

**What 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 will be the development of new knowledge of blood disorders, the provision of data which will underpin the development of novel therapeutic antibodies leading to the progression of these new therapies into clinical development and ultimately onto the market bringing benefit to patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 5,500 adult rats and 5,000 adult mice over 5 years for this project

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

The animals used in this licence will be used to develop me antibody therapies to treat haematological conditions. This will include establishing the pharmacokinetic (how the organism effects the drugs and how long the drug is effective) and pharmacodynamic (how the body reacts to treatment with an agent) of therapeutic antibodies in both normal and diseased animals. Animal used under this licence will also be used to establish if therapeutic antibody therapies are effective at treating animal models (of anaemia and haemophilia) of haematological diseases and to establish if they are better than present therapies at treating these models. The majority of the adverse expected under this licence (e.g. weight loss, pallor and hunched posture) will be associated with the anaemia and inflammation associated with these models. This anaemia and inflammation may have effect the whole animal, and can affect the general welfare of the animal. We have put in place measures to closely monitor the effects of this anaemia and inflammation on animal health enabling us to monitor the impact on the animal. Animal condition will be checked daily and a closer inspection and weighing of the animal will be carried out three times a week any changes in condition, weight and / or behaviour will be noted and animals deviating from normal condition and/or behaviour will be assessed. If necessary, following consultation animal care technician and /or the veterinary surgeon, mice maybe further closely monitored or an intervention such as the supply of dietary supplements or animals may be killed. Adverse events can occur, following the administration of compounds depending on the intended mechanism of action of the molecules being tested. Where adverse events are noted, if necessary, following consultation with the veterinarian and appointed animal technician, mice maybe further closely monitored or an intervention such as the supply of dietary supplements may be given, or animals may be killed. When mice are anaesthetised there will be close and continuous monitoring to ensure that there is no possibility of mice recovering consciousness until procedures have finished. At the end of the experiments, animals will be killed, and tissues taken for further analysis. This analysis is an important and integral part of the project as this data will help us to decide which types of inflammatory response are likely to respond to our therapies and therefore which patients are most likely to benefit.

## Application of the 3Rs

### Replacement

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

#### Replacement

Haematological diseases involve complex process involving many cells types which interact with each other. This interaction is not just between involves many types of cells in different organs. It is not currently possible to model these aspects of complex interactions without the use of animal models, as we cannot reproduce the overall complexity of the types of cells involved and how they interact with each other in an in vitro system. We will do this as we test our lead monoclonal antibodies in vitro first, only picking those that have the right characteristics to progress to in vivo models

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will regularly consult the most current papers on the subject to make sure we have the most up to date scientific knowledge in the area of research we are working in. We are also working in collaboration with a laboratory REDACTED who are working on improving the reproducibility and translatability of these models. We will initially run studies with as few animals as possible to make sure that our experimental technique is correct and the models we have chosen are the most appropriate. In this way, for each project, only a few antibodies will need to be tested in vivo. We will also use statistical methods to ensure that we are using the fewest animals per experiment to obtaining meaningful data.

In addition, we will be taking many samples that will tell us about the changes to the immune system at the end of each study to ensure that we gain the maximum amount of information from each animal and minimise the number of experiments that need to be carried out

We will also develop imaging techniques to enable us to monitor inflammation longitudinally which will enable us to use fewer animals and time points.

### Refinement

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

## **Refinement**

Mouse models have been successfully used for the development of therapeutics for a wide range of haematological conditions. It is well established that antibodies can be used to treat patients with haematological diseases. It is also well established that rodent models can be used to develop treatments for haematological diseases and that these rodent models are translatable to the clinic.

Rodents will be housed in state-of-the-art conditions with care and welfare provided by an excellent and highly trained team of technicians.

We will ensure that only agents that have passed stringent analysis for quality will be used in rodent studies. When conducting studies, we will select the protocol where we are using the lowest concentration of challenge possible during the study to meet our experimental requirements. We will actively monitor the anaemia and the impact that has on condition of the animal to ensure that no animal suffers unduly.

We will continue to meet with local and international groups that work in the field of haematological conditions to refine experimental techniques and bring the best advice to bear on our projects so that we can always obtain the best information from the studies we conduct

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 105. Collagen (I) homotrimer and cellular stress in ageing and disease</b>
<b>Key Words</b>	brittle bone, collagen, osteogenesis imperfecta, cellular stress
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Collagen (type I) is the most abundant structural protein in the body and a major component of bone and joint tissues. It forms fibres that surround cells and make tissues resilient to mechanical loading. It is degraded and reformed by cells using biological enzymes and this process allows skeletal tissues to adapt to changes in mechanical loading. When the tissue structure is inadequate to resist external loads, tissue injury including fractures and ruptures can occur. This leads to diseases including osteoporosis (weak bone), osteoarthritis (cartilage loss and bone overgrowth) and soft tissue injuries. Over-production of type I collagen (termed fibrosis) furthermore restricts tissue function leading to disability and increased morbidity and mortality.

An abnormal form of type I collagen, termed collagen (I) homotrimer, is present in both degenerative and fibrotic diseases. This abnormal collagen alters the properties of collagen fibrils and is resistant to breakdown. Collagen (I) homotrimer may therefore affect the ability of tissues to respond to changing mechanical loads and to counteract fibrosis.

The effect of abnormal type I collagen on tissues has been well-studied in a spontaneously occurring mouse model (oim) of the brittle bone disease 'osteogenesis imperfecta'. The genetic mutation in these mice appears to have side-effects that detrimentally affect the bones, but is very similar to a mutation causing brittle bones in humans. The side-effects are related to cellular stress, which has been previously implicated in osteogenesis imperfecta. A mouse line engineered to produce collagen (I) homotrimer alone, without the genetic side-effects does not have brittle-bones.

The project aim is to characterise the side-effects that cause brittle-bones in the oim model and determine how abnormal collagen itself affects collagenous tissues such as bone and tendon. To do this the oim and engineered mouse lines will be compared for the objectives below.

Objectives:

- 1 – to monitor type I collagen production and degradation, and assess collagen fibre structure and organisation
- 2 – to measure the mechanical strength of bone and tendon
- 3 – to analyse the fine structure and mineral content of bone
- 4 – to monitor the development of osteoarthritis in joints
- 5 – to identify and classify the genetic side-effects of the oim mutation.

In parallel, in vitro cell culture studies will be carried out to identify molecular regulators of abnormal collagen synthesis and identify potential new therapeutic targets. In a separate project a computational model of collagen synthesis is being developed to predict conditions driving abnormal collagen production.

**What 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 relate to both identifying the genetic side-effects (cellular stress) in the mouse model of brittle bone disease and in understanding the effect of abnormal collagen (I) homotrimer on tissues. Identifying and classifying the genetic side-effects would be expected to make a substantial contribution to the evidence supporting future pre-clinical and clinical trials of pharmaceuticals known to target cellular stress. Human brittle bone disease occurs in 1 in every 10,000-20,000 live births with 3,000-4,000 affected people in the UK. Patients have brittle bones but joint laxity, hearing loss and other connective tissue problems also occur. An effective treatment for osteogenesis imperfecta could transform the lives of up to 500,000 people worldwide. If abnormal collagen (I) homotrimer itself is found to cause cellular stress this would also support trials of pharmaceuticals known to target cellular stress in age-related human musculoskeletal, cardiovascular and fibrotic diseases. These common conditions affect quality of life, cause pain and disability and are costly to the NHS. If abnormal collagen (I) homotrimer solely has adverse effects on tissue structure and strength, the parallel non-animal studies should identify drugs to block its production, which could be new pharmaceuticals or repurposed existing drugs. The research could ultimately benefit the ageing population, in particular those suffering from osteoporosis and osteoarthritis, as well as the patients' families and supporting healthcare systems.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

It is expected that a total of 1,500 mice would be bred over a period of 5 years, of which 250 would have brittle bones. Of these up to 72 could be injected with a dye to monitor bone production and degradation, up to 72 fasted for up to 18 hours before being put down. and up to 48 placed in a metabolic cage with a wire base for up to 24 hours (a maximum of three times less than once per week) to collect urine to measure collagen degradation. A quarter of those injected, fasted or metabolically caged could have brittle bones. Similar numbers of mice that produce collagen (I) homotrimer alone, without the genetic side-effects, may be injected (up to 72), fasted (up to 72) or metabolically caged (up to 48).

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

Expected adverse effects are spontaneous fractures, bone deformities, brittle teeth and a smaller size. Mice with brittle bones are expected to sustain at least one long-bone fracture with associated limping and abnormal gait. Mice with extensive fractures preventing movement or standing will be put down (using a legally-defined appropriate humane method). The maximum expected level of severity is severe for those with fractures. Mice will be bred and maintained until after weaning (8 weeks of age) and up to adulthood (18 weeks) at which point they will be put down. As well as obtaining tissues for this study, other tissues will be preserved so that future projects can be carried out without breeding more mice. Injection is expected to produce mild adverse effects such as mild transient pain. If localised irritation or infection occurs, those affected will be put down (using a legally-defined appropriate humane method). Fasting may potentially increase aggression in which case animals would be separated. Mice prone to fractures will be monitored in metabolic cages and removed if mobility is affected.

## Application of the 3Rs

### Replacement

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

### Replacement

The effect of abnormal collagen on tissue cannot be evaluated in other systems due to the complex structure of collagenous tissues. Non-protected animal alternatives, 3D culture systems and computational models are not sufficiently developed for this purpose. However the project will also encompass cell culture experiments to investigate the early stages in collagen production and a separate project is generating a computational model.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Sample size calculations were carried out by a qualified chartered statistician. Tissues from the hind limbs of each animal will be used for different analyses to halve the numbers involved. Procedures to measure bone degradation are incorporated to reduce future breeding.

## **Refinement**

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

### **Refinement**

The 'oim' mouse is the only model of human brittle bone disease where abnormal collagen is present and all models of the disease have brittle bones. The comparison to the engineered mouse line, in which genetic side effects are not present, is the only means to pinpoint the source of the bone fragility. To reduce pain and suffering, non-steroidal anti-inflammatory pain relief will be provided in drinking water or as a self-medicating gel. Mice will be checked regularly and those with extensive fractures preventing movement or standing will be put down. Otherwise stronger pain relief (e.g. morphine) will be given as required with veterinary advice. Handling will be minimised and gentle capture methods will be used. Easily accessible soft food and water, soft bedding and non-tangling nesting material will be provided. Mice will be housed socially where possible to provide distraction and given floor-level tunnels and refuges. For bone degradation assays, injection will be carried out with gentle handling and/or anaesthetic. Mice will be closely monitored during any fasting or metabolic caging and relocated as appropriate.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 106. Training in Specialised Therapeutic Procedures</b>
<b>Key Words</b>	Minimally invasive, New procedures, surgery, Training
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>No</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>Yes</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project will train surgeons in new, advanced therapeutic minimally invasive (keyhole surgery), surgical procedures.

**What 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 many cases minimally invasive surgical procedures (keyhole surgery) are significantly better for patients than open procedures as they are associated with less post-operative adhesions, less time in hospital, faster recovery, less pain, easier post-operative care and much faster return to active life. Consequently many new minimally invasive procedures are being developed to replace larger, open procedures - particularly in response to the Governments new screening programmes for bowel cancer and aortic aneurysm among others. These screening programmes are identifying 30-40% more patients requiring surgical intervention for their conditions and the number of surgeons qualified in the new procedures is very limited. Untrained use of these new procedures results in unacceptable death rates and long term side effects. We will teach surgeons these new procedures, in terminally anaesthetised animals, to ensure rapid competency and safety. These courses will ensure an adequate supply of appropriately trained surgeons who will be able to fulfil the needs of our increasing numbers of patients using new minimally invasive procedures safely and effectively.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

100 pigs and 100 sheep over the course 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 levels of severity? What will happen to the animals at the end?**

As all protocols are non-recovery, at the end of the procedures, animals are given an anaesthetic overdose and then all possible organs are harvested for use in other studies being conducted by a range of scientists at the institution.

## Application of the 3Rs

### Replacement

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

### Replacement

Simulators will be used as part of the training but, as yet, there are no simulators that truly represent the full physiological state necessary to teach these procedures. Current simulators are unable to replicate the blood and lymph flow of tissues and are also not able to replicate tissue responses to stimuli, muscular activity in bowel, effects of surgery affected by temperature, size models representative of the human anatomy or tissue changes relative to procedures. We will endeavour to develop better simulators as these courses progress.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

By carrying out a number of procedures in one animal we can reduce the number needed and, as all animals will be deeply and terminally anaesthetised, there will be no suffering or adverse effects. Using 2 animals per 3 or 6 surgeons depending on the course also reduces the number of animals needed.

### Refinement

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

### Refinement

The pig and sheep have been chosen for these courses as we need to represent the same size and physiology as humans, in particular with regard to blood system, lymph system, tissue response and general anatomy. Principally, animals are terminally anaesthetised and therefore insentient throughout. They are carefully

monitored using staff trained, skilled and experienced in ensuring effective prolonged anaesthesia in these species.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 107. A Nipah virus vaccine to eliminate porcine reservoirs and safeguard human health</b>
<b>Key Words</b>	vaccine, Nipah virus, pig
<b>Expected duration of the project</b>	3 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Nipah virus (NiV) poses a significant epidemic threat because of its broad host range and widespread distribution of fruit bats, which act as a natural reservoir. Humans may become infected indirectly from bats by consumption of contaminated raw date palm sap or through contact with infected livestock species. NiV infection of humans or pigs can result in severe respiratory disease or fatal encephalitis. Direct pig-to-human transmission was responsible for the first and still most devastating NiV outbreak. Despite the importance of NiV as an emerging disease with the potential to cause large outbreaks with significant mortality, no vaccines or therapeutics are currently approved for human or livestock use. The first aim of this project is to conduct a head-to-head comparison of existing NiV vaccine candidates by assessing their ability to induce immune response in pigs that are associated with protection against NiV. Based on these data and a trial conducted by overseas collaborators to assess whether the most immunogenic vaccines can protect pigs against NiV challenge infection, we will select a single candidate for further development. This will include our second aim of comparing the duration and strength of immune responses elicited by one and two-shot immunisation regimes. In addition to providing a solid basis for developing a vaccine that can be used to protect pigs against NiV, the work done under this licence and by our collaborators is likely to inform work being done by others to develop a vaccine for 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)?**

The proposed project, in conjunction with overseas work with our collaborators, will complete the initial stages of development of a NiV vaccine. We will be well placed to proceed to the next stages of development of a commercial vaccine for use in pig populations to prevent NiV infection and transmission thereby reducing the risk to public health. The vaccine will also reduce the major risk NiV poses to both the pig industries and poor livestock keepers in low and middle income countries. The vaccine will enable the discrimination of infection in vaccinated animals and therefore provide an important new tool to aid the monitoring, control and elimination of NiV from these reservoirs in South and Southeast Asia. The project will also provide a solid basis for the further evaluation of the vaccine for protection of humans against NiV infection.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

96 pigs (*Sus scrofa*) 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 levels of severity? What will happen to the animals at the end?**

Pigs will be immunised by injection up to three times. Blood samples will be collected at intervals over the course of vaccination to assess the immune responses stimulated by the vaccine candidates. All animals will be euthanized at the end of the experiments and tissues collected post-mortem to assess local immune responses. It is expected that animals will typically experience transient distress from restraint for injections and blood samplings, transient pain from the insertion of a needle and some localised transient pain and swelling at the site of vaccine injection. The likely/expected level of severity for the procedures is 'mild'.

## Application of the 3Rs

### Replacement

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

### Replacement

*In vitro* cell culture-based assay systems can be used to support vaccine development by assessing the immune recognition of vaccine candidates and the innate immunostimulatory properties of vaccine formulation/delivery systems. However, the biological complexity of the immune system means that there is no alternative to the use of animals to evaluate the performance of vaccines. Both the NiV vaccine candidates and the formulation/delivery systems we propose to evaluate have been demonstrated to be both safe and immunogenic in mice and other animal species. We can therefore proceed directly to assessing their performance in the target species, pigs.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The animal studies are designed to maximise collection of biological materials/data from each study. A power calculation based on published immunogenicity data from an experimental NiV vaccine trial conducted in pigs has allowed us to select the appropriate number of animals to obtain reliable and meaningful results. Animals will be randomly allocated to experimental vaccine and control groups and the identity of these treatment groups will be blinded to the laboratory investigators until the immune response data has been measured and analysed.

## **Refinement**

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

### **Refinement**

As both pigs and humans are readily infected with NiV, the pig is a robust and appropriate model for investigating vaccines to protect against both porcine and human disease. Like humans, pigs are outbred, and are physiologically, anatomically and immunologically similar to humans. General measures taken to minimise welfare costs to animals include the use of vaccines and adjuvants with demonstrated safety profiles and reducing sampling frequency and volumes to a minimum.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 108. Craniofacial development and associated birth defects</b>
<b>Key Words</b>	Cleft palate, submucous cleft palate, craniofacial, Birth defect
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

In the embryo, the upper lip and the oral palate form from tissues that initially develop on both sides of the early face. These structures complete when they fuse together along the midline. A failure in fusion will lead either to a cleft of the lip or palate or sometimes both. These defects are considered relatively commonplace, affecting about 1 in every 700 babies worldwide. Clefts have a very serious impact on that baby's future quality of life, starting immediately with feeding difficulties. Lip and palate repair is by surgery, usually performed between 4-12 months. However, as they grow, patients may require a number of additional surgical procedures, in some cases into late teenage years. Apart from surgery, patients usually require specialist care for hearing, speech and language as well as orthodontic work and psychological therapy.

This project focuses mainly on a particular palate defect that is called a submucous cleft palate. This is where the palate initially fuses but bones that normally form in this structure, fail to grow properly. These bones are important as they anchor muscles at the back of the palate that are required for swallowing and speech. The palate therefore doesn't work effectively and the child can have many of the same problems with feeding, speech and hearing as a child with an open cleft palate. Whilst it is possible to correct the problem with surgery, this is not always as effective as for an open cleft palate since the palate muscles tend to remain inefficient even after repair. Little research has been carried out in this area and it is not clear if the persistent loss of muscle function is a primary (i.e. something affecting the muscle structure itself) or a secondary defect, perhaps through their prolonged lack of connection to the usual bones.

One well-established cause of cleft palate is the inheritance of a genetic mutation in the *TBX22* gene. *TBX22* is found on the X-chromosome, which means that associated defects mainly affect boys. Sometimes this results in an open cleft palate but more often in a submucous cleft. At the moment it is still not possible to predict which type of cleft will occur. We have shown that mice lacking a normal *TBX22* gene provide an excellent model for submucous cleft palate, as this is also the predominant feature. This project therefore sets out to investigate mice lacking *TBX22* as a model to learn more about palate development and, in particular, better understand why a submucous cleft palate results.

In this project, we will use a variety of methods to study both normal and abnormal palate development and to investigate the exact role of the *TBX22* gene. In addition, we will investigate novel methods to study palate closure using a culture system. This will provide the means to test potential drugs or manipulations to the tissues without using live animals. These results might later be applied to humans with a view to improving palate muscles repair and lessen the need for subsequent operations.

**What 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 sets out to improve our understanding of how the palate develops normally and what factors can lead to a cleft. Submucous cleft palate is a common human defect but has received very little attention from researchers. We will look closely at the relationship between an open cleft palate and submucous cleft palate. We will investigate the potential to develop novel therapies that could improve palate muscle function and complement what is currently achievable with surgery alone.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Because of the similarity between mouse and human palate development and the relative accessibility to investigate palate closure in the mouse, it makes an extremely valuable model. We will use both wildtype and mutant strains including mice with mutations in *Tbx22*, *Tbx1* and *Chd7* genes since these are genes that have been associated with submucous cleft palate in humans and the mice recapitulate the human cleft condition very well. We expect to use no more than an estimated 2000 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 levels of severity? What will happen to the animals at the end?**

Each of the named animal models are already well characterised. Submucous cleft palate has been shown to be a sub-clinical phenotype in mice. Nevertheless, all breeding will be conducted and carefully monitored by appropriately trained staff. For X-linked traits, i.e. mutation of a gene carried on the X-chromosome and only

adverse in males, the colony is maintained as heterozygous females (who have one mutant and one normal X-chromosome). Breeding to maintain the colony will also result in live born male Tbx22 mice (X-linked; hemizygous), however, for this gene, adverse affects are generally mild and not expected to exceed “moderate” in severity. Heterozygous females are completely unaffected. For autosomal genes, animals are maintained as heterozygous males and females ( carrying one mutant copy and one normal copy). Experimental homozygous animals (those with two mutant copies) are produced by setting up a cross between heterozygous male and heterozygous females. These are only investigated at embryonic/fetal stages. The Dam is killed prior to embryo removal and embryos are subsequently killed prior to experimentation.

## Application of the 3Rs

### Replacement

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

#### Replacement

Craniofacial syndromes affect complex structures and multiple cell/tissue types and are therefore difficult to model in other ways. We can and will use alternatives such as cell culture wherever possible, however, we can only fully reproduce certain physical characteristics in an animal model.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We always aim to reduce the numbers of animals we use and include power analyses based on effect size in order to identify the minimum number of animals that we need, in order to answer the specific questions being posed.

### Refinement

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

#### Refinement

Cell culture models cannot easily represent dynamic growth of the head and face. It is critical to be able to measure the effects of genetic changes not only at the cellular level but also in the whole animal. The possibility of genome manipulation makes the mouse an invaluable model to study the genetics of complex syndromes in a live

mammalian system. During this project we also take advantage of other in vitro disease models such as standard laboratory cell lines.

In terms of welfare, animal colonies are maintained as asymptomatic heterozygotes. The experimental (symptomatic) animals are investigated at embryonic or fetal stages, thus minimising the welfare costs to the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 109. Safety Testing of Chemicals, Plant Protection Products, Biocides and Substance added to Food or Feed Products Using Small Animal Species</b>
<b>Key Words</b>	Regulatory, Safety Assessment, Small Animal
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

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 small animal species with the aim of evaluating the hazard profile of novel chemicals, plant protection products, biocides, food and feed additives and veterinary medicinal products in terms of general toxicity and potential life time exposure. Further aims include validation of new experimental conditions including the collection of tissues from surplus stock animals to support validation of non-animal alternative methodology.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the 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. The principal benefit of this project is the generation of safety data to allow regulatory decisions regarding human exposure. Without these studies, progression of new products could not occur safely. Validation and refinement of test methods may also be completed for specific techniques and may be published to the wider scientific community.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 year life of this Project Licence, it is estimated that 20,000 mice, 42,000 rats and 5,000 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 levels 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 in to the oesophagus. 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 or exposure to the test material for a number of hours each day for example by placing the material on the skin and covering it with a gauze dressing. The length of study depends on the likelihood of repeated human exposure and ranges from a single administration for example to assess accidental contact through to daily administration for 2 years to explore possible long term effects. Blood and urine samples may be taken to measure the level of test material or its metabolites with an animal’s circulatory system. These may also be analysed to detect any effects on 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. 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. Many toxicological effects of the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. 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

### Replacement

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

#### Replacement

At present there are no scientific and legally acceptable evaluations of systemic toxicity which will satisfy regulatory requirements and provide sufficient safety data other than use of animals. Validated *in vitro* tests for specific organs and biological pathways are available and used to replace or refine procedures wherever possible. As new *in vitro* methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace *in vivo* procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism information will be utilised to reduce animal use.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

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.

Wherever practicable, and by looking across studies, the combination of endpoints eg general toxicity, reproduction and developmental toxicity, mutagenicity etc in studies is considered, to reduce overall animal usage.

As most studies involve the examination of tissues following treatment opportunities for re-use are very limited. Tissues are collected to support drug and *in vivo* developments from any surplus stock animals.

### Refinement

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

#### Refinement

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 biologically appropriate species, and the species which most closely relates to man. Studies to assess the types of material covered by this licence are usually performed on small animal species, occasionally there might be a need for comparative data in larger animals such as dogs, minipigs.

Generally the rat is the rodent species of choice in safety assessment. There is wide knowledge of the response of rats to various substances and a wealth of background literature. Rats are large enough to provide repeated blood samples, thus requiring significantly fewer rats than mice to achieve the same objective. Mice may be used when considered a more appropriate species, for example, if they more readily absorb the test material, are more relevant biologically or improved tolerance depending upon objective of the study.

Rabbits may be used when considered a more appropriate species, for example non-pregnant range finding studies prior to conducting reproductive toxicology studies in pregnant rabbits or local tolerance testing.

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.

Socially compatible species are routinely group housed with environmental enrichment which encourages species specific behaviours without not adversely impacting study outcomes. Occasionally it may be necessary to single house animals for example to collect urine samples or for the administration of test substances. All such occurrences are conducted in accordance with project licence limitations and under the oversight of the local Animal Welfare and Ethical Review Body.

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, under guidance issued by the site's Animal Welfare and Ethical Review Body (AWERB).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 110. Minimally invasive surgery to alter limb growth</b>
<b>Key Words</b>	Limb length, Epiphysiodesis, Radiofrequency ablation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We aim to develop a new technique (called radiofrequency ablation) for stopping bone growth to treat limb length discrepancies in children. By applying this technique to young sheep, we will observe if it can stop bone growth predictably, and whether it is associated with any side effects (such as deformities of the bone, damage to the local tissues). Additionally we will see if the other bones in the leg compensate for the shortening of the primary bone treated, and to what degree they do so.

**What 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 this technique works in sheep, our work will support its use in children with anisomelia (different leg lengths). Compared with currently used methods of stopping bone growing, this new procedure requires less exposure to x-rays (both for the patient and the surgeon), less surgical time, less risk of infection, less pain and less scarring of the patient's skin.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will test the procedure on two sheep initially in a pilot study to check that the settings work on two sheep. Both will be monitored for 6 months after the procedure. If the procedure is successful, a further 10 sheep will have the same procedure performed (8 sheep having the procedure and 2 sheep having a sham procedure). If the procedure does not work on the pilot sheep then the pilot will be repeated with different radiofrequency settings, and the larger study performed after that. The total number of sheep to be used in the project is likely to be between 10 and 12.

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

The level of severity is moderate because these sheep will undergo surgery to briefly place a short needle into each side of the growing part of the bone. We expect the level of pain to be well controlled by pain relieving drugs and short lived, and the likelihood of any post-operative problems to be low. Should any animal have an adverse effect, that cannot be treated, then they will be euthanatized by a schedule 1 method. The animals will be euthanatized at the end of the study by a schedule 1 method to allow harvest of their tissues.

## Application of the 3Rs

### Replacement

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

#### Replacement

We must prove that this technique works in a biological environment similar to that it will be used in children, and this cannot be replicated outside of a living animal. The previous experiment which have been carried out using this procedure in other species have not provided enough data for us to be certain it is both efficacious and safe; this project will provide that.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will use a minimal number of animals to establish whether the procedure works effectively or not. We have used a power calculation based on existing data for a similar model to determine the numbers of animals we should use in the experiment.

### Refinement

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

#### Refinement

Sheep are the ideal animal model for the growth studies aimed at human treatment, as they offer the optimal size, both in terms of the physical size of the animal and the thickness of the growing part of the bone. Their welfare will be maximised through the provision of anaesthesia and postoperative analgesia and intensive clinical monitoring to recognise and allow a rapid response to any evidence of discomfort or lameness.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 111. Genetic Control of Early Mammalian Development</b>
<b>Key Words</b>	Mouse, Embryo, Developmental Biology, Transgenic
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No

(d) protection of the natural environment in the interests of the health or welfare of man or animals;

No

(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No

(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No

(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

During embryonic development cells go from being a uniform ball of cells to acquiring all the information that is necessary for them to perform the functions required in the different tissues and organs of the body. During this process an embryonic cell must go through a number of maturation steps before it can perform the complex tasks that are required from adult cells. Each of these maturation steps involves a decision point where the cell decides between multiple different paths. As development proceeds, the range of fates available to a cell decreases and therefore its potential becomes limited.

Very little is known about how the earliest cell fate decisions are taken and how a cell goes from having the potential to contribute to every terminally differentiated cell type in the body to having its potential restricted with every maturation step that occurs during development.

This project aims to identify how cells go from a naïve state of differentiation to a fully differentiated state. We will identify the pathways and genes that direct the first steps of this differentiation process.

**What 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 identify the steps that take a cell down the differentiation pathway and identify the genes that play important roles during early development. It has become clear that the genes acting in mouse development are conserved in

humans. Although first active in the embryo many of these genes are used again in the adult, especially in tissue regeneration and go wrong in cancer. Consequently, the genetic analysis of development in the mouse has direct relevance to the understanding of genetic disorders, as well as for studying cancer and defective tissue repair in humans. Furthermore, the information harnessed in this project is essential to for the safe and efficient use of stem cells for regenerative medicine.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 6500 mice per year will be used for this project.

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

The vast majority of them will have the following experience – after being born and weaned, they will have a small tissue sample taken from the ear. Later, they may be set up to mate with a mouse of the opposite sex. Females will generally be killed a few days after they have been mated, so that embryos can be dissected out of them. Males will be retained for use in further crosses and then, before getting old and infirm, will be killed. Most of these animals will only experience procedures of a mild severity but a small number of the mice bred will carry a mutation present in diabetic patients and will experience moderate severity. A small proportion of the female mice will have surgery performed on them under general anaesthesia, in order to act as recipients and foster mothers to genetically manipulated embryos. These animals will experience procedures of a moderate severity. A small proportion of mice will develop tumours and these animals will be killed before any overt suffering occurs. These animals will experience procedures of a moderate severity.

## Application of the 3Rs

### Replacement

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

### Replacement

We still do not understand enough about mammalian development to be able to model it using computers. The specific tissue interactions and movements involved in this complex process cannot be accurately recapitulated in cell culture. Therefore, this problem can only be effectively addressed by the use of animals. We use mice as they have been studied for a long time by geneticists, so a large body of knowledge exists on which we can build; mice are very amenable to genetic manipulation, which is used a lot in this project; mice are mammals and their development more closely resembles that of humans than other vertebrates like fish (which for instance, do not have a four chambered heart like humans and mice).

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We are very aware of the need to reduce the number of animals used. It should be emphasized that where possible we use in vitro models of development, such as embryonic stem cells. However, the use of stem cells in a dish can only take us so far in our attempts to understand development as it cannot reproduce the complex tissue interactions that occur in the embryo, and for this reason we always have to go back to the mouse embryos as our model system. Where available we will seek to import genetic mice instead of making them.

### **Refinement**

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

### **Refinement**

Mice are the ideal animal for this type of study for several reasons: mice have been studied for a long time by geneticists, so a large body of knowledge exists on which we can build; mice are very amenable to genetic manipulation, which is used a lot in this project; mice are mammals and their development more closely resembles that of humans than other vertebrates like fish (which for instance, do not have a four chambered heart like humans and mice). Mice are the lowest mammalian species it is relatively easy to make genetic modifications in. To ensure their well being, all animals will be housed in the newly built pathogen-free animal unit for the duration of this Project and beyond. Here a dedicated team of technicians and members of the laboratory work towards ensuring the wellbeing of these animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 112. The neural basis of cognitive function</b>
<b>Key Words</b>	Cognition, behavioural neuroscience, frontal cortex, basal ganglia, psychiatric
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim is to identify how chemicals in different areas of the brain work together to generate the thoughts and behaviours that are commonly referred to as 'executive functions'. These are functions such as planning, goal-directed thinking, attention, expectation and anticipation. These functions are compromised to varying degrees in many neurological and psychiatric disorders as well as in the course of normal and pathological aging. Rats also have executive functions, albeit not as well-developed as in humans, but there are many similarities. By testing rats, we can learn about the similarities and differences in the brains of different animals, and this will improve our understanding of the impact of human diseases and aging on these functions.

**What 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 expectation is that short-term benefits will arise from improvement(s) of pre-clinical models, with validation by cognitive assessment. We work closely with scientists in drug companies with the expectation that we can improve research techniques for preclinical testing of new drugs. We hope that this in turn might enable the medium term benefit of progression of a new drug to clinical trials for the treatment of psychiatric illness.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 year project, we will use up to 700 rats, bred for the purpose.

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

A typical experiment involves measuring the behaviour of rats as they perform a particular tasks, which might be spontaneous behaviour (such as foraging for food in a maze or arena) or trained behaviour (such as pressing a lever for food). We measure changes in the animals' behaviour as a result of interventions such as permanent or transient inhibition of different brain circuits (for example, using brain lesions, neurochemical depletion, or drugs), which mimic the effects of clinical pathology or of clinical treatment. Some of these interventions (e.g., those involving surgery) are up to a 'moderate' level of severity, assumed to cause transient pain or distress. As for human surgery, painkillers are administered to reduce post-operative pain. During behavioural testing, the effects of procedures are sub-threshold or, at most, mild. We limit access to food prior to testing so that the rat is hungry and motivated to perform a task to get food treats, but they are maintained at a healthy weight and always fed a normal quantity of food daily even if they do not perform a task for food. At the conclusion of testing, the animals are humanely killed and their brain tissue may be taken for analysis post-mortem.

## Application of the 3Rs

### Replacement

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

### Replacement

We are interested in the brain processes underlying behaviour. It is not possible to study behaviour in anything other than an awake behaving animal, which includes humans. However, we cannot investigate the brain processes underlying behaviour in humans because it is not possible to systematically manipulate brain function in the same controlled manner that is possible in other animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

To ensure the minimum number of animals we try to obtain as much behavioural data from one animal as possible, for example, by testing them multiple times in the same task to improve confidence in the accuracy of measurements, and under multiple conditions (for example, before manipulations ('baseline') and after, to measure change in behaviour as a result of a manipulation.

Because the behaviours we use are initiated by the animal and 'self-paced', we can use 'rate of work' to indicate effort and willing. This provides an important 'check' on welfare: the animal stops when it wants to. Although we use food reward to motivate the animals, the rewards are 'treats' (e.g., sucrose pellets or cereal pieces) and it is not necessary to deprive the animal of food to make them work. We control access to laboratory chow by feeding them after testing, so that they are hungry but never starving.

We collaborate with a statistics advisor who offers support and advice in design and analysis and supports continual professional development, particularly in statistics. We are currently working with him to develop a novel approach to our data analysis using Bayesian inference. This will enable us to gain more information from the data, so potentially increasing statistical power and enabling a reduction in numbers.

## **Refinement**

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

## **Refinement**

Rats are the most suitable animals for this project because they are inquisitive and learn readily. Because there are brain circuits that have been conserved during evolution, the differences, as well as the similarities, between animals (e.g., humans and rats) provides important information about how behaviour is organised in the brains of different species and how this gives rise to different, species-typical, behaviour.

Our objectives - which rely on being able to measure behaviour - can only be achieved by minimising animal suffering as the rat will not perform the behavioural testing if it is overly anxious or in distress.

In humans, psychiatric symptoms (for example, hallucinations or depressed state) are themselves distressing. It is not possible to know the experience of a rat with perturbation of the systems presumed to underlie psychiatric symptoms in humans. However, it is not our intention to 'model' the entirety of the psychiatric syndrome and it is unlikely that this would be possible anyway. By addressing individual symptoms or symptom clusters, rather than modelling all aspects of the psychiatric syndrome, we try to minimise the severity experienced by an individual rat.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 113. Processes and pathways underlying ageing</b>
<b>Key Words</b>	Ageing, Longevity, Age-related disease, Progeria, Dietary restriction
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this project is to identify overlapping/shared molecular and cellular processes that act to modulate ageing rate in mice using a range of experimental interventions that either extend or shorten lifespan and the period of life free from age-related disease (healthspan).

The first objective will be to determine whether there is commonality (overlap) in ageing processes across different mouse models that show slowed ageing (e.g. mice harbouring mutations in specific genes known to increase lifespan and healthspan, or environmental interventions such as dietary restriction or every other day feeding that similarly impact positively on lifespan and healthspan). If commonality/overlap exists in a particular process across different long-lived mouse models then it provides confidence that this pathway is an important driver of mammalian longevity and healthspan. Thus, this pathway should provide a realistic point of intervention to extend lifespan and increase healthspan in mammals.

Our second objective will be to identify whether candidate processes identified in our long-lived models (Objective 1) can have a positive impact on mouse models of ageing (e.g. normal (chronological) ageing that mice experience over their lifespan, or models where the ageing rate is increased, typically using high fat diet feeding or using mouse models of progeria; progeroid mice are genetic mouse models that carry specific mutations in genes that can accelerate the ageing rate. This second objective will further support the notion that particular candidate pathways play a critical role in the ageing process of mammals.

Our final objective will be to use the information collected in Objectives 1 and 2 to design realistic interventions (treatment strategies) capable of modulating these shared/overlapping pathways. Ultimately it is predicted that these interventions will slow down the ageing rate in the mouse models described in Objective 2, and lead to an extension in lifespan and in healthspan.

The ultimate purpose of this project is that by using a focussed and realistic approach in model organisms, the information generated should help identify tractable interventions that may ultimately be used to help delay both age-related pathology and progeria-induced accelerated ageing pathology in humans and potentially in companion animals (e.g. dogs and cats).

**What 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 likely to derive from this project are as following. The protocols described will enable a better understanding of how different experimental interventions act to regulate the ageing process in mammals. This information in turn should help identify feasible, realistic and achievable interventions ultimately capable of delaying the onset of, and minimising the impact of, multiple age-related diseases that affect the quality of life in humans. It is hoped that given the perceived overlap in the underlying processes across different animals, it would be hoped that this information derived from this project may similarly help generate interventions to improve late-life health in companion animals, such as dogs and cats. In addition, the use of progeroid mouse models may also help to design interventions strategies that may potentially improve the quality of those individuals that suffer from the rare, but debilitating effects of progeria in humans; typically these are diseases that cause rapidly accelerated ageing-like symptoms and a range of diseases in young children and teenagers.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use laboratory mice in this project, and expect to use approximately 2600 over the 5 year duration of this programme of work.

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

The manipulations we will use will primarily extend lifespan and improve health, and so typically our animals will not be subjected to unnecessary suffering or illness. The likely/expected levels of severity will be mild/moderate, with the majority of mice being used to provide biological tissues following humane killing that will be subsequently used in cell culture and/or molecular biological studies. As mice age, like humans, they experience age-related pathology and it is critical to make

informed decisions as to whether an individual is experiencing adverse pathological effects rather than simply displaying the characteristics of ageing, but is in otherwise good health relative to its peers. We have designed clear humane end-points for all our ageing studies, which enables us to studying ageing and lifespan whilst minimising animal suffering. In line with our humane-end points, mice will be screened for changes in parameters such as body weight, body condition, general appearance, behaviour and appetite as they age. We will ensure that all reasonable steps are taken to intervene appropriately through humane killing, to achieve our humane end-points by making informed decisions during any ageing studies, in order that we maximise animal welfare and minimise suffering. The frequency of these screening, by experienced staff, will increase as the animals approach the limits of their healthy lifespan, which will be strain and context dependent. The experimental protocols within this project are based upon well-established methods refined to optimize experimental design and minimize animal suffering. We will carry out extensive literature searches throughout the time-scale of this project in an effort to continually improve and refine our experimental techniques used, in order to avoid unnecessary repetition of experiments, minimize suffering and whenever possibly identify appropriate replacements.

## Application of the 3Rs

### Replacement

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

#### Replacement

Ageing acts at the whole-animal level and will depend upon coordinated interactions across multiple organ systems within live animals. Thus these fundamental biological processes cannot all be appropriately captured in any other manner, for example through cell culture or computer modelling, although both approaches can certainly help inform and support. We will carry out extensive literature searches throughout the time-scale of this project in an effort to continually improve and refine our experimental techniques, in order to avoid unnecessary repetition of experiments or induce undue suffering. Wherever possible we will identify appropriate cellular, system-biology or statistical approaches to supplement our animal studies.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

I have extensive prior data on the variability in relevant experimental parameters related to mammalian ageing and will use these data to inform power analyses to establish the minimum numbers of animals required to obtain a reasonable effect size (significance) for any particular treatment. We will actively liaise with departmental or college statisticians in order that our studies are always undertaken using the minimal number of animals but retaining appropriate statistical rigour throughout. Any pilot studies will be run in such a way that they will be rolled in to the main study wherever possible, so that they are not additional to the numbers ultimately required for the main experiment. Many of the relevant processes involved in ageing are unlikely to be apparent before age-associated changes occur, and so to understand age-dependent and independent changes in particular parameters will require the study of animals across their natural lifespan. For our ageing studies, we have defined a clear set of humane end-points that will minimise the risk of suffering. We will make appropriate arrangements to randomly assign animals to experimental groups and blind studies wherever possible. Experiments will be planned so they can be published in accordance with the NC3Rs' ARRIVE guidelines. The genetically altered animals will be mice, and all suitable lines will be obtained from existing colonies, from collaborators or from a relevant supplier. In every case we will measure production and breeding performance and ensure the minimum numbers of animals are used in the programme.

### **Refinement**

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

### **Refinement**

Rodents are the lowest sentient animal model suitable for the study of mammalian ageing and this work builds on my previous research characterising the responses of mice to experimental interventions that almost exclusively impact positively on lifespan. Mice have been shown to be highly effective model organisms to study the basic mechanisms of ageing and have generated seminal findings on the physiological and genetic factors governing longevity. In addition, there are several advantages of using mice for understanding human ageing, such as the ability to easily manipulate genetic and physiological parameters, short generation times and the fact that they are maintained in highly regulated housing environments which helps to minimize experimental variation. I have a set of highly refined standard operating procedures for all laboratory procedures, and all regulated procedures which ensure a consistent quality in the generated data streams. All equipment is

regularly serviced and calibrated ensuring accurate, low variability data are generated. All staff will be trained in the standard operating procedures to ensure consistency in the data output.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 114. Effects of chronic intermittent hypoxia on carotid body and cardiac function and the exacerbating effect of poor glucose control and obesity</b>
<b>Key Words</b>	Breathing, Heart, Blood glucose, Hypoxia
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

To investigate the effects of repeated low oxygen levels seen in obstructive sleep apnoea (OSA) patients on oxygen and carbon dioxide sensors in the neck and heart function and how this affects control of blood glucose 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)?**

OSA is a disease in which patients periodically stop breathing during sleep. It is linked to obesity and type 2 diabetes with multiple adverse health outcomes in common. High blood pressure (hypertension) is strongly associated and might be caused by changes in the function of the carotid bodies (sensors that respond to changes in oxygen and carbon dioxide levels in the blood as well as other substances). Chronic changes in carotid body function may cause higher background sympathetic nerve activity resulting in the development of heart disease. Additionally, poor control of glucose (seen in diabetes) is linked to damaging oxidative stress that also may contribute to the altered function of the heart or carotid bodies. This licence will help unravel the changes seen with repeated periods of low oxygen (when breathing stops in OSA) on the control and function of the cardiovascular and respiratory systems.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

1000 Mice/rats will be used over the course of 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 levels of severity? What will happen to the animals at the end?**

Exposure to repeated short periods of low oxygen (intermittent hypoxia) induces long-term changes to the cardiovascular and respiratory systems, however, we have never seen any observable adverse effects and the animals exhibit normal behaviour. Measurements made on awake animals are short-lasting and generally non-invasive (eg nothing greater than a needle prick). Tissue collection for *in vitro* experiments or measurements made on anaesthetised animals are all carried out under terminal anaesthesia and so the animal will not suffer any pain or distress.

## Application of the 3Rs

### Replacement

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

#### Replacement

To determine how the cardiovascular and respiratory systems work together and the nature of reflex responses requires a whole animal that has sensory inputs (carotid bodies) and relevant outputs (heart function or blood glucose levels) to understand control mechanisms.

We will continue to review the published literature so that we will be aware of any developments in this area of research where *in vitro* techniques could replace animal use. We will use the

NC3R's systematic review tool to help with searches

[www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf](http://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf)

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Use of isolated tissues will be made where possible to investigate mechanistic questions and this reductionist approach will generally allow smaller numbers of animals due to less biological variability. In whole animal experiments, collection of as much data as possible from each animal will allow greater interpretation of the data and reduce the need to carry out multiple separate experiments to answer the scientific question. We will use the NC3R's EDA to help design our experiments and we will publish in peer reviewed journals that support the ARRIVE guidelines to share out findings with the wider scientific community.

### Refinement

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

### **Refinement**

The integrative physiological nature of the research in this licence requires the use of whole animals, however, rodents have been selected as the lowest order of animals displaying the complexity required to interpret the results in the context of human physiology. Where the scientific questions can be answered without using live animals (eg in isolated tissue) this will be done. Where whole animals are used, these will usually be done under terminal anaesthesia to remove any animal suffering whilst allowing the maximal amount of data to be generated. In experiments where conscious animals are used (eg measuring ECG or breathing), steps are taken to minimise the stress on the animal. These include familiarising the animal to the chambers prior to carrying out experiments, transplanting some home cage bedding to the chambers to increase the familiar scent of the chambers, keeping the measurement duration as short as possible, keeping animals in group housing except when experiments require short periods of single housing for measurements. All procedures are kept under review and new refinements published will be incorporated whenever possible.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 115. Reagent production and screening: Immunological Toolbox</b>
<b>Key Words</b>	Immunological tools, Antibodies, Reagents, Vaccines, Immunity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The primary focus of this project is to address the acute shortage of experimental tools (called reagents) that are available to study veterinary immunology. By developing new tools with which to study avian and mammalian biology this will enable work within these fields to progress more rapidly, increasing our understanding of animal models and systems. We aim to improve immunological knowledge which will benefit vaccine and diagnostic reagent development. An important type of tool that we use to measure the immune response are antibodies: these recognise and bind to specific parts of cells or their products (targets). We can measure whether these antibodies bind to their targets as a way to determine whether certain cells or responses are present in animals that have disease or have been vaccinated.

The major output will be the generation of these highly specific antibodies. This will be of value to researchers within the veterinary immunology community. The requirement for, and impact of, these reagents will be assessed and prioritised by a steering committee overseeing Immunological Toolbox activity. This will ensure that the reagents made in animals will be of impact to the wider community. Alongside this we will engage with industrial partners for distribution and commercialisation.

**What 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 focus of this project is to address the acute shortage of veterinary immunology reagents currently available. By generating new tools and reagents we will increase the capability to understand specialised cell populations, their products

and functions. In the medium term (3-5y) this will allow us to determine how these cells and their products influence the interaction with, and control of disease causing microorganisms or the response to vaccines. These reagents may also be used to define immunological correlates of vaccine induced protection which can then be used to identify candidate vaccines for disease protection studies. Longer term (up to 10y), we expect the data from the short and medium-term goals to feed directly into the development of vaccines or diagnostic tests for strategically important diseases of livestock.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over 5 years we expect to use approximately 100 mice.

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

The procedures carried out under this project licence are of mild severity with no expected adverse effects as each animal will receive a small number of injections and blood samples will be taken. At the end of each carefully planned study animals will be humanely culled.

## Application of the 3Rs

### Replacement

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

### Replacement

The generation of highly specific antibodies cannot be achieved without the use of mice as the cells and processes required for their induction require whole body systems. We have processes in place to identify alternative methods by screening existing reagents or non-animal tools that may work in some circumstances. We will always investigate alternatives to the use of animals as our first step before progressing to experiments in animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Good principles of experimental design will be used to ensure that we used the minimum number of animals to achieve robust and reliable results. All of our experiments are officially reviewed by a named vet, animal care technicians and a statistician before they can be undertaken. These study protocols include aims,

numbers of animals, treatments, assessment of adverse effects, end points and our data analysis methods which are carefully scrutinised.

We will archive samples to allow additional analyses to take place without the need to repeat experiments in animals.

### **Refinement**

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

### **Refinement**

Mice are routinely used for the generation of monoclonal antibodies and the procedures used are well established in this species. This results in robust, reliable outputs.

The procedures will be of mild severity and animals will be closely monitored during, and after, experimental procedures by experienced staff. This will enable us to minimise harm.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 116. Development of Medical Countermeasures for Licensure</b>
<b>Key Words</b>	Licensure, High Severity Pathogens, Treatments, Vaccine, Antitoxin, Toxin
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This licence aims to produce data for regulators so that new medical treatments for high severity pathogens and toxins can be licenced for use in humans. It enables safety checks for the production of these new treatments, and checks of pathogens and toxins in order to determine the hazard they present.

These treatments may include vaccines or antimicrobial agents (such as antibiotics for bacterial infections) or antitoxins (for toxin poisoning).

There are no routine clinical populations for these pathogens and toxins, and so animal studies are required as a measure of effectiveness of the new 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)?**

These studies will support the granting of licences for use of new vaccines and medical treatments for humans exposed to high severity pathogens or toxins by ensuring that they are safe and effective before being used in humans. These studies also support validation of in vitro alternatives to animal studies, which could replace some current animal studies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to 17400 mice and 4850 rats over the whole period of the licence (5 years).  
 Vaccine or antimicrobial studies: up to 6000 mice. Antitoxin potency checks: up to 5000 mice. Pharmacokinetic studies: up to 1000 mice and 350 rats. Antitoxin efficacy studies: up to 3500 mice and 3500 rats. Toxoid checks: up to 400 mice. Agent toxicity/potency checks: up to 1500 mice and 1000 rats.

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

There will be five types of study: 1. Potency – this allows comparison between different batches of treatment. 2. Efficacy – a direct measurement of how effective the treatment is against the disease. 3. Toxoid inactivation – this is the production of a non-toxic product which will still be recognised as the toxic material, for the generation of antibodies to the toxic material. 4. Pathogen/toxin checks – there may be occasion where it is necessary to demonstrate the hazardousness of the infectious agent/toxic agent. 5. Correlates of protection studies - studies to inform the way in which the animal's body responds to the test substance. Following exposure to infectious agents or toxins, animals will display signs of disease/intoxication associated with them. These may include weight loss, changes in posture, slowing of movement, changes in breathing patterns, fluffing of the fur, closing of the eyes, hunched posture. Wherever possible, these signs will be used as 'humane end points', preventing the animals from suffering for prolonged periods. It is likely that animals will undergo Severe signs following exposure to pathogens or toxins, but the proportion will decrease as our understanding of the course of disease/intoxication improves and the Humane End Point is refined. While every effort has been made to ensure that animals do not die as a result of infection/intoxication, the novelty of the infectious agents and toxins, the experimental nature of the medical countermeasures, and the requirements of some of the experiments (lethal dose determination studies, if required) may result in animals dying during the study. As the animals will be exposed to infectious diseases or toxic substances, it will be necessary to humanely kill them at the end of the study.

## Application of the 3Rs

### Replacement

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

### Replacement

In order to demonstrate that the medical countermeasures being developed are effective, a complete physiological system is required. This requires the use of whole animals.

Wherever they are available, non-animal tests will be used instead of whole animal studies, and the information which is generated from this work will be used to develop non-animal techniques. An example of this a non-animal potency test which will be compared with data from this project to demonstrate that it could be used to replace animal work.

Unlike most diseases, there is no patient population where these treatments could be tested.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The number of animals used is based on the size of effect, and based on advice from a statistician. The group size will be the minimum necessary to achieve the requirements of the study.

Wherever possible, the studies will use a factorial experimental design. In a factorial design, the information each study produces is maximised, and reduces the need for unnecessary additional animal studies. Wherever possible, a single control group (animals receiving no treatment) will be used across multiple studies.

## **Refinement**

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

### **Refinement**

For the diseases and conditions that are under examination in this licence, the majority of studies will use mice. The mouse is the 'lowest' species which can demonstrate the same signs of disease and progression of disease as the human. Rats will be used instead of mice for some studies, for example where test substances need to be delivered over a period of time into a blood vessel (intravenous infusion).

Humane end points have been determined which will allow an animal to be euthanised before experiencing the most severe effects, and these will be improved by increasing the number of animal checks at the times they are most likely to show signs of disease.

The rats and mice will be acclimatised for at least 5 clear days prior to experimentation and will be handled during this period. Enrichment will be provided in the cages eg Dome homes, bedding materials.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 117. The development and function of ectodermal appendages</b>
<b>Key Words</b>	Hair, Feather, Skin, Regeneration, Development
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project aims to understand how the skin of mammals and birds develops and sustains its ability to heal itself.

The first aim is to understand how the embryonic skin produces different structures, such as hairs, feathers and scales through different signals that pass between cells. Some of these signals have been identified, but how they work at different stages of development and in different species is not known.

The second aim is to understand which adult body parts different embryonic structures develop into so that an understanding of the construction of the skin's different components is achieved. Some tracing of these relationships have been done, but new tools allow this to be done much more accurately and trace the boundaries between different regions in a more refined manner.

The third aim is to understand how skin sustains and heals itself by tracing the origin of the cells involved in healing. This process has been well described for mammals, but the means by which birds heal skin wounds is little understood at a cellular level.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

From this study of the basic biology underlying the development of the skin and how it is healed, we will provide knowledge that can be used: - to understand, and perhaps design treatments for, conditions humans are born with that affect the skin. This is through study of communication between cells and the potential to mimic or block these signals to help skin develop along the normal route. -to improve breeding of farm animals, particularly the chicken, a species in which feathering has important effects on heat tolerance. If we can understand how feather number and type is controlled we can use this information to breed birds with the appropriate number of feathers for their conditions. -to improve our understanding of wound healing in birds, potentially aiding in poultry production and welfare, due to the frequency of breast

skin lesions occurring in commercially produced chickens and turkeys. In particular, understanding whether feather follicles aid in healing or not will be useful to veterinarians managing and deciding treatments for birds with skin wounds.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use, in breeding and in experiments, up to 1000 mice per year, 100 rats per year and 110 chickens per year over the entire 5 year course of the project.

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

A majority of the experiments will have mild effects on the animals, primarily involving either innocuous labelling of cells so that they can be detected and tracked, or altering the structure of the hairs, feathers, glands and possibly teeth of the animals. Creating a small (up to 1 cm) skin wound in a chicken, to determine how it heals, carries a low risk of infection, which we will minimise by use of good surgical technique. Pain relief will also be given to chickens in these studies. To understand the relationship between embryonic and adult body parts we will do some transplantations of small pieces of tissue between chick embryos at a very early stage of development. The transplanted tissue can become a normal part of the embryo, which we can then identify later. If the chick did not develop normally it would be culled humanely. All animals will be humanely culled at the end of the experiment in which they are used.

## Application of the 3Rs

### Replacement

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

### Replacement

We can not use non-vertebrate animals as the vertebrate skin has a unique structure and appendages, like hairs and feathers, that simply do not form on other types of animal. The skin and its associated structures are composed of many different types of cells interacting with one another throughout development. This complex environment can not be mimicked by a culture system. However, as far as possible we will perform experiments on cultured skin collected from culled animals, rather than the intact animal itself. Also, we will complete many experiments using embryos only, rather than manipulating adults directly.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Experimental designs will be developed with advice from our institute statistician. We will reduce variation in our experiments by maintaining animals in a constant controlled environment and by maintaining, as far as possible, inbred lines of animals so that genetic variation is reduced. This will allow effects of experiments to be detected with fewer animals used compared to populations in which there is higher genetic or environment-derived variation. The number of animals required to maintain each experimental line will be kept to a minimum.

### **Refinement**

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

### **Refinement**

The mouse, chicken and rat already have suitable genetic resources, that is, mutant animals or genetically modified animals in which cells can be tracked, to allow us to address the scientific questions that we have set.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 118. Identification and characterisation of therapeutic targets for cardiac disease</b>
<b>Key Words</b>	Heart, cardiac function, cardiac structure
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The leading cause of premature human death in the UK is cardiac disease. A common end point for many cardiac diseases is heart failure.

This programme of work aims to investigate the changes in gene expression that occur after a range of cardiac diseases that lead to heart failure. This information will be used to determine the implications of these genes for cardiac structure and function and thereby their therapeutic potential to prevent heart failure.

**What 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 increasing our understanding of the science underlying the functional and structural changes to the heart that occur after a range of cardiac diseases we can inform the development of therapeutic strategies to prevent heart failure. Such treatments would reduce the socioeconomic burden of heart failure in the UK and improve the quality of life of patients living with heart disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 24,500 mice, 15000 rats and 600 rabbits over 5 years may be used.

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

Rats/mice/rabbits will be kept in a tightly controlled environment with excellent welfare conditions. Mice/rats/rabbits may undergo either surgical occlusion of arteries in the cardiovascular system to induce heart damage, administration of substances that

alter the function/structure of the heart or viral infection. The adverse effects of these procedures may include heart failure. Minimally/non-invasive animal imaging and assessment of heart pump function and heart rhythm disturbances will also be performed but have little adverse effects. The techniques to assess cardiac function and structure may involve injections, imaging and general anaesthetic.

Organ/tissue/cell assessment will then be performed in order to establish the gene changes that occur after disease, functional consequences of the intervention and detailed structural and biochemical analysis. Some of these procedures require complex microsurgery and would be classed as a major procedure but our expertise and care of the animals ensures that any pain, suffering and unexpected deaths are minimised. The duration that the animals will be exposed to cardiac disease will be the minimum required to obtain sufficient data about the acute and chronic changes in cardiac structure and function. At the end of the procedures the animals will be killed humanely by anaesthetic overdose or cervical dislocation/concussion.

## Application of the 3Rs

### Replacement

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

#### Replacement

It is difficult to obtain viable human heart muscle including suitable non-disease human heart muscle. There is considerable variation in age, medication and underlying pathology of any obtainable human tissue and there is the likelihood of progressive disease being present. It is also not possible to investigate the processes at well-defined time points after a single incidence of damage. Substantial prior and continuing organ/tissue/cell experiments will inform and limit the number of animal experiments required and where possible as much information from one animal will be obtained.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We have considerable expertise in minimising the number of animals required for experiments whilst ensuring generation of robust data as evidence by our publication track record. We will use advice from statisticians in our institute where required. Samples sizes will be set from our knowledge of the literature, pilot experiments, previously performed experiments and statistical analysis

The flexibility of being able to use mouse/rat/rabbits is aimed at reducing and refining the number of animals used rather than increasing them. Experiments will not be repeated in both species where unnecessary. The decision as to what species is to be used for a particular set of experiments will depend upon a clear decision at that time as to whether the use of the species tissue with the particular technique maximises the ability to detect a difference between experimental groups for each measure and hence decreases the numbers of animals used.

### **Refinement**

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

### **Refinement**

The rat, rabbit and mouse represent the lowest mammalian vertebrate group with which the scientific community have been able to fully characterise that the alterations in cardiac structure (including ventricular remodelling) and function in disease models such as coronary artery ligation. The models chosen closely resemble the pathophysiological changes in human heart disease. There is no alternative to using these models however, we will continue to utilise our current laboratory animal and organ/tissue/cell data to inform whether for particular aspects of the project severe procedures are required or whether the information we seek can best be obtained using protocols of lower severity.

We will constantly review the literature for ways to refine the severe disease models.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 119. Regulation of DNA replication and damage by small protein modifiers</b>
<b>Key Words</b>	DNA replication, genomic instability, ubiquitin, DNA damage, SUMO
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overarching aim of this project is to understand the regulation of DNA replication by small protein modifiers (e.g. ubiquitin and SUMO). We especially focus on understanding how the final stages of DNA replication are executed, as our understanding of this stage is, at present, very limited. Ultimately, we would like to use generated knowledge to inform research on potential targets for 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)?**

Problems during duplication of our genome are a major reason of development of cancer, neurogenerative disorders and aging. It is essential that we understand in depth how this process operate to be able to target its components for cancer and other disease therapy.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use female *Xenopus laevis* frogs to lay eggs which we use for preparation of egg extract. Egg extract provides us with a cell-free simplified model to study biochemistry of DNA replication. DNA added to such extract can undergo a whole round of synchronous DNA replication, which is regulated in a manner analogous to human cells. We stimulate frogs to produce eggs by hormone injections, collect the eggs and return the frogs to the tank. Frogs can be stimulated to produce eggs again after 3 months. We will stimulate frogs to lay eggs only when we need to prepare next batch of egg extract. We expect to maintain maximum of 100-150 frogs laying eggs every 3-4 months (or less) for the duration of this license.

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

The procedures carried out have no expected adverse effects and are of mild level of severity. After producing eggs frogs will be kept in tanks for at least 3 months before next egg production. Finally, when the quality and/or quantity of produced eggs declines frogs will be culled by schedule 1 method. Practically, we are likely to use the same frogs for 2-3 years and then exchange them for younger ones.

## Application of the 3Rs

### Replacement

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

### Replacement

*Xenopus* egg extract provide the only higher eukaryotic cell-free system capable of efficiently supporting cell cycle progression *in vitro*, it is therefore indispensable for a biochemical studies of DNA replication process. Due to its unique characteristic we can carry our analyses impossible using immortalised human cell lines. However, once we carry out such procedures we will continue our research in human cell lines.

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.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Reduction in animals use is achieved by maximising egg yield in the procedure by optimising husbandry and handling. Re-use of females is requested on the basis that the procedure itself is considerably less stressful than the act of importing them into the animal house. It allows also females to settle into the egg laying cycle increasing the egg yield

### Refinement

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

### **Refinement**

The hormone injection procedures have been refined to ensure only minimal and transitory discomfort to the animals.

As we require cell-free system for our biochemical analyses of higher eukaryotes DNA replication, *Xenopus* egg extract is the only such system available to date.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 120. Neuroendocrine mechanisms regulating appetite, body weight and growth.</b>
<b>Key Words</b>	obesity, body weight, appetite, hypothalamus
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The body has an internal clock that controls our basic physiological functions. It is well known that disruption of this clock, such as shift work, can lead to severe long-term health problems including obesity. The clock is located in the hypothalamus of the brain and synchronises circadian rhythms throughout the body. The hypothalamus is also the brain centre that is important in regulating appetite and body weight. The objectives of this study are to investigate how the hypothalamus regulates long-term changes in energy balance, how this is linked to the internal clock and why circadian disruption can lead to disturbances in appetite regulation.

Using an animal model which is able to naturally and reversibly vary its body weight and food intake by simply changing the daylength, we will investigate how the hypothalamus changes in the natural response to daylength, and compare these to a stress response, such as high fat feeding (objective 1). This will help us to untangle the mechanisms involved in the regulation of body weight (related to growth) from those involved in diet-induced obesity. We will investigate how photoperiod can influence the structure of the hypothalamus (objective 2) and how disrupting the biological clock, e.g. by using light cycle phase shift to mimic the experience of shift workers (objective 3), affects the hypothalamus. These experiments will allow an understanding of the mechanisms involved in the control of body weight regulation and will give new insights to help us understand why disruption of the biological clock can lead to obesity and how this can be reversed. Behavioural changes in response to photoperiod and/or diet will allow us to develop a better understanding of the mechanisms that underlie our motivation to eat (objective 4) and link this to the molecular and physiological responses identified in objectives 1 to 3.

The overall aim of the project is to help us understand what goes wrong in obesity and how this may be reversed and will allow us to identify new medicines for potential use in the treatment of obesity and related diseases (e.g. diabetes).

**What 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 work will be to identify the mechanisms involved in the regulation of body weight and energy balance. This will give new insights to help us understand how environmental factors such as diet and light disrupt the control of energy balance in the obese brain and how this may be reversed.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats, approximately 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 levels of severity? What will happen to the animals at the end?**

Expected adverse effects associated with brain surgeries are loosening or blockage of brain cannula or wound infection and these will be minimised by using good, aseptic surgical practise. Wounds will be checked daily and infections will be treated as advised by the veterinary surgeon. After surgeries animals are checked hourly until full recovery. Animals showing signs of distress after surgeries will receive veterinary attention or will be humanely killed. We will investigate drugs related to body weight and appetite regulation and these might result in mildly obese phenotypes or animals might be less hungry and this might result in weight loss. Body weight and food intake will be checked at least three times per week. Injections and infusions into the brain have a moderate risk of increased brain pressure, haemorrhage or development of meningitis. This will be minimised by careful examination of the available literature to identify potential adverse effects before drugs will be used the first time. Animals will be closely observed during and after injection or infusion of drugs. If animals show distress or other abnormal responses, administration will be stopped and veterinary assistance will be sought. Discomfort or stress due to implantation of minipumps for drug delivery will be minimised through deployment of experienced staff and through the appropriate use of pain relief medication. We do not expect adverse effects after photoperiod manipulation. The behaviour tests that the animals perform will not have any adverse effects. Expected adverse effects for dietary studies include mainly weight gain or loss. Our experience with this rat model indicates that in the proposed type of studies rats rarely experience symptoms beyond mild discomfort. The likely/expected level of severity of the procedures is mild to moderate. At the end of the experiments animals will be killed humanely and tissues will be fully utilised.

## Application of the 3Rs

### Replacement

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

#### Replacement

The purpose of this work is to produce an understanding of the mechanisms of obesity and of its links to diseases, and has the potential to produce new insights to develop effective interventions to prevent and treat these conditions. In order to achieve the goals of this proposal it is vital to study the effects of nutritional, endocrine and/or pharmacological manipulations on physiological systems. These manipulations can only be performed *in-vivo* as there are no alternative methods available at present. In all instances, cell culture and slice culture will be used as much as possible and animals will only be used if preliminary work supports further investigations.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The project has been designed based on preliminary data conducted in my laboratory and the number of animals for each study has been carefully calculated based on power calculations conducted. From experience we estimate that 6 to 9 rats will be sufficient to perform the proposed experiments. If we gain significantly relevant information with fewer animals over the course of the experiments, the study design will be changed accordingly.

### Refinement

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

#### Refinement

We will use the photoperiodic-sensitive F344 rats which show pronounced changes in body weight and appetite by simply changing the daylength. This is a natural response to photoperiod and allows us to investigate energy balance regulation in a healthy model. Non-photoperiodic rats will be used to confirm the broader relevance of our studies. Anaesthesia and analgesia will be used to limit suffering. Daily handling of rats prior to ICV injections of alert rats will minimise stress level and allow injections of non-restrained animals. Where appropriate, osmotic minipumps will be used to avoid daily injections over a longer period of time.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 121. Macrophage differentiation and the regulation of inflammation</b>
<b>Key Words</b>	Inflammation, Macrophage
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The key aim of this programme of work is to understand how inflammation and tissue repair are regulated in living animals.

Between 1 and 2% of the UK population suffer from the autoimmune disease Rheumatoid Arthritis (RA), which is characterised by joint pain, chronic inflammation and tissue damage. Chronic inflammation is the driving force behind many other important human diseases including cardiovascular disease (CVD), which is caused by the development of hotspots of inflammation in the major arteries which lead to heart attacks and strokes.

New medicines that directly target the inflammatory component of RA such as anti cytokine antibodies are in widespread clinical use for patients with the most severe symptoms. However, there remains an unmet clinical need because less than 50% of RA patients treated with anti-cytokine antibodies exhibit sustained improvements in their disease symptoms.

An important scientific unknown is how is the physiological process of inflammation is regulated. One of the main objectives of this programme of research is to answer the question 'What are the molecules and pathways that regulate the magnitude and duration of an inflammatory response in vivo?' In this 5-year programme of work we have designed experiments to critically test one or more pathways to see if they play an essential role in regulating inflammation and tissue repair in living systems.

Macrophages are a cell type that is important in both the initiation and resolution of inflammation. Macrophages also orchestrate tissue repair, a process that is currently

not well understood. In our experiments we will identify molecules that enhance macrophage tissue repair processes by using cell-based assays in the laboratory. We will then test candidate molecules in simple models of tissue inflammation in mice before selecting one or more molecules for further development. The ultimate goal of this project will be to identify drug-like molecules that might find applications in clinical and veterinary medicine. We have established a good team to realise this goal over the next 5 years.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Advances in basic science knowledge derived from this project of work will help the scientific community to identify (and exclude) conserved pathways that regulate inflammation. Our experiments are designed to identify and validate novel targets for the development of new anti-inflammatory drugs. There is a clinical unmet need to develop more effective anti-inflammatory drugs to treat chronic inflammatory diseases such as rheumatoid arthritis and psoriasis. Many human diseases have a strong inflammatory component including atherosclerosis, a disease process in arteries that directly leads to heart attacks and strokes. Pharmaceutical companies are testing different classes of anti-inflammatory drugs alongside statins to see if they can reduce the burden of cardiovascular disease in an increasingly elderly population. Our basic science studies of macrophage differentiation and inflammation biology could lead to the development new therapeutic strategies for improving tissue repair processes. Our work may identify new treatments for chronic non-healing wounds that currently affect ~1% of the UK population. An important emerging idea in the scientific literature is that a failure to appropriately regulate inflammation can lead to changes in behaviour, notably an increase in anxiety. In addition to studying classic inflammation readouts like white blood cell recruitment and activation in our experiments we will carefully monitor changes in animal behaviour. It has been estimated that 8% of the UK population suffer from debilitating anxiety at some point in their lives and there have been no new classes of drug for the treatment of anxiety have been developed in the past 20 years.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

All our studies will be performed with wild-type and genetically altered mice. We expect to use up to 30,000 mice over a period of 5 years.

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

The majority of the animals in this study will be used in breeding programmes to generate genetically altered animals to perform critical tests of how inflammation is controlled in vivo. We anticipate that ~80% of procedures will be at a mild or sub-threshold level of severity with the rest being at a moderate level of severity. The

anticipated adverse effects will be transient pain and brief discomfort following injection of cells or substances into the bloodstream or into the peritoneum. Some animals (no more than 10% of total over 5 years) will undergo general anaesthesia for a period of 10-15 minutes or less for imaging, injections under the skin or implantation of slow release devices for continuous drug delivery. Animals may suffer a brief period (1 to 2 minutes) of disorientation following inhalation anaesthesia. All our animal behaviour experiments will use non-invasive techniques and over 50% of experiments will look for changes in behaviour without using aversive stimuli. Any animals that display discomfort or abnormal sickness behaviours will be killed by a humane method. All animals will be humanely killed as soon as possible after we have obtained all the required data outputs needed to complete the study.

## Application of the 3Rs

### Replacement

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

#### Replacement

In the past 5 years we have identified novel features of the host inflammatory response using genetically modified animals that could not have been predicted from the existing literature and the use of cultured cells. However, we will continue to use primary cells derived from human blood and mouse bone marrow to perform *in vitro* experiments to study gene expression and macrophage biology, for instance cell migration assays. In recent years we have adapted our cell biology assays and we now make greater use of bone marrow derived macrophages rather than cells from living animals.

We will use literature searches, internet data resources such as the ImmGen gene expression database, chemical data mining resources and information from organisations such as NC3Rs to inform our experimental design.

We recently initiated local collaborations to study highly conserved processes such as uptake of bacteria and debris in fruit flies, but there are significant differences between inflammation in mammalian and non-mammalian systems.

To extend our findings from *in vitro* studies using cell lines and model organisms we need to study inflammation *in vivo* in mice in order to extend our studies towards application in man.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Every effort will be used to reduce the number of mice used. We will use the most efficient breeding strategies to ensure that we do not generate an excessive number of genetically altered mice for our planned *in vivo* experiments. We will also explore the use of new gene editing techniques to make genetically altered mice with changes in two or more genes at the same time. This could greatly reduce the number of animals used in breeding programmes.

Where pilot data exist we will perform statistical power calculations to calculate the minimum number of experimental animals to obtain statistically meaningful results.

Where possible we will use sperm and embryo freezing as alternatives to long-term colony maintenance.

## Refinement

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

## Refinement

Human diseases characterised by unregulated chronic inflammation such as rheumatoid arthritis (RA) cause long-term pain and suffering. Ultimately we want to develop new drugs to treat this important unmet clinical need. Pre-clinical RA disease models that directly mimic joint inflammation cause long-term pain and suffering to experimental animals. For that reason we have chosen to use a suite of experimental protocols that allow us to derive good datasets on leukocyte mobilisation and activation *in vivo*. The protocols in this PPL have genuine relevance to the basic biological processes that drive human inflammatory disease and our animal work will inform future translational research. The experimental approach set out in this PPL therefore represents a very significant refinement in terms of animal welfare.

The mouse is the most appropriate species to conduct this research programme as it is the lowest vertebrate species to give an inflammatory response similar to that seen in humans. The type of inflammation we generate in mice shares many features in common with acute and chronic inflammation in man.

We have modified our experimental protocols so that implanted mini-pumps or *in vivo* gene delivery will replace repeated injections of proteins or drugs. This will reduce animal suffering and improve the quality of our experiments by reducing day-to-day variation in drug dosing.

For all our experimental protocols we have listed clearly defined action points and humane end points to reduce animal suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 122. Regulation of Glomerular Barrier Function in Health and Disease</b>
<b>Key Words</b>	
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall focus of this research is to determine how the kidney filters are maintained in health and disrupted in disease. The experiments I propose will improve our understanding about the biology of kidney filters before applying this knowledge to test therapies that will prevent or stabilise the effects of kidney disease in animal models. These studies will be the prelude to human studies of new treatments for kidney disease.

- We aim to find early biomarkers for kidney disease. We have already shown that mice with kidney disease show very early changes in the kidney filters using a powerful technique called proteomics and we can now test whether these biomarker changes provide an early warning about disease.
- We aim to find the molecular mechanisms that cause kidney disease to progress in severity. We have shown that disrupting the ability of the kidney filters to respond to mechanical forces can affect the progression of glomerular disease and we now want to find ways of protecting the kidney filters from these forces.

Using the knowledge we acquire from the above aims we expect to identify and test new therapies for kidney disease in zebrafish and mice.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Chronic kidney disease (CKD) is a huge public health concern, affecting more than 10% of the global population and substantially increasing their mortality. When kidneys fail, renal replacement therapy with dialysis or transplantation is necessary

but costs are escalating and replacement therapies are not universally accessible. Strategies to improve early detection of CKD and targeted therapy to prevent disease progression would have significant impact on improving human health. This research programme aims to identify early disease biomarkers and also new therapies for early intervention in CKD. As such this research could have significant impact on the early detection and treatment of kidney disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice 7000 over five years 4000 adult zebrafish

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

The project has a 'Moderate' severity level. We will use breeding programmes so that, typically, clinically healthy parents (each carrying a mutant gene) are mated to produce litters containing animals with two mutant genes. The latter animals will have kidney disease. Glomerular injury will be induced by removing kidney tissue or the administration of substances such as chemicals, peptides or antibodies. Therapies such as chemicals, peptides, antibodies or non-harmful virus vectors – which deliver genetic material into cells, will be delivered to animals. In experiments, when the interventions are mild or moderate, we will cautiously follow the progress of mice in the 8 months after birth. Should signs of ill health become apparent, the animal will be killed by a humane method.

## Application of the 3Rs

### Replacement

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

### Replacement

We aim to determine whether new therapies can prevent or treat kidney disease. Cell culture models alone provide limited insights into mechanisms of kidney disease and response to therapies. Currently there is no alternative to using live animals for preclinical models. In addition the administration of treatments to whole animals will ensure that we can detect any (albeit unanticipated side effects) on other organs.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

In order to minimise the numbers of animals in our experiments we will use carefully determine the number of animals that are needed for experiments together with REDACTED. In the maorinty of cases we will need to start with pilot studies using less than five animals in each experimental group. Important experimental results will be repeated or validated via an alternative follow-up experiment to minimise the likelihood of spurious nonreplicable results.

Sources of variability will be considered at all stages of the experimental design. For mice we will consider the genetic background and sex of the animal carefully when designing the experiment and choose animals that are appropriate to address the specific research question for a particular experiment. We will also consider the variability of experimental observers and where possible will allocate one observer to each animal experiment.

## **Refinement**

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

### **Refinement**

At present, the mouse represents the best or most refined species with which to test the efficacy of new therapies for glomerular disease. It has a kidney of similar structure and anatomical complexity (e.g. with glomeruli and branching collecting ducts) to human organs. However our inclusion of zebrafish studies will allow us to refine the number of studies in mice. Our experiments are proposed in mice after they are born when they will be closely monitored. Particular attention will be paid to

their weights and behaviour. Should these parameters deviate markedly and/or persistently from normal, mice will be humanely killed. For all experiments in animals we will use good experimental conduct with the appropriate use of post operative analgesia for surgical interventions and the appropriate species specific management of animals during and post anaesthesia.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 123. New targeted nanomedicines for cancer therapy</b>
<b>Key Words</b>	Cancer therapy, Gene delivery, Tumour targeting, Delivery systems
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this work is to develop new delivery systems able to carry anti-cancer therapeutic DNA and drugs specifically to the tumours, without secondary effects to normal tissues.

The objectives of this study are:

- to characterise novel drug and gene delivery systems *in vivo*
- to determine the efficacy of anti-cancer therapies delivered by these systems
- to determine the ability of these systems to reach the brain following intravenous administration

**What 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 least 1 in 3 people in the UK will be diagnosed with cancer during their lifetime. Therefore, improved treatment of cancer would greatly reduce suffering and save many lives. The efficacy of conventional therapy is often limited by its difficulty to selectively reach tumours after intravenous administration, without secondary effects to normal tissues. Developing novel targeted treatments for cancer will not only kill the tumour cells but minimise the death of normal cells in the body. They will therefore reduce the painful side effects associated with conventional therapies and improve the likelihood of patient survival.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice are the species of choice for these studies because they demonstrate many features of the human diseases and the genes involved are common to both species. We expect to use a maximum of 630 mice per year. The number of animals to be used is the minimum that will give any statistically significant results. If fewer mice are needed to get the results required, then lower numbers of 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 levels of severity? What will happen to the animals at the end?**

New delivery systems will be extensively tested in cell culture and only those of proven efficacy are advanced to in vivo studies, first to establish suitable dosing, then to evaluate their biodistribution. The delivery systems showing suitable biodistribution will then be tested for efficacy in a tumour-bearing animal. Efficacy will be measured as tumour growth delay, by calliper measurement of subcutaneous tumours. A pilot study with just a few animals will indicate if further work would be appropriate. In all experiments the mice will be monitored closely to ensure that no unforeseen adverse reactions cause distress to the animals. Tumours will be established in mice by a single injection of cancer cells and allowed to grow until they reach a suitable size for distribution and therapy studies. Tumour growth will be measured before and after treatment to determine the response to the novel therapy. The mice will be continually assessed for any (rare) signs of distress. We will take every measure to avoid any animal suffering. Following humane killing of the animals, tissues such as liver, lung and tumours will be removed for analysis.

## Application of the 3Rs

### Replacement

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

### Replacement

In order to avoid as much as possible the use of animals, the new nanomedicines will first be thoroughly tested in vitro. However, a total replacement of *in vivo* experiments cannot be achieved in our research project, as the overall aim is the development and evaluation of improved drug formulations for the delivery to distant tumours and metastasis after intravenous and other ways of administration. We have fully considered alternative approaches such as computer modelling and using non-protected species such as nematodes, however a whole mammalian organism is necessary to verify the delivery of these therapeutics to their target, the absence of

any unspecific distribution, the general toxicity which could eventually occur, as well as any changes in the behaviour of the animal as a result of the treatment.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

This research project pays due regard to using the minimum numbers of animals required to meet the objectives of the programme of work. The sample size will be adjusted based on the experimental outcome. The aim is always to be able to detect differences between treatment groups with the minimum number of animals necessary.

A small pilot study with just a few animals can indicate if further work would be appropriate.

For imaging experiments, in order to minimise the number of animals involved, it may be more appropriate in some cases for the control to be based on the animal itself, e.g. pre-treatment vs post-treatment or pre-contrast agent –contrast agent-wash out.

## **Refinement**

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

### **Refinement**

The mouse has been chosen for these experiments, as it is a well-characterised model for biodistribution, gene expression and therapeutic efficacy studies. We have extensive experience of this animal model acquired during our previous experiments.

Athymic mice will be used for the majority of the experiments as most of the tumours will be derived from human cancer cells such as the epidermoid carcinoma A431. The athymic mouse is the model of choice for these studies as the animal has a depressed immune system, and therefore can grow tumours of human origin. The mice will be kept in suitable barrier housing to protect them from the environment.

Animals will be housed in groups in cages with soft bedding and environmental enrichment (i.e. plastic houses). Good husbandry, daily monitoring and care by a team of well-trained animal technicians will ensure that animal welfare is paramount.

Methods which cause the least harm to the animals and which are the most likely to produce satisfactory scientific results will be chosen in priority. The use of imaging techniques such as bioluminescence / fluorescence to monitor tumour development and to evaluate the targeting of new therapeutic systems to tumours is a significant refinement of experimental technique.

Anaesthesia and analgesia will be used whenever appropriate and possible to minimise the pain, suffering, distress or harm caused to the animal. The *in vitro* study of the cytotoxicity efficacy of the new therapeutic systems on cancer cell lines, prior to any *in vivo* experiment, will allow obtaining essential data for choosing earlier endpoints, reducing the administered doses and the injection frequency, in order to cause the least suffering, distress and lasting harm to the animal. The animals will be checked at regular and frequent intervals. At the end of the experiment, the most humane method of euthanasia will be chosen.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 124. Role of Frontotemporal dementia-associated mutations of CHMP2B in regulation of synaptic function</b>
<b>Key Words</b>	CHMP2B, FTD, NMDAR, AMPAR, LTP
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Nerve cells communicate and transmit information across structures called synapses. They receive incoming signals by specialized proteins present within them, called AMPA and NMDA receptors, responsible for nearly all of the fast communication in the brain, as well as processes like learning and memory. Frontotemporal dementia (FTD) has various forms. In one, people have a different version of the protein called CHMP2B (involved in sorting and transporting various cargo to and from synapses, including AMPA and NMDA receptors). We show that early in the presence of mutated CHMP2B, a potentially inappropriate form of NMDA receptors gets stuck within the synapse, while simultaneously AMPA receptor responses are decreased, both indicating deteriorating cell function. We will thus explore CHMP2B's role in synaptic activity (including memory-like synaptic processes). To achieve this, we will combine top-notch electrophysiology (to record the electrical activity of synapses) with a powerful confocal microscopy to see tiny details within nerve cells (and even to track single receptor molecules moving in and out of synapses). We are good in both methods, so we can minimize the number of animals used. Understanding CHMP2B's role in early events could help us fight FTD while it is still potentially reversible.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Though knowledge of causes of dementia has advanced, the role of many factors operating normally in brain development is still unclear. Frontotemporal dementia (FTD) is as common as Alzheimer's disease before the age of 60 years. Its slow and early onset in life, the limited treatment options and negative outcome make it a challenging condition for helping patients. We are convinced that the potential for improvement of therapy lies in basic research, like the one we propose. This will help us devise a new strategy in the fight against dementias and their devastating consequences.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice, 2400

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

The animals will be wild type (as a control reference) and genetically modified as follows: 1) CHMP2B<sup>Intron5</sup> mice is a transgenic mice expressing mutant CHMP2B found in human Frontotemporal dementia. This means that the human protein called CHMP2B carrying abnormalities found in human Frontotemporal dementia (CHMP2B<sup>Intron5</sup>) is expressed in this strain. The mice in this strain have previously been assessed for alterations due to the expression of the mutant protein. At 6, 12 and 18 months of age it has been shown that only subtle changes in how they relate to other animals, and a subtle motor impairment that appeared only at 18 months old animals. In both cases, these changes are expected as the development of the Frontotemporal dementia progress, and they do not compromise the animal welfare. In addition, we will only use 12 months animals or younger, and therefore, before they develop any of those adverse effect. 2) CHMP2B knockout mice. In this strain the endogenous CHMP2B protein expression is suppressed in order to address the normal function of this protein. It has been established that animals of this strain lacking both copies of CHMP2B (called homozygous CHMP2B knockout mice) may develop an abnormal gait from 4-12 months of age, due to developmental changes in their hip. however, those animals having only one copy of the gene (called heterozygous CHMP2B knockout mice) do not have any observable manifestations or adverse effects. Thus, we will normally breed heterozygous animals for this strain (which do not have abnormal gait), to avoid having homozygous mice with potential harmful manifestations being ordinarily used under the breeding protocol. When, however, under specific circumstances a large number of homozygous mice are required for a specific experiment, we will use homozygous knockout mice for breeding under the following conditions: -We know that a small proportion of homozygous knockout mice may develop gait problems at 4 months and that the proportion increases as the mice age. If mice are shown to have such issues in hip development affecting their normal locomotion, they will be culled immediately. In addition, all homozygous breeders will be monitored for abnormal gait/hip

development issues from 4 months of age. The animals will be sacrificed using Home Office-approved methods. Importantly, the tissues from these animals will be used only after death for the imaging and electrophysiological studies. Anaesthesia and analgesia will be used as necessary and any animal experiencing an unexpected adverse effect will be treated as advised by the NVS or will be killed humanely.

## Application of the 3Rs

### Replacement

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

#### Replacement

Currently, there is no satisfactory alternative model for investigation of the mechanisms of synaptic changes in dementia models that does not require the use of brain tissue acutely removed from animals.

The project is based on the use of the previously validated transgenic mouse strains engineered to evaluate the role of CHMP2B and its mutations in the development of frontotemporal dementia. Therefore, this requires maintaining viable breeding colonies.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Using the preliminary data, we have used validated statistical procedures to calculate the minimal number of animals necessary to produce meaningful data, without compromising the scientific validity of the study. In addition, the tissues will be shared with other groups to ensure that neuronal and non-neuronal tissue from the animals is used to the fullest extent possible.

Animals of both sexes would be used for experiments.

### Refinement

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

### **Refinement**

We chose mice as the species widely used in transgenic animal design, while also simultaneously validated as the species of choice by current scientific literature. Further, there is a wealth of correlative studies between mouse and human which indicate that the results gained by the animal use are translatable.

All of the procedures I propose: a) are validated in current scientific literature b) will be performed according to the relevant legislature and c) will be performed by trained staff.

Mice will be monitored on a daily basis and for any animal that shows signs of adverse or unexpected responses, depending on the severity, either the advice will be sought from the local NACWO and/or NVS or the mouse will be culled immediately to limit any additional discomfort.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 125. Physiological significance of organ plasticity</b>
<b>Key Words</b>	Sex differences, reproduction, intestine, obesity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

It is becoming increasingly recognised that adult organs such as the intestine are dramatically different between people and that, even within same person, they can grow, shrink and change the way they function. We do not fully understand how they become different, how they decide to change and why. We will systematically investigate the way in which adult organs differ between individuals and how they change; we will describe the changes organs undergo during adult life, and will use genetic approaches to establish why these changes are important, with a focus on their possible effects on weight gain/loss and fertility. The genetic mechanisms that we will identify may help us, in future, develop drug targets for the treatment of obesity, diabetes and/or infertility. They may also shed light on why some patients respond to drugs better than others, paving the way for personalised 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)?**

Western societies are currently experiencing an obesity epidemic with direct and very significant medical costs. Increased obesity rates as well as delayed reproductive age are also reducing fertility rates and increasing the need for costly, and often ineffective, fertility treatments. In other parts of the world, malnutrition affects over 700 million people, and poses further therapeutic challenges, partly because it can lead to persistent deficiencies in how organs such as the intestine assimilate nutrients. If we find organ changes that are key to reproduction and/or weight gain/loss, characterisation of the mechanisms involved will have diagnostic and therapeutic potential in the context of obesity, undernutrition and reproduction. Our findings will also contribute to advancing our current knowledge in several scientific disciplines (metabolism, physiology, neurobiology, developmental biology). Such findings will be disseminated both amongst scientists and to the general public.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice: 2500/year

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

Our protocols will be of a mild to moderate level of severity. In brief, we will typically 1) use animals that have been fed a certain diet or have undergone a change in internal state (e.g. reproduction) or 2) use animals with a genetic modification that may prevent or mimic how adult organs change in response to certain diets or internal states (e.g. reproduction). We will then determine how that affects the physiology and/or metabolism of the animal. Consequently, only mild or moderate adverse effects are expected to occur at the level of, for example, weight gain or loss, blood sugar control or progeny size or viability. Many of the experiments will be carried out *ex vivo*, in which case the animal will be humanely killed so that its organ(s) can be harvested. In the case of *in vivo* experiments, animals will be humanely killed at the end of the experiment, typically followed by further experimental analyses (i.e. anatomical or molecular). Some animals will undergo routine surgery. This may be required, for example, to remove the ovaries to investigate a possible contribution of sex hormones to any changes of interest. In these cases, appropriate analgesia and/or general anaesthesia will be used in order to avoid/minimise pain. Because they involve general anaesthesia, these surgical procedures are considered to be of moderate severity. Animals will be terminated by a humane method at the end of the project period. Animals exhibiting any unexpected developmental abnormalities or behaviour will be humanely killed, or in the case of individual animals of particular scientific interest, advice will be sought from NACWO, NVS or the local Home Office Inspector. If the animal fails to respond to treatment or its condition deteriorates, it will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

Most of our work will continue to make use of non-protected alternatives such as the fruit fly *Drosophila* or cell lines. However, the regulation of complex metabolic processes such as weight gain or reproduction is dependent on communication between many different organs, some of which are not present in flies or in isolated cells. Mice are closer to humans, suitably complex from a metabolic perspective, but also genetically amenable. We will always use simpler systems such as the fly to identify potentially important candidate genes, and then we will test their functional relevance in mice.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will only embark on *in vivo* experiments in mice once we have obtained evidence for potential functional importance of certain genes/processes in flies and/or cell culture systems. Prior to generating genetically modified mice, we will ensure that lines with the desired genetic manipulations are not available elsewhere. We will design our mouse experiments to obtain the maximum amount of data from a single animal (e.g. by generating multiple sections from a single organ for different experiments). We will design our breeding to maximise the use of offspring, and will perform power calculations to ensure that the minimum number of animals is used to obtain scientifically meaningful results. Spare animals will also be made available to other scientific projects. When appropriate, we will cryopreserve mouse lines that are not required for extended periods, rather than maintaining stocks. We are used to controlling for sources of variability such as genetic background, diet, age and gender in flies, and will continue to do so in mice.

## Refinement

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

### Refinement

Mice are one of the lowest vertebrates in which genetic manipulation can be successfully achieved and where metabolic/reproductive studies are well documented. In addition, the availability of transgenic mice provides powerful tools for tackling these scientific questions. All the procedures in this licence are classified as either mild or moderate and will be performed under local, general or terminal anaesthesia, where appropriate, to minimise stress and suffering of the animals. Pain relief will be provided as appropriate and as advised by the veterinary surgeon. Animals will be regularly checked for unexpected adverse effects, more frequently following surgical procedures.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 126. Understanding persistence and pathology in RNA viral infections</b>
<b>Key Words</b>	Pathogenesis, Virus, Coinfection, Ageing, Integration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

One large group of viruses have smaller genomes that are highly variable in nature. Our aim is to understand how these types of viruses are able to stay within cells in the body and cause disease. These types of viruses appear to have multiple ways of hiding from the body's immune system while provoking a response that damages the organs they are in. Examples in humans include the HIV virus and the Hepatitis C virus. These types of viruses are especially important in people who have weakened or improperly functioning immune systems. We now know that highly variable viruses that cause sudden infections in healthy adults can stay hidden in the bodies of newborn or elderly humans or animals. Even the Ebola virus has been found to persist within the body of people who have survived Ebola virus disease. However, the pathways that these viruses affect are not fully known. Because these virus infections affect the immune system they also allow other infections such as a second bacterial infection (so called coinfections) to occur. By allowing these infections to occur, not only does chronic disease develop, but there is also an increased chance for the virus gene to become part of the host genome. If this happens there can be long term negative effects for human health, such as the persistence and recurrence of infection. This research aims to understand how this happens so that it can be prevented.

**What 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 pathways by which this group of viruses cause disease, we may target the damaging responses in ways that can be used as new therapeutic

strategies eg specific drugs that block these damaging pathways may be developed. This will help increase healthy ageing and prolong disease free lifestyles in the UK increasingly ageing population.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice, all ages 8600 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 levels of severity? What will happen to the animals at the end?**

Animals will be infected to study the way in which viruses cause disease. A variety of routes and virus strains may be used that can cause either acute or chronic infection. Animals may develop disease (adverse effects) such as weight loss, ruffled coats, lack of movement. They may show diarrhoea and some difficulty breathing. Animals may be coinfecting with eg parasites to investigate the effect of the virus infection itself and the presence of pre-existing conditions or coinfections. Animals may receive injections of substances that alter the immune system or the microbiome by standard routes such as orally, intramuscularly, or intraperitoneally as well as by more invasive methods eg into the brain of young mouse pups (under anaesthesia). Animals involved in breeding may undergo surgery eg vasectomy or implantation of embryos. Animals are given anaesthetics and pain relief for surgical procedures. Older animals may be used to investigate the effect of age on infection and disease susceptibility. However at all times animals will be closely monitored and adverse effects arising related to either age or study effects are taken into account in determining the humane endpoint of the study concerned. All animals are humanely killed at end of study and their tissues, blood, spleen etc used for further analyses.

## Application of the 3Rs

### Replacement

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

### Replacement

The complexity of the interaction between the virus and the immune system cannot be recreated in non protected animal alternatives which do not have living tissue. Whenever possible we will use cells or organs generated from living tissues and cells in a lab setting to answer some of our scientific questions before commencing work in animals

This, in some instances, can provide information to help reduce the number of animals used and to refine the methods to become less harmful.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Experiments will be carried out in group sizes that are calculated from previous or published data providing solid statistics. Groups will be arranged such that controls can be compared to multiple groups and experimental groups can be compared to each other. This improves the experimental design and also decreases the numbers of animals needed overall in the project.

### **Refinement**

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

### **Refinement**

Mice are excellent models that reproduce disease uniformly and consistently, thus minimising the likelihood of repeat experiments. Mouse genetic (DNA) make up can be altered such that very specific areas can be changed and this enables study of particular mechanisms involved in disease processes and immune responses to be studied in greater detail than would otherwise be possible. To aid in the delivery of the experimental work with the minimum adverse effects for animals concerned, animals are carefully monitored for clinical signs of infection and clearly defined humane endpoints applied. Pilot experiments with small group sizes will be carried out when entering new areas of research so that any unexpected adverse effects can be detected and characterised in smaller numbers of mice before deciding whether to move onto a full study with greater numbers of animals. This approach can also lead to an overall reduction in animal numbers based on data from pilot study outcomes.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 127. Molecular Neuroscience of Ligand-gated and G-protein coupled Receptors</b>
<b>Key Words</b>	Receptors, Neurology, Neurophysiology, Neuropharmacology, Genetics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We will study the properties of key proteins called receptors that reside in the membranes of brain cells, which are fundamental to brain physiology. These receptors are the gatekeepers for activity and information transfer that occurs between all parts of the brain and ultimately controls our behaviour. There are many different families of receptors.

-Paramount amongst these are those that control the balance between brain excitation and inhibition. This occurs between specialised brain cells called neurons.

-The receptors we study are closely associated with diseases such as anxiety and stress, depression, epilepsy, and intellectual disability. Many of these diseases manifest because of genetic abnormalities in these receptors (mutations).

-Our work aims to increase our understanding of how these receptors operate in neurons to permit both normal behaviour and how their dysfunction causes 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 aim to increase our understanding and knowledge that can be used by academics, industry, and clinicians to pursue related work, and to provide leads for the pharmaceutical sector to explore new drug therapies. -For example, we have mapped on one receptor, a site which interacts with natural brain molecules that can increase the function of the receptor. In doing so the receptor physiologically contributes to the relief of anxiety and stress in individuals. -We will use this new map to design novel molecules with potentially increased therapeutic usefulness for treating debilitating diseases in society such as epilepsy and depression.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

In order to undertake our studies over the next five years, we will need to use rats, mice and frogs. -Our neurophysiology-related questions can only be addressed by using animals (up to 2500/year; typically 10:1 mice:rats) as we need to emulate the neuronal environment that only exists in brain tissue. -In order for us to better understand the impact of disease mutations on receptor function, the main animal of choice is the mouse because of its genetic usefulness. In this way we are able to generate disease models. Rats and frogs constitute only a minor part of the project, and we also are able to share tissue use between groups (~20/year).

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

The vast majority of the procedures we wish to perform come under the mild category defined by the Home Office, as this will mainly constitute tissue collection after humane termination in order to study brain proteins and their function. -Some mutations will be introduced into mice, to replicate diseases. From our single cell work, we envisage the effects of these mutations will not be overtly harmful. As a result the generation and breeding of genetically-altered mouse models is also considered to be mild. Very occasionally we will inject substances into the muscle or the CNS of rodents, which because it is more invasive, will be categorised as mild-to-moderate. Equally, we will chemically induce moderate epilepsy in rodents which they retain until they are used shortly afterwards following humane termination. The induction of these disease states is also considered to be mild-to-moderate. -A very limited number of our procedures will involve manipulating animal behaviour by altering protein function using light probes implanted in the brain. This will be transient and moderate in its nature.

## Application of the 3Rs

### Replacement

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

## Replacement

We extensively use 'secondary' cells (from tissue libraries world-wide) to characterise the behaviour of receptors for much of our work. These secondary cells are immortal and are used to reconstitute receptors for study. However, to examine our hypotheses, we also have to study the same receptors in neurons and the brain, and ultimately its impact on the behaviour of the whole animal.

-There are currently no other tissue or cell-based models that can emulate real neurons and all the proteins therein. This is especially relevant if we wish to apply our findings to diseases which are common to the brains of animals and humans.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

To limit animal numbers, we share tissues between members of our laboratory and with other laboratories. One example of this is the collection of frog eggs (oocytes) for studying receptor behaviour in isolation following a genetic mutation we have made.

-Currently, many local laboratories share oocytes taken from the ovaries of one humanely-terminated frog in order to do similar experiments. We can also do this with collected brain tissue from rodents. We use statistical power calculations to estimate how many animals (often 3-5) are needed to ensure that the effect we see is 'real'.

-If we are performing experiments monitoring the behavioural response of a rodent to the injection of a drug, we know exactly the single most effective dose of that drug to give because we will have previously studied the same drug, and how effective it is, in cell-based experiments.

-Equally, for experiments where we observe behaviours without administering a drug, we can use the same animal in multiple different tests for behaviour as they are benign.

## Refinement

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

## Refinement

Mice are the species of choice for studying behaviour and for inserting mutations into proteins because they are very amenable to genetic manipulation. We do this to study the effects of mutations on the function of our target receptors with regard to brain physiology and whole animal behaviour. Mice also breed reliably.

-Rats are occasionally used to facilitate the dissection of small brain regions from young animals. By improving visualisation and identification of brain structure we succeed with a greater proportion of our experiments.

-Some of our proteins, especially those that are genetically altered, can be difficult to reconstitute in cell types. We would normally do this by injecting DNA (which encodes for the receptor) into a cell and using the cell to express the receptor. Not all cells are capable of doing this, however, the frog oocyte is very faithful and reliable for this. In order to minimise harm to our animals we ensure that all of our staff and students who undertake experiments have undergone rigorous continued training, and re-assessment.

-This training is carried out by personnel who run our animal facility. It is undertaken according to Home Office approved guidelines, and each individual must be signed off as competent before they can practice a technique. All our lab personnel take responsibility for maintaining and using their animal colony, which fosters good husbandry practice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 128. Improving the outcome of Lung Cancer therapy using adjunct treatments</b>
<b>Key Words</b>	Lung Cancer, Adjunct therapy, Immunotherapy, imaging
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Lung Cancer is a cancer of unmet need with an extremely poor 5-year survival. Even with new advanced therapies (such as immunotherapy) a response is only seen in 10-20% of patients. This work will determine if i) the use of additional treatments can improve existing lung cancer treatment, ii) what the mechanisms of these additional treatments are and iii) to develop imaging compounds to determine who and when will they benefit from additional treatments.

The animal studies will be designed in such a way as to have as direct as possible relevance to patients to help inform clinical studies. This includes using drug treatments which have relevance to human lung cancer and in tumours that are already present prior to treatment. To understand how these drugs are working if the tumours shrink, some animals (<15%) will undergo live imaging of the tumour during therapy to 'see' the movement of inflammatory cells within the tumour. This requires the insertion of 'optical windows' to allow the microscope to visualise the cells at high resolution. These windows have been routinely used by collaborators.

**What 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 are likely to be additional drug combinations for human use to improve the outcome of lung cancer treatments and developing imaging compounds that will help us decide the best timing of treatment. This work will also allow us to understand how these combinations of treatments are working.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

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 levels of severity? What will happen to the animals at the end?**

Animals will be cared for with the highest standards of care with social and environmental enrichment where possible. All animals will be carefully monitored and those showing deviation from normal (assessed by several criteria including humane endpoints) will be humanely killed. For tumour bearing animals the tumours will be regularly assessed and will not be allowed to exceed 15mm. These tumours are superficial tumours, easy to measure and are not likely to spread elsewhere in the body. Treatments given, where possible, will be using drugs previously given to humans which minimises toxic effects and has direct relevance to patients. Any surgical procedures or imaging will be done with appropriate general anaesthesia and pain relief will be provided. The overall expected severity is moderate and at the end of all studies the animals will be killed humanely.

## Application of the 3Rs

### Replacement

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

### Replacement

Lung cancer is a complex process and no laboratory experiments can truly mimic the complexity of the disease process. Therefore, to test if, how and why certain treatment combinations are going to be of benefit in lung cancer an animal model must be used. Several laboratory experiments are being undertaken in cancer samples resected from patients with drug treatments in the laboratory to help decide which drug combinations will be helpful, and this will reduce the number of animal experiments needed. These additional experiments will characterise the cell types involved in lung cancer and their assessment in the laboratory to drug treatments and will go on to inform which animal experiments will be beneficial.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

With the animal experiments proposed I will also be using high resolution imaging techniques to understand what is happening in the tumours during therapy, which will reduce the number of animals required as these imaging experiments can be which can be performed repeatedly in the same animal and it allows the use labelled cells.

I will also use the existing scientific literature to decide the best drug doses to administer and the experiments are designed to ensure the results are valid, and are correctly compared to appropriate control groups (where treatments may not have been given or they received placebo). All work will be undertaken by trained and competent individuals.

## Refinement

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

## Refinement

The animal models I propose to use are directly relevant to the types of lung cancer humans develop and the model will mimic the response to treatment we see in patients. The murine model proposed closely resembles the most common type of lung cancer found and tumours of these types are not able to be modelled in fish or invertebrates. The results will be more applicable to managing human lung cancer. Where possible, clinically approved drugs will be used to minimise harm to animals and to provide patient relevance to the results. By placing tumours under the skin in animals I can closely monitor the development of the tumour, I can image the tumours as they respond to treatment with a microscope and do this repeatedly as treatment progresses and therefore this method will minimise harm compared to other possible models (where for example additional surgery is required or the same animal cannot be imaged repeatedly). I will also use some animals with genetic alterations which reduced the overall numbers required to establish the mechanism of any findings.

All animals will receive the highest standard of care with social, environmental and behavioural enrichment. Animal with 'optical windows' inserted to allow imaging of tumours will be done with general anaesthetic for insertion and the provision of pain

relief following surgery and these animals will be cared for with modifications to the environmental enrichment to minimise interference with the windows. All animals will be carefully monitored for clinical signs including visual inspection of the tumour development site and if any animal is demonstrating suffering then clear humane endpoints will be applied.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 129. Regulation of immune response during infection and inflammation in rodents</b>
<b>Key Words</b>	Immunoregulation; immunopathology; infection; autoimmunity; inflammation.
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The immune system has developed to be highly specialized and effective in eradicating a wide variety of pathogens. The adaptive arm of the immune response, consisting of antigen-specific T and B cells interacts with innate immune cells to mediate an effective response to infectious pathogens. This interaction is tightly controlled by many mediators to achieve an appropriate response with minimum immunopathology. The adaptive immune response is enhanced upon secondary exposure (memory) as in vaccination, but in tuberculosis (TB) this is ineffective. Common to infections such as TB a chronic, persistent infection follows, and their control is mediated primarily by T lymphocytes and innate cells that activate T cells, which can be suppressed by the pathogen. In order to protect or cure individuals against infectious agents causing diseases such as tuberculosis (TB), a disease of major morbidity and mortality in man, particularly when antibiotics are ineffective, an understanding of the immune response is badly needed to intervene and induce immune enhancers and/or immune modulators, to achieve maximum protection with minimum pathologies. Moreover, the same molecules/immune modulators and/or enhancers that bring about protective immune responses against a pathogen, may also result in immune and autoimmune pathologies, such as rheumatoid arthritis (RA) or multiple sclerosis (MS), or inflammatory pathologies such as asthma. This, and the multigenetic complexity of inflammatory and autoimmune disorders has made therapeutic intervention in these diseases also very difficult, and many of the current drugs have multiple side effects. Likewise, immune responses required for the clearance of pathogenic micro-organisms can sometimes result in immune

damage to the individual. Molecules that protect against immune damage, like IL-10, on the other hand, can contribute to chronic infection.

We aim to understand the immune molecules that lead to a balanced immune response so as to test novel strategies of preventive and therapeutic immune intervention. First, we will identify molecules/pathways leading to over-exuberant responses, which may result in disease during infection, inflammatory or autoimmune diseases, or cancer, and identify molecules that regulate them to prevent immune pathologies. Second, we will identify immune molecules resulting in protection or chronicity during infectious diseases in mouse models refining them to more accurately reflect the human counterparts. We will identify mechanisms by which pathogens such as *Mycobacterium tuberculosis* and other bacteria, viruses or parasites, act to subvert these responses, and how certain infections lead to over-exuberant responses and host damage. Using this knowledge we aim to identify therapeutics to control 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)?**

An understanding of the molecules enhancing and regulating the immune response, may help to design therapeutics in order to manipulate inflammatory and autoimmune disorders specifically, effectively and with minimum side-effects such as the development of chronic infectious diseases. In addition, this could lead to discovery of immune modulators to protect against chronic infections. To achieve both these goals we will require a very thorough understanding of the molecular basis for the regulation of the immune response. Hence, this research area is still highly active internationally and our laboratory is actively pursuing this line of research to understand the immune response to identify novel molecular and cellular events and novel or immune modulators, to protect against inflammatory, autoimmune and infectious diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We plan to use wild type and genetically modified mice for in vitro and ex-vivo experiments; and for in vivo experiments in mouse models of immunomodulation, infection; inflammation; allergy; autoimmunity; and cancer – the main emphasis will be on infectious disease models but for understanding mechanisms of immune regulation the other models are employed, albeit to a lesser extent. Experiments will range from 1 – 3 days for certain infections; to a maximum of 180 days for others (mainly TB models). Over the five years we anticipate breeding and maintaining

30,000 genetically altered animals (with or without associated wild types); 1000 for phenotyping, tissue provision and long-term monitoring; 5000 for experiments *in vivo* for defining mechanisms of immune modulation in innate and adaptive immunity and in allergic, inflammatory and cancer mouse models of disease; 10,000 in models of infectious diseases to determine protective yet regulated pathways; 500 in autoimmune disease to define immunomodulators.

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

Genetically modified animals with mutations in molecules potentially involved in the immune response will be used throughout the course of the project license. Many of them may be immunocompromised, and hence will be kept under specific pathogen free conditions. Furthermore, they will be monitored from birth so that any defects leading to mouse discomfort may be stopped by killing the animal. In some cases where therapeutic molecules are required to be tested these mice may be maintained alive for the minimum duration required for such intervention and/or analysis. We anticipate that breeding and maintaining 30,000 genetically altered animals (with or without associated wild types) will be of mild severity; 2500 mice are approximated to reach severe signs (2000 through infection; 500 through autoimmunity); 6000 to reach moderate severity (through infection) and 5000 to reach moderate severity (through immune modulation); 2000 to reach mild severity (infection). All mice will be strictly monitored within each protocol to ensure that the defined severity is adhered to.

## Application of the 3Rs

### Replacement

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

### Replacement

Firstly, this work will include *ex vivo* studies using immune cells obtained directly from either normal or genetically manipulated mice, to dissect the mechanisms underlying the activation and effector function of immune cells *in vitro*. This approach cannot be replaced by cell lines that have been maintained in long term culture, since they invariably do not maintain their true fidelity. Although this may result in the use of large numbers of mice, this first step together with findings we are making in clinical studies will **replace** the initial need for *in vivo* manipulation of mice. Such *ex vivo* studies may reveal the molecular basis for enhancement or suppression of immune responses. However, these findings will still need to be verified *in vivo*, in whole organisms where multiple complex interactions take place resulting in the overall response.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

For most of the experiments quantitation is required and we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own or from the literature). Pilot experiments will use between 5-8 mice per group, which should be sufficient if a significant result is obtained and experiments will be designed to use the minimum number of mice that will provide statistically reproducible results which are set using power analysis, generally using a significance level of 5%, a power of 80% and at least practicable difference between groups of 20%. Once a desired effect has been obtained it may be necessary to use a greater number of mice per group in order to facilitate obtaining rare immune cells involved in the response for function analysis.

## **Refinement**

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

### **Refinement**

We will use the laboratory mouse as the model organism. The mouse is the best characterised model for these studies, with many features applicable to human infection. Their immune responses are well defined and the technology enabling sophisticated manipulations of the haematopoietic and immune system is highly developed. Mouse transgenic and knockout techniques are well established; mice have a relatively short generation time; the haematopoietic system of the mouse has been extensively studied and, in addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this project to the same extent as the mouse. All mouse models used will be assessed such that the severity will be reduced to the minimum in terms of infection or inflammation burden required to show effects and obtain meaningful results. Mice will be monitored closely to ensure that the numbers are maintained at the minimum severity possible to obtain meaningful results that may inform our knowledge to advance therapeutics.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 130. Translational Studies in Prostate Cancer</b>
<b>Key Words</b>	Prostate cancer, treatment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

In the UK, prostate cancer affects 1 out of 8 men. It kills more than 11,000 men each year, exceeding the number of breast cancer related deaths. Hormone treatment, which has been used for more than 50 years, controls prostate cancer in a palliative manner. We urgently need better and more specific treatment options. Better knowledge on how prostate cancer spreads (or metastasise) and survives (or resists) treatment (such as hormone, chemotherapy and radiation) will help researchers worldwide to formulate new ideas and approaches to defeat prostate cancer. During these studies, better tests to detect aggressive cancer and/or to predict how cancer will respond to treatment can be developed. Our project builds on our extensive expertise and resources, linking laboratory to clinical (surgical and oncological) practice, to study cancer metastases and treatment resistance. In addition, we are well placed to begin efforts to test the usefulness of novel treatment agents.

**What 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 likely benefit will come from knowledge on why cancer spreads and resists treatment. This information will shape ongoing and future efforts in drug development for patient benefit. Data from work carried out within this project will be tested using resources from clinical prostate cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

All studies use mouse, with ~50,000 over 5 years. Relatively high numbers of mice are required for the breeding protocols. One of the reasons is that only male animals will develop a prostate gland and are used as cohort animals. Also, there is a need for extensive breeding regimes, and as such, only ~25% of the mice bred will harbour the desired genetic alterations required for our studies. Hence, at least 50% of mice will not undergo scientific procedure but are used to generate the required

mice. We estimate that around 8000 mice will be studied as transgenic models and around 7000 mice (including immune deficient and immune intact but genetically compatible) from this project or other sources will be used in our transplantation models.

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

The majority of animals used (~70%) will not have any observable clinical signs. They are used for breeding programme only and will be humanely culled once their genetic statuses are known. Experimental (cohort) animals will carry patient-specific genetic events to recapitulate human cancer. Such animals will develop prostate cancer. When cancer develops in the prostate, animals may experience abdominal distension or develop urinary symptoms. We have refined the required surgical procedures (to administer treatment and/or facilitate imaging studies) described in this project, typically using a small lower abdominal incision, which is closed when the procedure is concluded and mouse recovery will be closely observed per protocol. Some of the study animals will be administered substances/therapeutic agents, or fed altered diet (e.g. high fat diet). We have refined the technique to produce prostate cancer in its natural environment by implanting cancer cells directly into the mouse prostate. This is a very useful method to test the growth behaviour of cancer cells with certain genetic contents. We will also use such technique to test the usefulness of new treatment agents in the project. By implanting cancer cells in the prostate gland or injecting into the mice (using different routes to mimic clinical metastasis to bone and other organs), we hope to study the reason for prostate cancer to spread and/or resist treatment. All animals on treatment or anaesthesia will be carefully monitored for discomfort, recovery or development of relevant clinical symptoms. Animals will be humanely killed at the end of the experiments and tissues collected at post-mortem to maximise data obtained.

## Application of the 3Rs

### Replacement

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

### Replacement

Prostate cancer is closely controlled by male hormone function. Communication between the prostate and other organs/cell types in the body (including immune cells, adipose tissue and liver) are very important in determining the way cancer behaves. For some research objectives, there are currently no alternatives to the use of appropriate mouse models. However, our research group is active in trying out new ways of co-culture systems whereby prostate cancer and host cells (fat, immune and other cell types) can be studied in the laboratory, including the use of three

dimensional spheroid (or mini-organ) cultures. We also have extensive expertise to generate cell lines from our mouse models which will replace the use of the whole mouse for some exploratory experiments.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Statistical significance and power analysis are applied to design our studies to ensure our studies are informative using the least number of animals. We will also use our transplant models to reduce the use of transgenic mice and the overall study periods. We have extensive experience and support within our Institute to use the minimal number of mice to answer specific research questions. We have also pioneered the use of ultrasound scan and other non-invasive imaging methods such as magnetic resonance imaging and functional scans (e.g. positron emission tomography) to allow serial (multiple) monitoring which reduces numbers.

## **Refinement**

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

### **Refinement**

We have developed expertise in a range of mouse models that are selected to best answer individual research questions. For instance, if we were to test the usefulness of a new treatment, we can apply our implanted prostate tumour model which will minimise the need for breeding and substantially reduce the number of mice needed and duration of the entire experiment. We have developed expertise in non-invasive imaging using ultrasound scan to monitor tumour growth so animals can enter our studies at the optimal time to ensure robust comparison between mice and minimise suffering. Evaluation of new therapeutic agents will be tested using small number of mice (3-4) in the first instance. Analgesia will be applied to ensure the welfare of the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 131. Immune responses in the intestine</b>
<b>Key Words</b>	Immunology, Intestine, Lymph, Cell migration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We are exposed to large numbers of micro-organisms through our intestines. Many of these micro-organisms can cause infections. Our immune system is usually able to prevent such infections, but sometimes fails. When the immune response fails, either the infectious organism causes disease, or the immune system itself can cause large amount of damage. When the immune system damages the intestine, this causes inflammatory bowel disease, a chronic, incurable, life-changing condition that affects approximately 1 in 200 people in the UK. The project aims to improve knowledge of how the immune system in the intestine is controlled, so that we can develop ways to improve protection against infections, and ways to prevent or treat inflammatory bowel 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 improve understanding of the way intestinal immune responses are controlled. These studies are likely to impact on the field of immunology generally. Collaboration with local doctors who treat people with inflammatory bowel disease, and other diseases where the intestinal immune system can cause damage, will help us to transfer of any relevant insights from the animal studies described here to our other studies involving samples donated from human volunteers. This may eventually lead to the design of better treatments for inflammatory diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use up to 12,000 mice and 1800 rats during the course of this 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 levels of severity? What will happen to the animals at the end?**

Many of the animals we use will not experience more than mild discomfort. They may be bred to carry genetic modifications that do not affect their health, and may then receive treatments that are added to their drinking water, or delivered by injection, before they are humanely killed. Such animals would only experience procedures of mild severity. Over the period of the license, up to 4500 mice and 150 rats will undergo surgical procedures, either to deliver cells or molecules directly into their intestinal tissues, or to collect specific tissues or cells from the animals. They will experience procedures of moderate severity. Any discomfort will be minimised by the use of both local and systemic analgesics, under veterinary advice. Any animals showing signs of distress are humanely killed before suffering approaches carefully-defined limits. Up to 500 of the transgenic rats we use to study inflammation will themselves develop arthritis and intestinal inflammation as they age, and up to 2500 mice will be given substances that induce intestinal inflammation. These animals will be closely monitored. All animals will be humanely killed at the end, including any animal showing effects approaching the moderate severity limit of these protocols.

## Application of the 3Rs

### Replacement

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

#### Replacement

The immune system is highly complex, involving the orchestrated interactions of multiple different cell types. At present while it is possible to model many immune interactions using cells obtained from animals without performing regulated procedures, these experiments cannot recapitulate the full range of interactions that determine the outcome of an immune response. For example, immune cells are highly responsive to changes in their microenvironments; this sensitivity is crucial for their ability to detect infection. Even removing these cells from their environment rapidly induces changes in their functions. Thus, while we will continue to make the maximum possible use of cells from animals that do not undergo regulated procedures, and cells obtained from human volunteers (not covered under this license), it is currently not possible to perform the experiments described in this application without performing procedures on living animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will design our experiments to use the minimum number of animals, and the smallest amount of suffering, to achieve our experimental objectives. To ensure a

sound statistical basis for our experiments, the following will be considered during their design.

Many experiments will need only two groups; one experimental, one control. For others, three or more groups will be used. Where yields of cells from one animal may be too low for analysis samples from multiple animals may be pooled.

Treatments will usually be given to groups containing no more than four animals. The effect will then be measured and statistical analyses performed. Power calculations will then enable us to estimate the number of animals required to generate statistically-significant results, and these predictions will guide the decision making process. Experiments will need to be performed on at least three occasions to ensure reproducibility. Statisticians have been consulted in designing specific experiments. Expert statistical advice will continue to be sought to maximise our scientific output and minimise the number of animals required.

Bias is minimised by counting measurable units (e.g. cell numbers) rather than subjective scoring (e.g. animal health). Mice will be randomly assigned to groups by animal care staff who do not know which treatments the animals will receive.. Where possible, the researcher will be blinded to sample identity during data collection. Most experiments will be completely randomised.

One element requires that we collect specific cells that from from bone marrow, and then grow them in the laboratory. If we treat mice before collecting these cells, we can collect more cells from each mouse. This substantially improves the yield of the necessary cells and enables us to achieve our objectives using fewer mice.

## **Refinement**

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

## **Refinement**

Rodents are the lowest form of sentient species that can be used for the studies we propose on the functions of the immune system. Because mice and humans are mammals, their immune systems share many similarities. Because mice and rats are

commonly used by immunologists, there are many useful materials that have been developed by other immunologists that are available for our work.

In this license we describe four experimental protocols, with differing levels of severity. When designing every experiment, we will use the procedures that cause the least possible level of harm. For instance, where possible, animals will be infected with bacteria that do not generate any ill-effects, and experiments will be terminated as soon as the required immune responses have begun, rather than waiting for signs of infection to occur.

To collect the necessary material for our experiments we will perform invasive procedures which cause some short-term suffering to the animals. This is alleviated by the use of analgesia, as recommended by the veterinary staff. This approach is necessary because it permits the study of immune cells under near-physiological conditions. Since beginning to work with these techniques fifteen years ago, we have made significant refinements to our procedures and have, for instance, developed techniques that remove the need to restrain animals after surgery. This process of refinement of our techniques continues. We are, for instance, actively working to improve standards for aseptic surgery.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 132. Late effects from inhaled carbon nanotubes</b>
<b>Key Words</b>	Carbon nanotube, Late effects, Inhalation, Inflammation
<b>Expected duration of the project</b>	2 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Globally, carbon nanotubes (CNTs) are increasingly being produced and used in many aspects of everyday life. At the same time, public concerns on potential adverse health effects of CNTs are increasing, driving the emergence of nanotoxicology as a fast growing science discipline. The safety data generated so far provide little context for the hazard component of risk assessment, largely due to the lack of mechanistic understanding of CNTs' biological effects in relation to detailed physicochemical properties of CNTs. This hampers the development and validation of risk assessment methods/models that can be generalized to all CNTs. Compared to "traditional" environmental and occupational hazardous materials evaluated within the current risk assessment paradigm, CNTs have unique physicochemical characteristics at the source and can undergo dynamic changes when interacting with biological systems (e.g., agglomeration and aggregation). Therefore, more meaningful risk assessments for CNTs can be accomplished if the hazard assessments are based on mechanism-driven toxicological studies linking specific biological effects to specific physicochemical properties of the CNTs.

This aim of this work is to enhance our understanding and perception of the potential health detriment resulting from the inhalation of CNT. The animals used have been previously exposed to an aerosol of CNT and displayed only minor, short-term biological response to their exposure. Extending the study period of these animals will allow the collection of valuable additional information on potential late effects and health outcomes.

**What 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 outcomes of this study will support or question the manufacture and use of CNT, provide information for the development of improved strategies/policies/recommendations on the use of CNT and identify possible adverse health effects

**What types and approximate numbers of animals do you expect to use and over what period of time?**

60 rats within a period 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 levels of severity? What will happen to the animals at the end?**

Currently all the animals (exposed and control) in this project are healthy but possible adverse effects from the inhalation exposure to CNT include chronic inflammation and fibrosis in tissues of the respiratory tract. All rats will be killed during the lifetime of this licence and it is anticipated that most of the rats will be euthanised for reasons unrelated to their exposure to carbon nanotubes.

## Application of the 3Rs

### Replacement

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

### Replacement

Whole animal studies are necessary at this stage in the research because the deposition, translocation, organ biokinetics, metabolic fate and excretion of inhaled nanomaterials involve the interaction of many biological processes and there are no non-animal alternatives available to study the described inflammatory processes in the in-vivo situation. Whole body systems e.g. an intact circulation, the presence of cell-to-cell contact and cellular interactions is essential to mimic the situation following human exposure to CNTs. These factors are essential for the inflammatory processes to reach the full pathological. However, as part of an overall programme, we are developing in vitro procedures (bioassay and cell-culture studies) focusing on appropriate cell lines (or bioassays) relevant to the secondary target organs.

Rats and/or mice have been the animal model of choice at this establishment and elsewhere for similar studies after inhalation of aerosols of larger sized particles (0.5 to 5  $\mu\text{m}$ ). Many studies have been performed to characterise the deposition, clearance, and temporal organ distribution and excretion parameters of materials in

these species. Substitution of rodents with animals with lower developed senses is not always possible, because of dissimilarity with the respiratory tract of mammals.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The animals used in this study have been previously exposed to a carbon nanotube aerosol under a previous project licence. Extending the study period of these animals will provide valuable additional information on potential late effects from the exposure to CNT materials by inhalation and thereby help reduce the need to conduct further animal studies with these materials.

Based on previous experience, and studies of this kind reported in the literature, the number of animals in a group is generally between four to six animals for each time point and this was the group sizes used for the initial, short term study. However, because these animals will provide invaluable information on the late effects of exposure to CNT, and individual animals within a group will become unwell and sacrificed at different times, the minimum number of animals per group will be reduced to three. Any surviving animals will be killed at the end of the project period.

The health status of the exposed animals will be compared against that of a clean air exposed control group. The analyses performed uses techniques that are non-biased.

## **Refinement**

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

### **Refinement**

Experience gained during the exposure, handling and assessment of these animals on the previous project will be applied here too.

Animal suffering will be minimised by identifying potential adverse effects and ensuring that humane endpoints are developed and applied under these

circumstances. Trained staff will handle the animals during euthanasia so as to minimise stress.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 133. Developmental and Reproduction Safety Testing of Chemicals, Plant Protection Products, Biocides and Substance added to Food or Feed Products Using Small Animal Species</b>
<b>Key Words</b>	Regulatory, Safety Assessment, Developmental, Reproduction
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

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.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the 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 through 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.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

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

## Application of the 3Rs

### Replacement

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

#### Replacement

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 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.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

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

### **Refinement**

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, whilst 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).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 134. Control of Ovine Pulmonary Adenocarcinoma</b>
<b>Key Words</b>	OPA
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Ovine pulmonary adenocarcinoma (OPA) is a common naturally occurring disease of sheep and is caused by Jaagsiekte sheep retrovirus (JSRV). Once clinical signs are seen the disease is always fatal. OPA is one of the top 20 causes of death in sheep in Great Britain (APHA & SRUC, 2017) and is an increasing problem in the sheep industry as highlighted by a recent opinion piece in the farming press (p8 Scottish Farmer, 7 Oct 2017). Control of OPA remains an unmet need. For this reason the principal aim of our research is to develop better diagnostic tests for pre-clinical OPA. Understanding how the virus causes the disease will be important towards developing better diagnostics or vaccines and may also deliver important information relevant to human cancers.

**What 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 pathogenesis of OPA and the immune responses to JSRV is the only way we can move forward in developing vaccines or diagnostics as the “usual suspects” have already been tried and failed. During this project we will follow up on any promising leads to assess the efficacy of potential new diagnostic tests. In the future, vaccine development may also be pursued. We are looking for a potential product that will benefit farmers economically and will improve sheep health and welfare. This is worthwhile to support sustainable farming and food security in the UK and elsewhere. Hopefully anything we learn will also have added value in understanding some aspects of human lung cancers and developing treatments. The use of transthoracic ultrasound scanning is currently being trialled on farms for pre-clinical diagnosis of OPA. It is important to find out whether this technique is effective. Therefore it is necessary to follow up a proportion of tested sheep with post mortem examination (PME) of the lungs because this is the only way to definitively

diagnose OPA. Ultrasound will not detect the very earliest OPA tumours, nor JSRV infection prior to tumour development, therefore we will also continue to try to develop an improved laboratory test. Blood and nasal swabs collected from the sheep prior to PME will provide an archive from known OPA positive or negative sheep in order to validate any new tests.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to 100 sheep each year naturally affected with OPA at different stages of disease plus up to 50 unaffected cull sheep as negative controls will be used. These sheep will be kept for the minimum time. Typically they are received from the farm of origin and euthanized on the same day. Blood or nasal swab sampling will be done on farm for up to 400 sheep during the last 3 years of the project. Two experimental infections of up to 30 sheep (including negative controls) are anticipated during this project. These sheep will be kept for no more than 12 months.

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

Transient discomfort will occur during blood sample collection. Some sheep will then be euthanised and subject to post mortem examination. Those bled on farms will remain on the farm of origin. Sheep experimentally infected with JSRV will be euthanised at the end of the study (up to 12 months) or earlier if signs of OPA disease are seen.

## Application of the 3Rs

### Replacement

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

### Replacement

The majority of the animals used in this programme will be naturally affected with OPA and are donated by commercial farms. Clearly it is not possible to replace these since PME is necessary to verify ultrasound scanning results and to provide samples for other aspects of the OPA research programme. In vitro techniques (see below) are proving useful for some aspects of studies on pathogenesis but cannot replace the use of animals for understanding host responses to infection.

The use of ex vivo lung tissue culture techniques enables us to do many in vitro experiments looking at initial infection and transformation that could previously only be done in vivo. It also allows us to pre-screen virus constructs or delivery systems in order to select for in vivo experiments only those most likely to deliver the required

outputs. Similarly the use of cell lines is appropriate for some studies such as proteomic and genomic techniques to elucidate the transformation process.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Each study is carefully considered with expert statistical advice in order to use the minimum number of animals possible to give statistical power, i.e. useful results. Approval by the local Experiments and Ethical Review Committee is only given where the animal numbers have been demonstrated to be the minimum consistent with deriving statistically-significant results and where statistical advice has been demonstrated.

## **Refinement**

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

### **Refinement**

Sheep are the only model we can use for experimental infection as JSRV cannot infect any other species except for goats. Using naturally affected animals means we can collect the majority of samples needed without doing experimental infections. Where unaffected negative controls are required for PM these are usually sheep that have been selected for cull anyway.

We aim to minimise any stress or discomfort to experimental animals. For example, wherever possible sheep are housed and transported in groups of 2 or more. At any signs of distress or disease the animals will be examined by a vet and treated as appropriate or euthanised.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 135. Strategies for Brain Repair</b>
<b>Key Words</b>	Brain repair, transplantation, neuroscience
<b>Expected duration of the project</b>	0 year(s) 6 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project seeks to develop novel strategies for treatment of brain damage, whether caused by injury or disease, with a particular focus on the development of novel cell and gene therapies for Parkinson's disease (PD), Huntington's disease (HD) and stroke

**What 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 underpins clinical trials of fetal tissue transplantation in HD and PD taking place now, and provides the biological foundations for the next generation of major new applications using more efficient sources of cells, including pluripotent stem cells.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats and mice. The project will use approx. 300 rats and 500 mice over 6 months.

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

The project involves surgical, anatomical, physiological and behavioural procedures of mild, or at most, moderate severity, including breeding genetically modified animals, that express modest impairments of motor and cognitive disability, that are the targets for structural repair and functional amelioration. The experimental procedures are reliable, and serious adverse effects are rare and not expected, but procedures are in place for rapid alleviation of distress in the case of unexpected adverse events being detected. All animals are killed at the end of each experiment by the most humane methods appropriate to the species.

**Application of the 3Rs**

## **Replacement**

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

### **Replacement**

Motor and cognitive behaviours are complex features of the living sentient animal, dependent upon the intact functioning of a complex living nervous system, and impaired in human neurodegenerative diseases. The survival, growth and connectivity of cells in this complex environment cannot be adequately modelled in vitro or in simulation. Thus, in order to develop effective new cell-based therapies for devastating human conditions, the experimental use of live animals is the only way to model the disease processes, to determine the survival integration growth and connectivity of cell repair processes, to test the effectiveness of alternative cell therapy procedures, to develop the transplantation technology and to test protocols for safety and efficacy prior to human application.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

All protocols are designed for maximum sensitivity, and experiments are designed to maximise power to detect significant results with the smallest numbers of animals achievable. Non-animal alternatives e.g., tissue culture are used to optimise all cell preparation protocols prior to assessment in animals, but ultimately the in vivo situation cannot be avoided if the goals for human health are to be achieved.

## **Refinement**

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

### **Refinement**

The organisation of motor and cognitive functions and of the brain systems that underpin them are relatively consistent among mammalian species but differ progressively from non mammalian brains. Rats and mice are used as the least sentient mammals to model the relevant systems and functions disturbed in human neurodegenerative disease. These species tolerate well living in the laboratory environment, and provide the most extensively validated models for addressing the physiological, anatomical and behavioural functions under investigation. All animals are housed in licenced facilities and cared for by professionally trained staff following procedures designed to optimise health and welfare, operating under a rigid

inspection system to ensure compliance with full and continuous attention to welfare regulation and best practice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 136. Estuarine Fish Monitoring to Inform Conservation</b>
<b>Key Words</b>	Acoustic tracking, European Seabass, Conservation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

<b>Yes</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>Yes</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

From 2013-2016 the International Council for Exploration of the Seas (ICES) reported a dramatic decline in North Atlantic European Seabass (*Dicentrarchus labrax*) stocks, and recommended corresponding severe reductions in landings. European Seabass are highly dependent on coastal nursery habitats throughout infancy. In 1990, 34 Bass Nursery Areas (BNA) were designated, these include - estuaries, power plants and shallow embayments, within which targeted commercial fishing is prohibited for all or part of the year. This project proposes to monitor the movement patterns of European Seabass within BNAs and adjacent coastal habitats. The project outputs will be used to inform management of fishing practices within and adjacent to these protected areas.

The primary project aims are to record the frequency and duration of European Seabass (*Dicentrarchus labrax*) (>25cm total length) habitat use within and adjacent to, Bass Nursery Areas (BNA) of UK.

Specific research questions are:

- What proportion of time (frequency and duration) is spent in unprotected coastal habitats compared to Bass Nursery Areas?
- How European Seabass distribution related to habitat, environmental variables (temperature/salinity), season and/or year?
- Record if there are significant inter-annual differences in individual European Seabass movement patterns?

**What 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 provide empirical data on European Seabass movements within coastal and estuarine habitats, as well as provide evidence of the effectiveness of BNA legislation at protecting European Seabass. The project results will be provided directly to local fisheries management and could lead to increased sustainability of local European Seabass fisheries.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

200 European Seabass (*Dicentrarchus labrax*) (>25cm total length) will be tagged and monitored over a period of 1.5-5 years using acoustic telemetry. Acoustic telemetry is a tracking technique which relies on attachment of transmitter tags to host organisms. Unique pings can then be recorded by strategically placed receivers.

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

All surgical procedures have the potential to result in health problems for fish such as disturbance of physiological function, or more subtle behavioural or immunological effects. However following a short period of perturbation following capture and tagging no significant effects on swimming behaviour are typically observed in fish following this procedure. Handling stress will be improved by rendering the fish unconscious (anaesthesia) during the operating procedure and were advised by REDACTED analgesia will be provided to minimise surgical pain. Fish that do not successfully recover will be immediately killed.

## Application of the 3Rs

### START HERE Replacement

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

#### Replacement

Live fish are required for the project so that their movement patterns can be monitored. No suitable non-protected animal would serve as a viable substitute.

The use of wild fish is preferable because the swimming capabilities and behaviour of hatchery-origin stock may be biased by the condition, and learned behaviour of captive fish. The validity of results based on non-wild fish may be challenged.

#### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Within the project 50 individual European Seabass will be tagged and tracked per designated POLE (200 total). 50 has been selected as the minimum number of animals required, because individual fish are likely to display variation in movement patterns. This number also allows for fish within the project to be lost from the study due to capture within commercial and recreational fisheries or to die as a result of natural causes. The loss of fish due to fishing will be minimised through the placement of visible external tags asking for immediate release.

### **Refinement**

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

### **Refinement**

European Seabass (*Dicentrarchus labrax*) are a species of conservation concern and this study will inform management and regulatory objectives.

Whilst invasive, surgical implantation to the peritoneal cavity represents a secure form of attachment from which individuals have been shown to recover rapidly with no lasting harm. The use of an anaesthetic agent to sedate the fish during transmitter insertion offers effective pain control plus additional analgesia on recovery. Analgesics will also pre-operatively applied to the surgical site to minimise pain distress and suffering. Minimal fish handling and the employment of fish husbandry welfare techniques will ensure minimal stress to the fish prior to and following the surgical procedure.

To ensure minimal disturbance, extensive validation exercises will be conducted to ensure appropriate tag: fish sizes are used. Fish will also be monitored prior and post-surgery. Brightly coloured external ID marker tags will also be attached to each fish to advertise it's inclusion in the study if caught by recreational or commercial fishermen. To reduce drag, external ID tags will be constructed of a complimentary design and be the smallest possible size. Acoustic receivers will be attached to buoys throughout each Bass Nursery Area included in the project. All these marker buoys will be visible at all states of tide as to reduce the risk of boat collision.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 137. The genetics of wild-type chicken lines.</b>
<b>Key Words</b>	chicken, genotype
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This Licence will allow the sampling of chicken lines to determine their genetic characteristics which will allow the selection of appropriate birds for breeding programmes or use in scientific research.

**What 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 accurate determination of genetic types of chickens held in the research units will inform the breeding strategies so that the eggs and birds have known genetic characteristics. These are made available to the wider research community for use in their research projects that may then lead to scientific, veterinary and medical advances in many fields.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

It is anticipated that approximately 12000 birds of various chicken lines will be sampled to determine their genetic type over the five year 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 levels of severity? What will happen to the animals at the end?**

. Sampling birds to look for their genetic types involves a small blood sample or plucking a feather, this causes minimal pain, with a slight risk of bruising afterwards at the site of the needle insertion, but this quickly heals. Birds may be transferred to other Licences if authorized or will be culled by a humane, authorised method.

## Application of the 3Rs

### Replacement

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

### **Replacement**

The aim of the project is to be able to maintain and select individual birds to keep particular chicken lines as a resource required by scientists working in relevant research fields. These projects require the use of live embryos or birds or tissues derived from them and so the lines must be maintained to allow this supply.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Birds will only be bred if they are deemed to be required to maintain an efficient and effective flock or sampled if it is necessary for an accurate determination of genetic type to inform decisions on flock management and breeding strategies.

### **Refinement**

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

### **Refinement**

It is essential in some cases to determine the genetic type of the birds and this means that the birds themselves may be sampled using a regulated procedure such as a small blood sample or a plucked feather. We collect the smallest volume of blood and smallest feather that will provide us with enough DNA to analyse.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 138. Investigating the biology of musculoskeletal tissues in health and disease.</b>
<b>Key Words</b>	cartilage, dwarfism, osteoarthritis, biomarkers, therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We propose a detailed study of disease mechanisms leading to abnormal cartilage growth (chondrodysplasias) and osteoarthritis (OA) using genetically tractable mouse models. This will lead to the identification of potential biomarkers and of novel treatment.

Cells in our bodies exist embedded in a scaffold of various proteins, so called extracellular matrix (ECM) which they lay down during the course of their lives. ECM determines the biomechanical properties of tissues and contributes to the diffusion of nutrients and signalling molecules, and thus tissue health. Mutations in genes encoding cartilage components or modulating the levels of these components in the tissue lead to disease (for example increased matrix deposition leads to fibrosis and increased breakdown of cartilage matrix leads to osteoarthritis). The purpose of this project is to determine the mechanisms by which changes in genes encoding or modulating the cartilage components lead to chondrodysplasias and how they contribute to the more common complications such as osteoarthritis and lower-limb weakness. Numerous cartilage mutations leading to musculoskeletal diseases have been previously described; however, the mechanisms leading to these conditions remain largely unknown. REDACTED we have elucidated several important mechanisms of cartilage disease progression that allowed us to propose and test a treatment that resulted in a world-wide clinical trial. Our research to date highlighted the role of cell stress and endoplasmic reticulum (ER, part of the cell responsible for processing and correct folding of newly made proteins) stress in the disease mechanism and validated some promising therapeutic targets allowing us to further our research into new therapies for these 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)?**

These studies will advance our knowledge of musculoskeletal biology and disease. They will provide further insight into how cellular and ER stress contribute to tissue integrity and disease. In addition, our experiments continue to identify potential therapeutic targets and we will continue to test small molecule compounds which could be used in treatment of patients in the future.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Species: Mouse (*Mus musculus*). Overall number of animals used during this licence is estimated at approximately 10,000 over the 5 year project of which 750 will be used for treatment of chondrodysplasias and 250 in osteoarthritis studies.

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

The mouse strains included in this application exhibit a mild short-limbed dwarfism and in our experience suffer no obvious pain or discomfort (sub-threshold). Most our studies involve analysis of tissues after humane sacrifice of the animal. For the study of osteoarthritis, the animal will undergo a small operation (under general anaesthesia) on one knee to destabilise the joint and cause a reproducible osteoarthritis over the course of 8 weeks; as a result it will develop a slight limp. The procedure may induce mild pain and inflammation which will be moderated via pain relief. Potential treatments will be administered by injection which can cause momentary discomfort or via surgical procedures which will be accompanied by a relevant pain relief and anaesthesia regime.

## Application of the 3Rs

### Replacement

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

### Replacement

The use of animals is essential in our investigations. Although several aspects of musculoskeletal biology can be modelled in cell culture, the systemic process of bone growth involves integration of many biomechanically responsive tissues and cannot be modelled in cell culture. Cartilage has a relatively poor blood supply and limited regeneration potential and as such it is extremely difficult to obtain enough age matched human material to study. Studies of disease progression and of cartilage degeneration using human material are near impossible as it can only be obtained via surgical intervention which is usually performed at terminal stages of disease. Obtaining age-matched unaffected control tissue is also difficult. Moreover,

human material is genetically more variable and transgenic mouse models allow us to genetically dissect various disease pathways.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We have applied advanced statistical analyses to ensure we extract maximum insight from the minimum number of animals used. We have also implemented longitudinal studies of bone growth in order to reduce the number of animals used and we harvest all tissues from each humanely killed animal and store them in our biobank in order to maximise the material available from our animal models.

### **Refinement**

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

#### **Refinement**

We have extensive expertise in cell culture and tissue engineering and use cell culture models where possible, to dissect the disease pathways in parallel with the animal studies and to biochemically test the efficacy of specific drugs prior to their use in mice. We also use state-of-the-art systems biology and in silico modelling techniques to elucidate the pathways of interest using legacy data and publically available data. This allows us to refine the models needed for our research and reduce the amount of animals used in our protocols. We use solely repurposed drugs for which there is publically available data on side effects and symptoms, and only select the drugs that have negligible side effects.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 139. Safety Testing of Medicinal Products Using Non-Human Primates</b>
<b>Key Words</b>	Regulatory, Safety Assessment, Non-Human Primates
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project licence authorises the conduct of studies in laboratory non-human primates to evaluate the safety, quality and effectiveness of medicinal products for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening conditions in man, in terms of general toxicity and whole body system exposure.

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, Alzheimer'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 humans 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of blood/tissues to support drug development and the validation of non-animal alternative methodology.

As a specially protected species, the non-human primate is selected for safety assessment studies only after careful determination that it is the most biologically

appropriate species, and that there is no other acceptable candidate species. This is usually based upon the test material's mechanism of action, target systems/receptor profile etc and an assessment of the appropriateness of the primate model in general. This is typically achieved via the availability of data (e.g. *in vitro* metabolism, early pharmacokinetics, or other supporting information) demonstrating that the test material or metabolite is effective in primates, that primates are the most relevant model to man, and that the purpose of the programme of work cannot be achieved by the use of animals that are not primates. A record of the scientific rationale for the use of primates is always retained.

With respect to the high specificity of large molecule biotherapeutics (such as monoclonal antibodies and antibody-drugs conjugates) to the human target, non-human primates are often the only species exhibiting binding of the target and the desired pharmacological effect, and therefore, toxicology studies are most frequently performed in this single species.

Thus, the use of primates in carefully selected studies is an essential requirement in the successful development of new medicinal products.

**What 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 provision of robust safety data to facilitate sound decisions by national and international Regulatory Agencies regarding human exposure to medicinal products. Without these studies, progression of new medicines to early human studies and to patients could not occur safely or in the current regulatory framework. With the increasing use of advanced drug technologies, targeting the immune system to combat life-threatening and debilitating illnesses like cancer and autoimmune diseases, the non-human primate is often the only suitable test species due to similarity of the immune system. Validation and refinement of test methods may be completed for specific techniques and may be published to the wider scientific community.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 year life of this Project Licence, it is estimated that 4500 non-human primates will be used (900/year).

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels 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 are 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 to which the animal is exposed. These may also be analysed to detect any effects on 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. 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 the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. 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

### Replacement

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

### **Replacement**

There are currently no scientific and legally acceptable evaluations of systemic toxicity which will satisfy regulatory requirements and provide sufficient safety data other than use of animals, though validated *in vitro* tests for specific organs are used wherever possible. As new *in vitro* methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace *in vivo* procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.

As a specially protected species, the non-human primate is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species with relevance to man, and that there is no other acceptable candidate species that is not a primate.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

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.

In general, toxicity studies are initiated in rodents before progressing into larger animals. This approach, combined with background literature searches and looking across at other study types, can lead to earlier decisions on whether or not to continue development of a particular test material, refinement of study designs and reduced use of primates.

The number of small molecule new chemical entity drugs (NCEs) developed using non-human primates has declined in recent years but is being offset by the proliferation of large molecule biotherapeutics (such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs)). The non-human primate is often the only relevant model for testing such materials due to the similarity with the human immune system but a reduction in the numbers of animals used per study is being achieved through knowledge gathering and refinement of study designs at an industry/regulatory level.

As most studies involve the post-mortem examination of tissues following treatment, opportunities for re-use are limited. Nevertheless, this licence does include the potential to re-use animals, in compliance with Home Office guidance and, where possible, this is intended to help reduce the overall number of primates used at this facility.

### **Refinement**

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

### **Refinement**

Species choice and use of specific animal models is determined by the need to generate data that satisfies worldwide regulatory authorities. Where a choice of species is possible, care is taken to select the most 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, whilst 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.

Non-human primates are routinely group housed with environmental enrichment which encourages species specific behaviours without adversely impacting study outcomes.

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).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 140. Modulation of the immune system as a therapeutic target in cancer</b>
<b>Key Words</b>	Cancer, Immune Suppression, Treatment, Vaccination, Chemotherapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

One in two people are affected by cancer at some point in their lives. Further therapies are required to tackle the disease. The immune system is incredibly powerful and designed to detect and kill abnormal cells in the body. Therapeutic approaches which raise immune responses against established tumours, such as chemotherapy and cancer vaccines rarely represent cures for the disease. One reason for this failure is that tumours exploit mechanisms to suppress the immune system, which prevent it from attacking the tumour. We have started to understand the pathways exploited by cancer to achieve this, such as heme oxygenase-1, expressed in the tumour are capable of suppressing these immune responses. If we can develop therapies, aimed at interfering with these pathways, it is hoped that we can circumvent this immune suppression, which we believe would permit cancer vaccines and chemotherapy, to more effectively and efficiently attack and control the growth of an established tumour.

**What 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 a therapy, or combination, which can efficiently activate the immune system to identify cancer, alongside alleviating immune suppression, the combination could potentially lead to more robust response rates from these therapies. This would result in greatly increased survival rates for the disease, and in the best case, potentially even a cure for some cancers.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect this research to require 10,700 animals 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 levels of severity? What will happen to the animals at the end?**

We will use tumour models in mice which have already been established to be safe, and accurately mimic the human disease. These tumours will be closely monitored and will cause the animal minimal harm. We intend to administer immune modulating agents to the mice which we believe will generate immune responses and alleviate immune suppression in the tumour to result in robust responses on tumour growth. The primary candidates for these studies will be therapies with potential use in humans, and have a prior history of safe use, which as such, means that they will have low toxicities and cause no lasting harm to the animal. Mice at the end of the procedure will be humanly killed before the onset of any pain or harmful effects are felt.

## Application of the 3Rs

### Replacement

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

### Replacement

Mice have very similar immune systems to our own, and murine models of cancer, which have already been developed, share many characteristics of the human disease. The complexity of the immune response and its suppression in cancer cannot reliably be modelled in the laboratory, and as such the use of mice is an absolute requirement to address these important questions.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will conduct a study of human cancer tissue alongside our work in mice, this will be used to inform us of cells of interest and additional targets we may need to consider, which will allow us to conduct informed experiments, and use fewer mice. Further to this, we will ensure that the minimum number of mice will be used for this study, through the implementation of models which have previously been validated, and the implementation of in vitro studies where possible.

### Refinement

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

## Refinement

The mouse models of cancer which we are using have previously been established and validated. These models have been selected as they mimic the human disease, and with close monitoring, will cause the animals minimal harm. In addition, the therapeutic interventions to be tested are designed to be directly translatable into the clinic and are expected to cause the animal minimal harm. We have 5 years of experience, and data accumulated, in working with these models which will be used to inform experiments moving forward.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 141. Identification of determinants of pathology and protection in respiratory infections</b>
<b>Key Words</b>	Lung infection, immune response, tissue damage, epithelium, repair
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Infectious diseases are a leading cause of death worldwide, and respiratory infections like influenza and tuberculosis are among the biggest killers. Influenza kills up to half a million people in a normal flu season, and in historic pandemics, up to 50 million people were killed by influenza infection. Lung damage can be caused by the infectious pathogens directly, or by the activation of the body's immune response that should actually be protective. We try to understand what decides whether the immune response protects or damages the lung. We use the most advanced cell culture systems to study effects of influenza virus infection on lung epithelia, with the aim of directing and focussing our experiments in live animals to keep animal numbers down.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Given the huge clinical problem, understanding lung infections better is the first step to improving their outcome. We concentrate on two aspects: Which parts of the immune response cause lung damage rather than combat the infection? What factors hinder the lung repair that is crucial for recovery at the end of a lung infection? Answers to these questions will pave the way for novel therapies, to improve the course of infections in humans.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

To achieve our objectives we propose to use the laboratory mouse as the model organism, since infections in mice mirror human infections well, and many tools to analyse the mouse immune system are available. We estimate that we will be using

approximately 10,000 mice per year, a large fraction of which (~30%) will only be used for tissue sampling. We use advanced statistical methods and breeding schemes to make the most efficient use of our mouse breeding colonies.

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

Many but not all mice used in this project will be infected, and the mice will experience symptoms similar to those humans have during lung infections. Common to all infectious models is the assessment of virulence or pathogenicity. This will be assessed most frequently as morbidity, which is quantifying changes in physiological parameters relevant to each type of infection and clinical symptoms associated with all types of infection. Many of the pathogens proposed to be used in the project can cause disease, however, every effort will be made to limit infection-associated pathology to the absolute minimum required for answering our scientific questions.

## Application of the 3Rs

### Replacement

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

### Replacement

We try to do as much preliminary work as possible in tissue culture, even though the many parallel processes happening during a lung infection cannot be reproduced in culture entirely. We are constantly working on improving the tissue culture systems to make them more predictive of what happens in an infected organism.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Before we start working with mice, we test most candidate compounds and mechanisms in tissue culture, as far as this is possible. This allows us to test in mice only the most promising and potent molecules, and to investigate only the mechanisms we found to be in action in tissue culture. This greatly reduces animal numbers. We also aim to calculate the smallest number of animals required to obtain a clear answer from our mouse experiments.

### Refinement

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

## Refinement

The mouse is the best characterised model for infection studies, with many features that resemble human infection. Their immune responses are well defined and the technology allowing analysis and manipulation of the immune system is highly developed. In addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this project as well as the mouse.

In our experiments, we define specific end-points to monitor the effect of infection on physiology, instead of induction of pathology, and we will go to great lengths to minimise the possibility of severe pathology. This will be achieved by close monitoring of symptoms and physiological parameters during the course of the infection. During the infection phase, we take a range of measures to insure the mice do not suffer more than necessary, for instance helping the body temperature stay constant and avoiding dehydration. We will use genetically-modified micro-organisms for infections in order to take advantage of reporter signals – luminescence or fluorescence, for example – to monitor the course of infection and potential dissemination to different organs with non-invasive techniques. These and other longitudinal measurements allow to use fewer mice and obtain more robust data.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 142. Identification of determinants of pathology and protection in respiratory infections</b>
<b>Key Words</b>	Lung infection, immune response, tissue damage, epithelium, repair
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Infectious diseases are a leading cause of death worldwide, and respiratory infections like influenza and tuberculosis are among the biggest killers. Influenza kills up to half a million people in a normal flu season, and in historic pandemics, up to 50 million people were killed by influenza infection. Lung damage can be caused by the infectious pathogens directly, or by the activation of the body's immune response that should actually be protective. We try to understand what decides whether the immune response protects or damages the lung. We use the most advanced cell culture systems to study effects of influenza virus infection on lung epithelia, with the aim of directing and focussing our experiments in live animals to keep animal numbers down.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Given the huge clinical problem, understanding lung infections better is the first step to improving their outcome. We concentrate on two aspects: Which parts of the immune response cause lung damage rather than combat the infection? What factors hinder the lung repair that is crucial for recovery at the end of a lung infection? Answers to these questions will pave the way for novel therapies, to improve the course of infections in humans.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

To achieve our objectives we propose to use the laboratory mouse as the model organism, since infections in mice mirror human infections well, and many tools to analyse the mouse immune system are available. We estimate that we will be using

approximately 10,000 mice per year, a large fraction of which (~30%) will only be used for tissue sampling. We use advanced statistical methods and breeding schemes to make the most efficient use of our mouse breeding colonies.

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

Many but not all mice used in this project will be infected, and the mice will experience symptoms similar to those humans have during lung infections. Common to all infectious models is the assessment of virulence or pathogenicity. This will be assessed most frequently as morbidity, which is quantifying changes in physiological parameters relevant to each type of infection and clinical symptoms associated with all types of infection. Many of the pathogens proposed to be used in the project can cause disease, however, every effort will be made to limit infection-associated pathology to the absolute minimum required for answering our scientific questions.

## Application of the 3Rs

### Replacement

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

### Replacement

We try to do as much preliminary work as possible in tissue culture, even though the many parallel processes happening during a lung infection cannot be reproduced in culture entirely. We are constantly working on improving the tissue culture systems to make them more predictive of what happens in an infected organism.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Before we start working with mice, we test most candidate compounds and mechanisms in tissue culture, as far as this is possible. This allows us to test in mice only the most promising and potent molecules, and to investigate only the mechanisms we found to be in action in tissue culture. This greatly reduces animal numbers. We also aim to calculate the smallest number of animals required to obtain a clear answer from our mouse experiments.

### Refinement

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

## Refinement

The mouse is the best characterised model for infection studies, with many features that resemble human infection. Their immune responses are well defined and the technology allowing analysis and manipulation of the immune system is highly developed. In addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this project as well as the mouse.

In our experiments, we define specific end-points to monitor the effect of infection on physiology, instead of induction of pathology, and we will go to great lengths to minimise the possibility of severe pathology. This will be achieved by close monitoring of symptoms and physiological parameters during the course of the infection. During the infection phase, we take a range of measures to insure the mice do not suffer more than necessary, for instance helping the body temperature stay constant and avoiding dehydration. We will use genetically-modified micro-organisms for infections in order to take advantage of reporter signals – luminescence or fluorescence, for example – to monitor the course of infection and potential dissemination to different organs with non-invasive techniques. These and other longitudinal measurements allow to use fewer mice and obtain more robust data.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 143. Understanding mitochondrial turnover in vivo</b>
<b>Key Words</b>	Autophagy, Mitophagy, Parkinson's Disease
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Mitochondria are specialised structures that power the processes that occur within the cell. Mitochondria get damaged over time and so the cell produces new ones and destroys the old ones. Timely turnover of mitochondria in this way is thought to be essential for cellular health and development. Damaged mitochondria can be very detrimental to the health of the cell and impairment in their clearance has been linked to a wide variety of diseases including Parkinson's. However, we do not know how this turnover occurs physiologically. The aim of this research is to identify when mitochondrial turnover occurs and determine which tissues are most sensitive to this phenomenon. If we can understand this, then we can use the information gained to target diseases where mitochondrial turnover goes awry.

**What 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 provide a basis for a fundamental understanding of when and where mitochondrial turnover happens. This increased understanding will contribute to better treatment of diseases such as Parkinson's.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

A maximum of 7000 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 levels of severity? What will happen to the animals at the end?**

Many of the animals we use will only be bred and allowed to age and have nothing else done to them whilst they are alive. Less than a quarter may have food taken away for no more than a day on one occasion or have their diet changed (but the diet should not have any adverse effects on them). Some of this subset of animals may be given compounds that we think will change how mitochondria work or are turned over, so that we can see the effects on the cells. The compounds may be

given in food or water, by a tube into the stomach or by injection. We do not expect any of these drugs to cause the animals to be unwell and the methods we administer them by should not cause more than brief discomfort. Mice may be given access to running wheels to see what effect exercise has on mitochondrial turnover. Animals will have a free choice as to whether they use the wheels or not. All mice will be carefully monitored for signs of reduced weight gain or other signs of adverse welfare. On the whole, animals are not expected to show any significant signs at all. However, in using mouse models of diseases such as Parkinson's, neglect of grooming, reduced ambulation, signs of movement impairment and resistance to passive movements are to be expected. Animals displaying these early signs of deficit will be humanely killed immediately and tissue isolated for analysis.

## Application of the 3Rs

### Replacement

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

#### Replacement

Unfortunately, we cannot yet grow and replicate functioning organs in a petri dish as they are made up of a complex architecture of different cell types that cannot grow in isolation. Therefore to understand how mitochondria function physiologically in these cells, and how they become altered in diseases such as Parkinson's, we have to look at organs from a mouse model. We do not yet have an understanding of how the organ environment influences mitochondria and once we have this knowledge, which will hopefully be gained from this project, we can try and replicate it artificially.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Our work will use the minimal number of animals required to gain statistically meaningful results as too many animals could be wasteful and cause unnecessary harm, while too few animals would mean the work would have to be repeated. To ensure this, we will consult with a professional statistician before experiments are started. We will also consult extensively with previously published results to make sure we are using the optimal conditions for any treatments we use and if these are not available we will first carry out preliminary studies with small numbers of animals to identify the best conditions.

### Refinement

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

### **Refinement**

The mouse is the animal of choice for this project as it is a mammal and the majority of intracellular functions are highly conserved with humans. Additionally, many mouse models of disease that are relevant to our work are already available meaning we will not have to generate more.

Our studies do not involve any surgery and have been designed to cause minimal suffering. Also, our genetic manipulation and treatments should not cause lasting pain and distress to the animals. Regardless, animals will be monitored throughout their life for signs of distress and should any adverse effects on their health be observed, appropriate veterinary guidance will be immediately sought. We will constantly monitor our techniques for ways of improvement/refinement and be in close contact with other researchers/relevant new literature to be aware of any adverse effects and ways to circumvent these.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 144. Biological function of DNA modifications'</b>
<b>Key Words</b>	Cancer, Nucleotide metabolism, DNA modifications
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

During development human cells acquire different functions and form organs capable to perform specific tasks. Remarkably, all those cells contain the same genome. The way different cells manage the same genome is by “expressing” (ON) and “silencing” (OFF) distinct set of genes, which are useful for a particular cell type.

The mechanisms responsible for establishment and maintenance of ON and OFF states are managed by proteins, which act to orchestrate gene expression and to support normal homeostasis. Defects in these proteins lead to various degrees of chaos in cells, which manifests as cancer or certain neurological disorders such as Rett Syndrome.

Our first goal is to describe aspects of gene regulation, which operate during differentiation of blood cells and in neuronal cells. More specifically, we aim to address gaps in understanding of how exactly regulator proteins find target genes in normal cells and how defects manifest in cancer.

Mutations in the DNA cause cancer. Incidence of mutations is affected by natural mutational processes and environment. Based on our knowledge of metabolism, we predict that intake of certain foods can provide metabolites, which increase mutability of the DNA. We plan to test dietary components, which may affect incidence of bowel cancer. The similar mutational pathways, which cause cancer can be exploited to aid in killing cancer cells, since high frequency of mutations can be detrimental. The key here is to identify specific vulnerabilities in cancer cells, which increase their sensitivity to the mutagens. Our previous work led to identification such therapeutic window cancer, which have potential to benefit patients of pancreatic cancer. Thus we plan to further characterise activity of those compounds in animal models of pancreatic cancer.

The primary targets for the novel therapies will be Myelodysplastic Syndrome (MDS), Acute Myelogenous Leukemia (AML) and pancreatic cancer, although it may not be

limited to these specific diseases because our recent data indicates applicability to wider spectrum of cancer subtypes as well. For MDS European registry documents average incidence rate of 4.1 per 100 000 per year. 3-year survival rate for the patients is rather poor (35%). AML has a similar incidence of 3.6 per 100 000 per year with a 5-year survival rate of 23.4%. Pancreatic cancer 5-year survival is only 4%. Low survival rates indicate deficiencies in treatment strategies, calling for better understanding of the disease aetiology leading the development of novel therapeutics.

It is unclear how inflammation and hypoxia in non-pregnant individuals and during pregnancy impacts gene regulation and cancer incidence. We hypothesise that some of the effects are mediated by enzymes, which act on the DNA and require oxygen for their activity, since alterations of their activity can have long lasting effects. Animal models will enable to elucidate physiological roles and extent to which these enzymes contribute to disease risk. Since the long lasting effects have a potential to be transmitted to the next generation, we will investigate gene regulation in mice in the second generation after the exposure to inflammation and adversity.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Our research will advance both basic gaps in understanding of the gene regulation and directly evaluate use of specific compounds for cancer therapy. Evaluation of new compounds as cancer specific cell growth inhibitors have a potential to benefit nearly two thirds pancreatic cancer patients. As well it will establish a more general principle, which could be applied to other cancers as well. In a short term we will learn about the potency of the compounds, which will help to build a case for going into clinical trials. Identifying factors, which elevate cancer risk will help in cancer prevention efforts, by advising general public about risks associated with specific diets. The results illuminating functions of enzymes involved in regulating gene activity will be of interest to scientist in academia and industry. In a long term compounds may be developed for treating or preventing cancer. The proposed work in CNS will reveal basic facts about the differential use of gene regulation in neuronal cell types. The data will be a benefit to the fields of neurobiology and general molecular biology, with the perspective to be used by researchers working on molecular causes and consequences of different neurological conditions. Conclusions from lower oxygen and therapy exposures of pregnant mice will influence advice on treatment practices, which are safest for the mothers and children.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 10 000 mice over the 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 levels of severity? What will happen to the animals at the end?**

The proposed procedures reflect the human experience, since we want to understand molecular basis of gene regulation defects which cause diseases in human. Our proposed experiments employ well-characterised experimental techniques that should not cause more than moderate degree of suffering. Majority (80%) of the animals are likely to experience mild severity. Mice will be predominantly bred and organs used for post-mortem analysis. The animals can experience some discomfort due to subcutaneous or internal tumours. Tumour initiation strategies will be minimally invasive (injection of tumour cells under the skin or genetic pre-disposition). Once the onset of tumours is expected, animals will be closely monitored for signs of tumour growth. As soon as signs of suffering are observed, animals will be humanely killed. When evaluating new compounds for tumour therapy, small number of animals will be injected and closely monitoring for any signs of distress. Animals experiencing distress will be humanely killed and the lower dose will be used for evaluating tumour therapy. Intestinal tumours are associated with mild anaemia, which is comparable to the experience of humans with similar condition. Some animals will be exposed to reduced oxygen levels. The mice will either experience short periods of low oxygen or a gradual reduction over 1-2 days. This rarely causes the animals any distress although they may undergo transient weight loss. At the end of the procedure animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

Where feasible, we will use cell culture systems in our research. However, frequently there are no cell culture models that accurately replicate the temporal and spatial interactions of the signalling pathways involved in development and cancer, or that can mimic the cellular organization of tissues and organs. Mouse is widely used as the lowest species of mammals suitable for studying cancer development and disease and their biology and genetics are well characterised.

Lower animals are not suitable as their metabolism differs substantially from mammals and tumour behaviour can be different, impeding interpretation of the results and progressing to clinical trials.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We use statistical power calculation tools to obtain the minimum and sufficient number of animals to use for each experiment. Because variance in the measurements has the largest impact on the number of animals, we will breed mice using defined genetic background and house mice in individually ventilated cages to reduce variability due to genetic differences or sporadic infections.

Strains, which are not used for experiments will be cryopreserved to reduce the number of breeding animals.

We will use researchers unaware of the animals undergoing treatment to score the effects, reducing the biases in the assessment and increasing reproducibility of the study ("blinded" experimental design).

The experiment design is being discussed with different researchers in the group, to identify if the post-mortem animal tissues could be used to benefit multiple project aims.

### **Refinement**

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

### **Refinement**

Mouse is a critically important model for pursuing indicated studies as it is the only mammal where efficient generation of genetic alterations is possible. As well, an extensive publication history allows comparative studies, when testing effect of mutations or drug candidates. The techniques were researched using the various databases (NC3Rs, PubMed and others) and found to be most refined to achieve each of the specific aims. When exposed to reduced oxygen, animals will be allowed to habituate, before lowering the oxygen pressure. Subcutaneous model of cancer will be used where possible. For bone marrow transplantation, we will prioritise the use split lower dose of radiation, which is known to be better tolerated. For cancer therapies we will use pilot studies to determine the safe dose of compound. Animal monitoring will be increased during procedures and recovery. Appropriate analgesia and anaesthesia is administered during procedures to minimise the pain and distress. For early identification of internal tumours, we will use imaging techniques where possible.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 145. Evaluation of anticancer agents and combinations in orthotopic oncology models</b>
<b>Key Words</b>	Cancer, Pre-Clinical, efficacy, models, imaging
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

As the understanding of the mechanisms behind cancer progression continues to increase, so does the requirement to develop and validate relevant models in parallel to test new strategies. The aims of this project are to provide the scientific community with accessible expertise in terms of available clinically relevant cancer models, knowledge and technical capability to improve decision making on which agents should progress to the clinic and which patients will benefit from the treatment. This project focuses specifically on solid tumours arising in organs of the prostate, pancreas and bladder, all of which are very different in terms of their origin, growth rate, progression and response to treatment.

The objectives of this project are:

- 1) To develop, validate and optimise patient relevant organ specific pre-clinical models of prostate, pancreatic and bladder cancer to enable the testing of anti-cancer agents. .
- 2) To evaluate anti-cancer agents and combination therapies using models developed in objective 1.

**What 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 order to benefit the clients/collaborators who develop the anticancer agents and ultimately patients who are treated with these agents, the development of pre-clinical cancer models that exhibit greater patient relevance by implanting them in relevant organs will allow these novel agents to be tested in more relevant conditions where environmental factors such blood supply, spatial arrangement, interaction with supporting cells and structures will be better represented. These models require

expertise in surgery as well as generating the cells that emit light and then applying the imaging technology to capture the right data and analysis, which is not readily available in most institutions and companies. These models will enable decision on moving programmes forward into clinical trials or in some cases this may result in a specific anticancer programme being cancelled which may seem a negative benefit, but identifying anticancer agents that are either ineffective or unsuitable for further development can be considered a positive benefit in the longer term as it prevents the unnecessary progression of ineffective therapies to early phase clinical trials and allows the redirection of resources and patients to other projects. Once validated, all models are added to the proprietary databases; access to which is free to all users, as well as abstract submission to national and international scientific conferences.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice will be used for this project as they are the lowest species of animal that allow the modelling of human cancer, and offer the opportunity for genetic manipulation to generate specific models relevant to human cancer. Substantial numbers of cancer relevant models are already validated in-house (100+) including prostate, pancreatic and bladder cancer making this species most suitable for this project. Over the course of this project we'd expect to use 7,200 animals to model these cancer types, the different stages of cancer and novel anti-cancer agents and combinations.

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

The majority of mice will undergo surgery to implant tumours in the site of origin in the prostate, pancreas and bladder under anaesthesia which are then measured once/twice weekly (or up to three times weekly dependent on growth) throughout the study by imaging under anaesthesia to track internal size before it becomes too large. Imaging is non-invasive and not expected to affect the wellbeing of mice. Dosing of candidate anticancer agents by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response (as assessed by imaging) or humane endpoints as guided by imaging before the onset of any adverse effects. Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects is uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs will result in humane killing regardless of body weight measures. The highest level of severity will be moderate. All mice will be killed at the end of the studies with tumour, blood and tissue collected which will allow further characterisation of treatment effect providing additional information such as how the cancer has spread or whether the drug has reached its target.

## Application of the 3Rs

## Replacement

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

### Replacement

Research conducted in a test tube or artificial environment (In vitro) has replaced animal use in early development phases, particularly in the development of screening assays to refine compound selection, target identification, off-target toxicity or toxicity versus normal tissue cell lines, which increase our understanding of the target and candidate agent and therefore guide and refine the steps prior to moving into in vivo, and minimise subsequent use. However, there is still a requirement to use animals for this project as in vitro assays still do not optimally mimic all interactions between cells and tissues, such as blood vessel formation, specific organ environment, spread to other organs and thereby relevant drug access or the many homeostatic mechanisms in play in an in vivo environment that allows relevant tumour biology drug evaluation.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The use of in vitro studies can be used to identify lead compounds, evaluate dose ranges confirming target modulation/expression and relative off-target toxicity which can be used to inform on relevant doses for use in the equivalent animal models to evaluate drug distribution, target modulation and toxic effects. The use of complex 3-dimensional in vitro assays can be applied to pre-screen studies and compound selection prior to advancement into animal testing (thus reducing animal use). The model development stage of this project will be used to determine statistically powering so the minimum number of mice are used in a study design but still achieve scientific endpoints. The use of imaging technologies can also reduce the number of animals required to generate study outcomes as model variation can be improved by eliminating mice which do not develop the disease appropriately or refining the model so this is minimised.

## Refinement

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

### Refinement

Mice are the lowest species in which the knock out of the immune system allows growth of human tumours. Mice with a fully functioning immune system also provide

the opportunity to investigate the immune system interplay with a mouse tumour. The mice will have tumours implanted into the prostate, pancreas or bladder i.e. at the site of origin which are more relevant to patients but are more complex and require imaging to track the growth inside the mouse by using prostate/bladder/pancreatic cancer cells that are altered to emit light which is then captured by an imaging system specifically designed for small animals. Organ-specific models are known to better model cancer in patients as tumour grows in the correct environment which facilitates spread to other organs as seen in the clinic and show a reduced response to chemotherapy therefore providing more relevant information on the drug. The use of imaging is also a refinement as data from the internal tumours can be captured in real time, provided additional data that wouldn't normally be visible, only using animals that show tumour, and minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled killing of the mice, thus reducing the duration of model and regulated procedures.

The development of relevant pre-clinical models of oncology in objective 1 is a key stage for the evaluation candidate anti-cancer agents to ensure the right models are being used to answer the questions being asked in objective 2. The following will be undertaken to minimise animal suffering.

- Pilot studies for the establishment of new tumour lines and refinements to surgical techniques will be carried out on an ongoing basis under the advice of the vet and/or the named animal care and welfare officer will be sought in this respect.

All surgical procedures will be conducted in line with established welfare guidelines on aseptic surgery using suitable anaesthesia along with peri and post-operative analgesia.

- Any in-life sampling will be in line with established welfare guidelines and micro-sampling regimens will be utilised where study design supports this.
- The frequency of dosing will be such that animals fully recover between injections and will not suffer more than transient pain and distress and no lasting harm and there will be no cumulative effect from repeated injections.
- Use of pilot tolerability studies to ensure there are no unexpected adverse effects associated with new models or unexpected toxicity because of tumour: drug interactions and to ensure the drug levels used are not associated with any cumulative effects.
- Using cells that emit light to allow imaging to be used to recruit only those mice that have been identified to have the right tumour location and to reduce model duration

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 146. Ion channels and related proteins in the development and treatment of neurological disorders</b>
<b>Key Words</b>	gene therapy, epilepsy, migraine, Neurology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our aim is to develop new treatments for neurological disorders, like epilepsy and migraine. At present approximately 25,000 people in the UK have epilepsy which does not respond to current therapies, this is the clinical need we aim to address with our new treatments. Our programme of work involves understanding how cells in the brain change as people develop seizures, and how genetic changes in these cells can cause seizures or other neurological diseases (such as migraine, or weakness). We use this information to develop strategies (which we establish and refine in human cell lines) to treat these disorders. For example a mutation in a gene that causes epilepsy destroys the function of that gene, then we may try to find a way, using gene therapy, to deliver a healthy copy of that gene to the brain. As well as understanding what goes wrong and developing ways to repair those changes, we have two additional main aims for the long term: firstly to make better animal models of diseases to improve the clinical translation of our tests, and to ensure the models themselves are as well-tolerated as possible. In addition, we have a long standing interest in understanding how genes affect the function of the brain, which in the long term may help give insight into how genes may be harnessed for 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 have already published 2 potential new gene therapy treatments for epilepsy, and the success of these treatments means that part of our work will be building up

to our first-in-human clinical trial for gene therapy for epilepsy. This offers an exciting new possible treatment for the large number of patients for whom there currently is no effective therapy. Initially we will aim to treat the most severe patients, who are already scheduled to have surgery to remove the part of their brains that is causing seizures (and our aim is to allow them to be treated so that they do not need this surgery), but eventually we hope as our treatments and delivery improves, we may be able to treat an increasing number of people with epilepsy, and other neurological disorders. As our models improve, we will be able to determine whether our treatments are applicable, for example to different types of epilepsy or to migraine. As many treatments fail between bench and bedside, a big effort for our current work is to develop and test additional treatments and to validate them in additional disease models.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We use rats and mice only. We anticipate using ~4000 rats, and ~20,000 adult mice in this 5 year study. It is important to note the majority of mice will be used simply for breeding and will not have any stressful procedures carried out upon them. This work supports the research of over 30 scientists developing gene therapy treatments.

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

We make every effort to ensure none of our animals suffer pain. The animals used for breeding are given comfortable housing and toys. Occasionally animals fight and we must house them by themselves. Some animals will have seizures, but the seizures themselves are not thought to cause suffering. Some animals will have surgery, and we will do all we can to ensure a painless and comfortable recovery. Most of the animals are killed in a humane way at the end of the study, but some may be sent (only if they are healthy) to other researchers who could work with them to benefit studies of other disorders.

## Application of the 3Rs

### Replacement

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

### Replacement

The neurological diseases we study are products of interconnected neurons. Symptoms like seizures are difficult to model in non-animal systems. We do all we

can to use cells, and stem cells to test our treatments for safety prior to using them in vivo.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We carry out detailed data recording from animals over a long period when they live in their home cages so that we get as much information as possible from each animal. By using blinded, randomised trials we ensure our findings are not diluted by bias.

## **Refinement**

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

### **Refinement**

The type of neurological disease we study requires a full cortex which is only present in mammals (so we cannot use flies or c elegans). Both mice and rats provide detailed and rich behaviour that replicates human epilepsies, and allows for quantitative assessment of our anti-epilepsy treatments. We work to house, feed and maintain animals so that they are comfortable, and do not feel distress. We provide them with toys and special foods (e.g. Nutella) to keep them entertained.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 147.      Epidemiology of parasite infections in wild bird populations</b>
<b>Key Words</b>	birds, wildlife, disease, malaria, trichomonas
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>No</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>Yes</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this work is to improve our understanding of how parasites and diseases are transmitted in wildlife populations. We want to understand how changes in the environment – such as changing food availability or climate – affect parasite transmission, and how this can be managed for species conservation. There are four main objectives:

1. How do changes in climate or food availability affect the interactions between birds, their parasites, and the vectors that transmit parasites?
2. How does the diet of birds influence parasite transmission?
3. How does infection vary through the lifetime of individuals?
4. When do birds first become infected by parasites and, if infection happens in the nest, how does this affect the subsequent behaviour and survival of individuals?

**What 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 will provide novel insights into how parasite infections fluctuate within and between individuals in wildlife populations. The work will look at both vector-borne parasites such as avian malaria, and directly-transmitted parasites such as *Trichomonas gallinae*. The work will look at how parasite transmission is affected

by the distribution and availability of food resources, and will have practical implications for management of declining wildlife.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

In order to gain an accurate estimate of disease prevalence in avian communities, we need to collect samples from a large number of individuals although, where possible, we will minimise sample sizes through the use of statistical methods to aid analysis. We will sample a maximum of 200 of the majority of species (actual numbers will be far below this for the majority of species, and the numbers sampled will depend on those available at our study sites). We will sample a maximum of 500 individuals from 5 species over the duration of the project these are: yellowhammer, linnet, turtle dove, hawfinch, and mute swan

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

The protocols involved in screening birds for parasites are widely used and are known to cause minimal suffering. All samples will be collected from each bird within a short space of time, at the capture site. Each bird will then be re-released into the wild without delay. No adverse effects are considered likely.

## Application of the 3Rs

### Replacement

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

### Replacement

We wish to examine the dynamics of parasite infections in wild populations, so we cannot carry out this work without using wild animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

In order to gain an accurate estimate of disease prevalence and how this fluctuates within and between individuals, we need to collect samples from a relatively large number of individuals although, where possible, we will minimise sample sizes through the use of statistical models to aid analysis.

### Refinement

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

### **Refinement**

We are using species of bird within which previous work has identified that the relevant parasites are present in a large enough proportion of individuals that we can achieve our objectives.

The protocols followed in order to screen birds for parasites are well-established, and known to cause minimal harm.

Birds will be captured and handled by experienced individuals with the relevant licences, and will be released as soon as the required samples and measurements have been taken.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 148. New therapeutic approaches for inflammatory joint disorders</b>
<b>Key Words</b>	Inflammation, Arthritis, New therapies
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aims of this project are to:

- Identify efficacy of test drugs and side effects not predicted by cell culture based model systems.
- Study the effects of the drug on the body, and also the effects of the body on the drug.
- Demonstrate that anti-inflammatory activity can be shown at specified doses.
- Identify the best arthritis models to use pre-clinically that correspond with a specific therapeutic target.
- Test a drug's efficacy to affect leucocyte recruitment and identify associated mechanisms that may be shared with other autoimmune 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 target of this programme of work is to provide pre-clinical supporting information for clinical trial applications. A drug requiring evaluation will be supplied to us by clients in the biotech and pharmaceutical industry, along with evidence supporting the rationale for testing the agent. We aim to investigate the efficacy and mechanism of action of a drug to help our clients make a more informed decision on whether to proceed into clinical trials. This reduces the risk of later stage failures and hopefully predict on side effects associated with a particular therapy. Information supplied by

us will speed up the clinical trial process and make it less financially prohibitive. The benefit is, therefore, a reduced number of unproductive human volunteer studies (and a reduced risk of adverse effects) and most importantly the development of improved and more effective therapeutics targeting inflammatory joint diseases and potentially other inflammatory and/or autoimmune diseases with shared mechanism of action. The benefit to patients will be the identification of new anti-inflammatory drugs. This programme of work will help identify the best potential drugs early in the drug development process or aid in refining drugs that have not been efficacious in the clinic due to poor historic efficacy data and pre-clinical design.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We would expect to run approximately 100 studies on behalf of sponsors using approximately 3500 mice and 1000 rats over the 5 year duration 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 levels of severity? What will happen to the animals at the end?**

Animals injected with inflammatory stimuli to induce an arthritic disease state will, over time, develop inflammation in their hind and front paws which affect the digits, footpad area and sometimes the ankles. Joint swelling is an expected outcome of this protocol and is a primary measurement of disease progress or disease regress with a potential therapeutic. Animals may suffer from discomfort associated with inflamed joints but will be monitored daily for any additional, but unlikely, signs of discomfort associated with an arthritis diseased state such as weakness or a hunched appearance. General assessment of pain is not accurate but includes close monitoring of the animal's behaviour and feeding, the use of facial expression scoring system (grimace scale) and responses upon handling. The use of pain relievers by nature have anti-inflammatory effects which may compromise the underlying pathology of the disease, rendering the testing of potential anti-inflammatory therapeutics and the resultant need to use animals for the assessment of new therapies futile. Therefore, the use of analgesics to reduce pain and discomfort will be considered carefully and used where an analgesic regime will not interfere with the scientific endpoints being considered. General welfare checks and humane endpoints of a severe protocol will be observed at all times. Whilst no weight loss is associated with arthritis models, any animal which has lost 15% of its body weight will be monitored, and if this weight loss is combined with any other signs of discomfort mentioned above, the animal will be removed from the study. If the animal loses 20% of body weight, this is an endpoint and it will be removed from the study. Any animal which fails to put weight on one of its limbs for more than 72 hours will be removed from the study. A 72 hour window is a crucial to allow a potential therapeutic to manifest its effects against a control (non treated animal). Our experience with this model and historical data suggest that most therapies will

show statistically reduced disease parameters in the time period when disease is allowed to plateau (usually towards the end of the study design, which has been refined over the years). The white blood cell (leucocyte) migration models are short term moderate models where the injection of an inflammatory stimulus recruits cells to the site of injection. There is no expected peripheral effects associated with these models but, depending on the route of administration, there may be low-grade systemic inflammation. However, this is not expected to affect the wellbeing of the animal due to the length of these models. Animals will be monitored regularly for any unusual signs of discomfort such those mentioned above. Such symptoms very rarely appear with leucocyte migration models and therefore the risk of significantly affecting an animal's wellbeing is not expected. Any animal which has lost 15% of its body weight will be monitored, and if this weight loss is combined with any other signs of discomfort mentioned above, the animal will be removed from the study. If the animal loses 20% of body weight, this is an endpoint and it will be removed from the study. In all of the protocols and models described in this application, we plan to provide as much data as possible from every animal. This includes in-life assessment of disease progress (e.g. Manual measurement of joint swelling or imaging disease progress) as well as post-mortem analyses of whole organs and the cells/factors associated with an inflammatory response which may be specific to a particular organ (e.g. the local lymph nodes) or are systemic (blood, spleen, other organs). When possible and when confidentiality of data is not an issue, we aim to publish our results in peer reviewed journals and scientific conferences.

## Application of the 3Rs

### Replacement

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

#### Replacement

The programme requires that the models used are ones which closely mirror human disease. All compounds to be tested would have previously been screened in relevant cell culture based models to determine those candidates suitable for animal testing. Rodents (rats and predominantly mice) are suitable for these studies as the work cannot be conducted in lower vertebrates, invertebrates or cell lines due to the poor resemblance of these options to the clinical setting. Animal models address issues which current non-animal based tests cannot accurately determine.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Animal models will be restricted to the minimum number needed for a statistically valid result. The number of animals used will be the minimum safely necessary to allow meaningful statistical analysis of the data generated.

The most important aspect of the proposed programme of work that will reduce the number of animals used is careful selection of drugs, on the basis of preclinical and in vitro data. Only those potential drugs that offer a realistic prospect of therapeutic exploitation will be investigated.

Most importantly,. Such platforms are either cell culture based, relying on commercially validated and available immortal cell lines or human blood, artificial 3-D tissue equivalents, or more physiological platforms which are based on consensually or ethically derived human tissue. In fact, by installing such assays, I have managed to reduce the contract expectation under this programme from 100% use of animals (forecasted 5 years ago) to 40% (based on contracts from 2012-2017).

The investment of a cell analyser 5 years ago allowed for a more thorough assessment of the inflammatory pathways and cells associated with disease, thus bolstering statistical significance by offering additional readouts of drug efficacy and reducing the number of animals required. We have also recently acquired small animal imaging technology which may allow for monitoring of disease development in each animal over time, lessening the need to humanely kill satellite groups to examine disease progress internally, and thereby reducing total animal numbers. These techniques should maximise output and provide a more thorough assessment, with an aim to help in selecting the best models. Certain aspects of disease assessment, particularly in the in-life phase, are fairly subjective. Therefore, there is a demand to standardise and refine this. The use of the imager has potential to not only be beneficial in further assessment of the disease, but also in providing more measureable and standardised outcomes of disease progression, and the subsequent valuation of a therapeutic.

Rodents provide an effective and physiologically relevant platform in general for most pre-clinical testing. For the purposes of drugs targeting inflammatory pathways, the use of higher species is not required because there is a wealth of knowledge on different types of models in rodents, as well as historical in-house expertise with such models.

### **Refinement**

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

### **Refinement**

The majority of pre-clinical testing for arthritis and joint inflammatory disorders has been validated in mice, followed by rats. Only the most relevant models will be chosen based on the expected mechanism of action of a potential new therapeutic, or the information gained from testing that therapeutic in other parallel inflammatory disorders which has provided efficacy. More importantly, animals will only be used when all other non-animal based models will fail to provide the necessary information to progress the potential therapeutic into clinical testing stages. Models of arthritis, and joint inflammation, are expected to result in swelling of one or multiple joints in both the hind and front paws of animals. The swelling may prevent the animal from bearing weight on one or more paws, and refinement measures will be employed to allow the animal to be comfortable and able to access food and water. For example, drinking bottles with long nozzles will be provided, food may be placed at the bottom of the cage, and softer bedding will be considered. There are clear end points which allow for the least harm to be experienced by the animals such as weight loss (unlikely) or the inability to bear weight on a single limb for more than 72 hours. The animals will be handled minimally, but they will be monitored daily for changes in weight, well being, and joint swelling. The use of pain relief will be considered when it does not affect the outcome of the study.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 149. Mechanisms of normal and malignant haemopoiesis</b>
<b>Key Words</b>	Blood cells, leukaemia, age, therapy, cancer genes
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Leukaemia, a cancer of the blood, affects children and adults of all ages. The disease is not the same in children and adults. Despite this, children with leukaemia are treated the same as adults with leukaemia. Age, genetics, and the bone marrow microenvironment are all factors that can determine whether a patient responds or not to treatment. This project aims to investigate the impact of age, genetics, and the bone marrow environment on 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)?**

This project will provide a deeper understanding of the differences in leukaemia in children and adults. Identifying differences in the biology between paediatric and adult AML has the potential to identify better treatment for children and adults.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We have chosen mice to carry out our studies as the broad processes of blood cell development and blood cell cancer are well characterised and a wide range of reagents are available to address the biological properties of cells. Therefore our mouse models will allow us to determine human leukaemia cell responses to novel therapies, to inform clinical trials. This is a 5 year license and we estimate that 9000 mice including genetically altered and of all ages, will be placed on procedure.

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

Our work involves the introduction of mouse and human cells that may be manipulated to express the cancer genes into mice (whole animal bone marrow

transplant). We will generate mice that are born with a genetic alteration. The mice are monitored for abnormal blood cell development and disease. We then will assess the response of the leukaemia disease in the animal to clinically relevant chemotherapeutic regimes or to candidate chemotherapeutic regimes identified in our work. All mice are humanely killed at the end of the study. Precautionary measures will be taken to reduce any adverse effects. Due to the fact that some of the cells being introduced are being studied for function in leukaemia development in young and aged mice, particular attention will be paid to the appearance and general welfare of these mice, as the risk of developing cancers of the blood may be slightly elevated.

## Application of the 3Rs

### Replacement

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

#### Replacement

We will always perform experiments in cells in the laboratory when possible prior to using mice. This will replace the need for lengthy breeding of animals with more than one genetic alteration in most instances. While cell culture based studies can inform disease behavior in the animal (mouse and human), they cannot fully replace them due to the complexity of biological systems.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The number of animals used in each protocol in this project is estimated based on my previous experience and power calculations taking into account the number of experiments needed and number of mice per experiment required to obtain the appropriate statistical power.

Our replacement approaches reduce the number of mice required for some of the proposed experimentation. An additional way in which animal numbers will be reduced is by imaging mice that are carrying fluorescently labelled cells. With this technology, fewer mice can be utilised per treatment as the same group of mice can be imaged for the time course of the experiment as opposed to relying on different groups of mice per time point.

## **Refinement**

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

## **Refinement**

The mouse is the most frequently used model system to study the biology of stem cells and the best characterised mammalian model for blood cell development and blood cancers. Other advantages of the mouse model (apart from it being mammalian) include the availability of reagents that are suitable for use on both human and mouse cells. The protocols described will gain the maximal amount of scientific information from the minimal amount of mice, and the procedures are chosen to be the least invasive, thus minimising the suffering of the mice. For protocols involving bone marrow transplantation and leukaemia we have developed and successfully used a stringent distress scoring system which allows an immediate identification of mice with adverse effects. This system will allow us to efficiently minimise animal suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 150. Molecular mechanisms underlying chemical-induced adipogenesis in zebrafish</b>
<b>Key Words</b>	Zebrafish, toxicology, epigenetics, obesity, adipogenesis
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Humans are widely exposed to environmental chemicals, defined as any chemical that may reach the human body via any environmental route (i.e. via food, water, air, consumer products etc.). Increasing research in recent years has shown that exposure to environmental chemicals during the sensitive period of early development may play a role in obesity by influencing the process of fat cell development, also known as adipogenesis. We recently developed a zebrafish model of adipogenesis that allows us to visualize the development of adipocytes in living larvae as early as 12 days post fertilization. We have shown previously that developmental exposure of zebrafish to environmental chemicals leads to enhanced adipogenesis in zebrafish larvae. However we do not understand the mechanisms by which chemicals do this. **The main aim of this project is to determine the molecular mechanisms by which developmental exposure of zebrafish embryos to chemicals disrupts (epigenetic) regulation of genes and leads to increased adipogenesis.** As increased adipogenesis during early development may permanently establish an elevated fat cell number in adulthood, identifying and understanding these molecular mechanisms will allow me to better predict the long term effects of developmental exposures on 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 zebrafish is used in this project as a model for human adipogenesis. Our research has shown that zebrafish are a promising new animal model for studying adipogenesis and understanding the molecular mechanisms of action of chemicals. A major strength of the zebrafish model is that adipogenesis can be visualized in the living organism as early as 12 days post fertilisation through the use of fluorescent lipid stains or transgenic lines. The primary potential benefit of this research is related to new knowledge about chemical exposures in obesity. The research will advance the scientific understanding of the molecular mechanisms underlying obesity that can be influenced by chemical exposures early in life. The aim is to publish the findings in academic journals. Ultimately we aim to use this newly acquired mechanistic data to develop new computer models that will help us predict the effects of chemicals in humans in the future without the use of animals. Overweight and obesity are increasing all over the world, and are associated with serious health risks and huge economic costs to treat. This project will help characterize a new preventable risk factor in obesity, i.e. exposure to environmental chemicals.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

I will use both wild type and genetically altered strains of the zebrafish *Danio rerio*. In order to gain more knowledge, and to understand better the mechanisms of exposure to environmental chemicals, we estimate the total number of 15000 fish will be used (between 12 and 18 days post fertilization (dpf)) during the course of this project (5 yrs)

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

The main experiments will involve generation and breeding of (genetically altered) zebrafish lines, characterization of changes to genes and the epigenome, and determination of adipogenesis after exposure during early life stages to low concentrations of environmental chemicals. During the experimental procedures we will closely monitor the health status of the developing larvae. If larvae show any general signs of adverse effects, fish will be humanly killed. Fish will be observed regularly throughout the day for general signs of adverse effects, ill-health and injury, compared to other fish, with increased vigilance if signs are observed. Zebrafish will be screened for the following adverse effects e.g.: changes in swimming behaviour, scale loss and/or injuries, inactivity and lack of appetite, and overall poor body condition (compared to others in tank). We do not expect more than mild adverse effects in the fish. If any adverse effects do not improve during a 24 hour period, humane endpoint will be applied.

## Application of the 3Rs

### Replacement

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

#### Replacement

Many of the chemical-mediated effects that we will investigate in this project emerge from the interplay between biological responses at many levels in the body. Specifically, we will study effects that are initiated by chemical exposure during early life stages, but that become observable only later in life. To date, in vitro systems are not able to capture these complex responses.

Where possible, we will use in vitro data generated using cell lines to characterize the specific mode of action of chemicals at molecular level. Subsequently, we will validate this knowledge in vivo using zebrafish in order to understand the real-life relevance of those modes of action.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

For the breeding of zebrafish, we will ensure optimal husbandry methods to reduce natural losses.

For the generation of genetically altered fish, we will ensure that we use the most efficient and state of the art methods that optimize germline transmission and reduce the number of excess, non-genetically altered animals.

For the exposure experiments, we will design the most statistically robust designs in collaboration with our in-house mathematician/statistician. Power analysis will be employed to calculate appropriate replication and minimum number of fish required to adequately determine effects in each experiment.

## Refinement

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

### Refinement

I will use the zebrafish *Danio rerio* one of the most important models with which to study vertebrate development and an important model for human health. Zebrafish has also been used as a toxicological model for decades. The zebrafish genome has been fully sequenced and extensive genomic tools are available. Recent advances in forward and reverse genetic techniques and the establishment of transgenic lines allows for the detailed study of genetic pathways involved in (disrupted) development. The zebrafish possesses many structural similarities with humans that make it an appropriate alternative model for studying obesity. In addition to wild type zebrafish, we will use genetically altered zebrafish that we have obtained from established breeding facilities or generated ourselves, as these will provide us more accurate information on the role of regulatory genes in adipogenesis.

Exposures to chemicals are performed during early life stages to minimize any potential animal suffering. After 5 days, larvae are raised until 18 days post fertilisation to observe any potential effects of environmental chemicals on fat cell development.

We will minimise any potential animal suffering by using the best husbandry practices possible which are well established in our Animal Facility.

All environmental conditions, such as water temperature, water quality, day length and light levels will be optimised. Daily visual inspection of the fish by our highly trained staff, usually when they are being fed, will allow us to identify any possible welfare problems.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 151. Development and Homeostasis of the Enteric Nervous System</b>
<b>Key Words</b>	Gut, enteric nervous system, gut-brain axis, neural stem cells, gut inflammation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our aim is to characterise genetic, molecular and cellular mechanisms implicated in the assembly and maintenance of complex neural systems in animals. To address these questions we study the nervous system that controls digestive function and how it interacts with other tissues of the gut and the brain.

Neural networks throughout the body are receiving and processing information regarding the outside world or the internal state of the body (sensory system), are planning and co-ordinating movements (motor system), modulate the activity of various organs (autonomic nervous system) and support cognitive activity (brain). The neural system that controls gut physiology (called enteric nervous system-ENS) is critical for growth, digestion and metabolism and although far from the head, it is anatomically and functionally connected with the brain. Defects in the ENS result in severe and potentially fatal conditions and have been associated with chronic gastrointestinal disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Despite the importance of the ENS, we still know very little about the mechanisms that control its development and organisation and how it responds to gastrointestinal disorders, such as infections and chronic inflammation. We also have a limited understanding as to how defects in the ENS and gut function affect brain activity and behaviour.

Our aim is to fill this knowledge gap by identifying pathways associated with the development and function of the ENS, examine its response to pathological conditions and understand how it communicates and affects brain activity.

Specifically, our work has two objectives:

1. To discover genetic and molecular pathways that control the development and organisation of the nervous system of the gut and  
Characterise the response of this neural system to physiological changes or pathological 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)?**

Our work will advance fundamental biomedical science in an area that has great physiological importance but remains relatively underdeveloped. By advancing the understanding of basic and disease mechanisms, our work will promote the development of novel therapeutic strategies. All data, reagents and animal models developed by this work will be made available to the wider scientific community.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Our programme of works used mice (27000) and zebrafish (9000).

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

Prior to animal work, we carry out biochemical and cell culture experiments which, in combination with information obtained from publicly available databases, identify candidate genes that are essential for the formation and function of the nervous system. This information is then used to generate transgenic animals in which the expression of selected genes is modified. One of the main experimental approach involves histological and microscopic analysis of post-mortem tissues and physiological analysis of organs isolated from transgenic animals. Therefore, in the majority of cases, there will be no further intervention other than that required for breeding and genotyping. However, in certain cases we will need to inject substances or other agents in order to modify gene expression and mark cells. Substances will be administered by the most appropriate route, selected for the minimal invasiveness compatible with efficient delivery to the target tissue. To understand how the neural system of the gut functions and regenerates and how it interacts with the brain, we will manipulate these organs by altering the expression of genes and (in the case of gut) inducing pathology (inflammation) using chemical or infectious agents. A small number of animals may be analysed using behavioural

tests. It is expected that most animals will experience none or only mild adverse effects.

## Application of the 3Rs

### Replacement

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

#### Replacement

To understand how genes and cells control the development and function of the nervous system in the context of whole animal and in a way that is meaningful for clinical studies, it is necessary to carry out animal experiments. For example, gene function may be influenced by nutrients, oxygen, circulating hormones and other aspects of the complex physiological environment inside the body. It is not yet possible to recapitulate all of these parameters *in vitro*, nor to mimic the metabolic crosstalk between different populations of the ENS and gut-brain axis..

Nevertheless, our current and future research makes extensive use of alternatives to animals. *In vitro* cultures of mammalian cells/tissues can be used for many of our studies and are undoubtedly an important source of replacement.

Before embarking on any animal experiments, we will collect as much evidence as possible to determine whether a candidate genetic or environmental manipulation has a reasonable chance of success and is relevant to *in vivo* systems. Evidence will be collected from our own experiences and previous results as well as by surveying the mammalian and other literature. In addition, we will use non-regulated procedures to collect expression data from fixed non-GM mammalian tissues and functional/expression data from genetically and/or environmentally manipulated cell lines and/or early fish embryos.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

other cases, homozygotes will be generated from heterozygote intercrosses, with littermates genotyped as heterozygous or wild type used as age and gender

matched controls. Whilst most (~80-90%) of the experimental work will be *ex vivo* following breeding where any physiological or other interventions are required, we expect that 5-6 animals per treatment group will usually be sufficient to obtain robust results. For most of the quantitative experiments, design will be based on ARRIVE guidelines and sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 20%. Otherwise, we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own, or from the literature).

This programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. We will aim to collect organ samples from multiple body sites and to provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. This highly integrative approach will maximise the information obtained from the minimum resources. Cryopreservation of gametes, embryos, tissues and cells is routine and will ensure that the minimum number of mice is bred.

### **Refinement**

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

### **Refinement**

We are using refined GA mouse and zebrafish models, employing conditional and inducible technology where appropriate.

To minimise stress during breeding and maintenance, we will follow best practice guidelines and follow local refinements of husbandry such as cage enrichment and sufficient amounts of nesting material. On receipt or generation of a new line, we will minimize suffering by ensuring increased observation and monitoring until a detailed phenotypic analysis for each line is accomplished. If any welfare implications are identified, they will be acted upon and refinements considered in consultation with the NVS and NACWO.

The majority (~95%) of animals produced under the breeding protocol are not expected to exhibit phenotypes beyond a mild classification but a small proportion may exhibit a moderate phenotype - particularly if they are modelling a human disease. However, it is not possible in all cases (such as newly generated lines) to

predict fully the nature or severity of any potential defect and for that reason the limit has been set at moderate. For all types of mice, however, there will be careful monitoring of strain characteristics and the information will be collated and regularly reviewed to ensure that phenotypes do not exceed their usual features.

For all manipulations we will adhere to local or national guidelines that aim to minimize suffering. Most of the work as well as the administrations of gene inducers/repressors or other agents are standard and previous refinements from our own experience and from the literature will be used. If, however, there is insufficient information available, new manipulations will be pre-screened in small-scale pilot studies to obtain indications of the minimum dose and exposure time that is likely to be effective, thereby minimising any potential suffering.

Unless otherwise specified, all surgical work in this project will be undertaken in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010) or other such publication promoting best practice. Analgesia will be provided according to contemporary best practice and advice from the NVS/NACWO.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 152. Immunopathology of the skin</b>
<b>Key Words</b>	skin, inflammation, psoriasis, atopic dermatitis, microbiome
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The work described in this proposal aims at studying two common human skin diseases: psoriasis and atopic dermatitis.

Psoriasis, affecting up to 1 million people in the UK, and Atopic Dermatitis, which affects 1 in every 5 children and up to 3 % of adults in the UK, are dysfiguring skin condition due to skin inflammation. Patients suffering from either disease have a very significant reduction in their physical activity, low self-esteem, and overall poor quality of life.

Both disease are complex conditions and a number of factors appear important for its development including patient genetic susceptibility and environmental factors. No cure exists for neither of them, with current drugs only alleviating symptoms. Thus, further research into these two conditions is needed.

In particular, we are interested in: 1) studying the contribution of the environmental factors (e.g. microbial colonization of the skin, diet, drugs, trauma etc) that are known to trigger psoriasis/atopic dermatitis and 2) investigating novel strategies to treat this 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 output from this project will lead to a better understanding of the causes underlying psoriasis and atopic dermatitis, and to provide a basis for new therapeutic options that are urgently needed for both diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will breed up to 7000 mice over a period of 5 years; due to the complexity of genetic alterations we need to assess, the breeding of genetically modified mice will generate several animals that are not usable for functional studies. Mice undergoing procedures, including wild-type animals acquired by commercial sources, will be up to 7000 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 levels of severity? What will happen to the animals at the end?**

The work will use mice carrying genetic alterations that inactivate or change the function of specific genes coding for proteins of interest. As a results the mice may be more susceptible to develop disease or lack certain immune cells (=immunodeficient mice), and thus will be able to receive, under anaesthesia and analgesia (=pain relief), a human skin graft which will allow us to study how skin disease develops. This procedure is well tolerated and doesn't cause overt harm to the animal. In some experiments the animal, transplanted or not, will be exposed to chemicals, or microbial agents to induce inflammation of the skin with typical signs such as redness, scaling and swelling. In other experiments the animal may be subjected to a small cut of the skin (up to 3mm) which self-repairs in a few days. At the end of procedures mice will be killed by a Home Office approved method.

## Application of the 3Rs

### Replacement

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

### Replacement

While it is possible to generate some useful information about skin inflammation using patients tissues and cells, it is not possible to faithfully study diseases such as psoriasis and atopic dermatitis without using animals. In fact *in vitro* systems, while useful, do not fully replicate the complex interplay underpinning human chronic skin inflammation *in vivo* and it is essential to use appropriate and robust animal models to dissect these processes. Moreover, there is also no adequate *in vitro* model to assess the therapeutic value of targeting critical mediators and thus, we rely on appropriate animal models to develop therapeutic approaches with potential to alleviate human disease.

The mouse is the most appropriate species for these studies because, as a mammal, its skin and immune system is very similar to that of humans, and because of the extensive genetic modification technology that is available for the mouse, and

the wealth of pre-existing data and methodology will maximise the net benefit from these studies.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Numbers of animals will always be chosen to be the minimum compatible with sufficient statistical power to generate meaningful results. In some cases we can reduce the number of experimental mice by using imaging technology that allows studying a cohort of mice over several time points without the need of killing them for analysis.

## **Refinement**

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

### **Refinement**

To our knowledge no other species of lesser sentience can fulfil the requirements of this programme to the same extent as the laboratory mouse. The mouse models of skin inflammation we will be using have been chosen as they recapitulate key feature of human disease. Our procedures are mild or moderate and close monitoring of mice treated with any intervention will make sure they are not suffering excessive adverse effects. Mice will be housed in cages with environmental enrichments that allow them to burrow to maximise their wellbeing even under experimental procedures.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 153. Retrovirus-immune system interaction in mice</b>
<b>Key Words</b>	Retroviruses, immunity, cancer, T cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Infectious diseases are a leading cause of death worldwide, and are increasing in almost every nation. Infection also directly cause 1 in every 5 cancers. They are also among the biggest disablers. Protection against infection by viruses and other pathogens as well as the efficacy of vaccination crucially depend on appropriate activation of the immune system, a complex and vital network of cells and organs that fights invading pathogens. An understanding of these molecular pathways is essential for the design of vaccines for prevention and intervention in viral infections and cancer.

Our aim is to study the various molecules and mechanisms, which trigger the immune response to achieve long-term protection from infectious retroviruses or endogenous retroviruses expressed in cancer cells, with minimum pathology, and the broader interaction between retroviruses, both endogenous and exogenous, with their hosts.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The results of the research carried out under this project will be disseminated through publication in high quality peer reviewed journals and at meetings. The potential benefit is an improved understanding of the immune response to retroviruses and cancer. The knowledge gained from the vaccine experiments may be directly transferable to human vaccine studies or trials either through our institution or by other interested parties. The studies on immunopathology may lead

to a better understanding of the molecules and cells contributing to pathogenesis. This knowledge may help in the design of intervention therapies in clinical therapeutics in immune pathologies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

To achieve the objectives outlined above we propose to use the laboratory mouse as the model organism. We estimate that we will be using approximately 12,000 mice per year, the majority of which (~70%) will only be used for breeding.

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

We are proposing to use appropriate mouse models for clinically-relevant viral infections and cancer, in which the parameters of protective immunity can be studied and tested. Mouse models for infection with retroviruses will be studied in detail. In addition, genetic alteration of specific genes will reveal their precise role in the response to infection, autoimmunity or cancer. The most acute signs we expect to see in the experimental animals will be the result of tumour development. However, this is expected only in a small minority (~10%) of all the mice that will be used in this programme. Common to pathogenic murine retroviral infection or cancer models is the assessment of pathogenicity. This will be assessed most frequently as morbidity, which is quantifying changes in physiological parameters relevant to each type of infection or cancer and associated clinical. When clinical symptoms reach a predefined and closely monitored level or the physiological parameters reach the predefined values, mice will be humanely killed by a Schedule 1 method.

## Application of the 3Rs

### Replacement

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

### Replacement

Initially, the role of different mediators and compounds, which can affect the induction of an immune response to and/or growth of a pathogen, will be determined in *in vitro* studies. Ultimately, however, the immunological and immunopathological investigations, and particularly vaccine and antimicrobial effectiveness and autoimmune pathologies, cannot be carried out without the use of animals, since the host's immune system cannot be entirely mimicked by any *in vitro* assay. Furthermore, although all compounds will be selected for *in vivo* testing based on evidence of activity in relevant *in vitro* assays, this cannot replace the *in vivo* tests

under the physiological conditions of an infection, as potent *in vitro* activity might not translate into an *in vivo* activity.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The design of quantitative experiments will be based on our extensive prior experience with the animal models proposed in this application and will additionally be tested against power and sample size algorithms. This combination will allow us to calculate with accuracy the minimum number of mice required to obtain a scientifically meaningful result. Using too few mice would lead to inconclusive results.

Moreover, results will be reported according to ARRIVE (Reporting of In Vivo Experiments) guidelines published by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) (PLoS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412).

The use of dedicated computer databases for mouse breeding and management has been implemented at our Institute. This allows us to carefully monitor the mouse breeding programme and also share mice with selected genetic traits between investigators so that duplication is avoided. Cryopreservation of gametes, embryos, tissues and cells is also routine at our Institute and will ensure that the minimum number of mice is bred.

## Refinement

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

## Refinement

The mouse is the best-characterised model for these studies, with many features applicable to human infection. Their immune responses are well defined and the technology enabling sophisticated manipulations of the haematopoietic and immune system is highly developed. Mouse transgenic and knockout techniques are well established; mice have a relatively short generation time; its haematopoietic system has been extensively studied and, in addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this project to the same extent as the mouse.

In our experiments, specific end-points will be used to monitor the effect of infection on physiology, instead of induction of pathology, and we will go to great lengths to minimise the possibility of severe pathology. This will be achieved by close monitoring of symptoms and physiological parameters during the course of the infection. We will use genetically-modified micro-organisms as challenge strains in order to take advantage of reporter signals – luminescence or fluorescence, for example – to monitor the course of infection and potential dissemination to different organs with non-invasive techniques.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 154. Gluing behaviours together - 'chunking' in the normal brain and its disruption in Parkinson's disease</b>
<b>Key Words</b>	Basal Ganglia, Striatum, Neuropeptides, Behavioural Chunking
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Patients with Parkinson's disease have particular difficulties with performing habits or behaviours that they don't have to think about. One important aspect of habits or behaving in an automatic way is a process called 'chunking' – the ability to link a series of behaviours into a sequence or chunk that can be played out as a unit. Changing gear in a car with a manual shifter is a behavioural chunk which requires the driver to depress the clutch pedal, move the gear stick and release the clutch pedal. A part of the brain called the striatum, which is affected in Parkinson's disease, is considered to play an important role in establishing chunks. However, how the striatum does that has yet to be determined. We have recently made the novel proposal that certain chemicals in the striatum called 'neuropeptides' play a role in chunking, and have evidence that if you block the actions of one of those chemicals, substance P, then rats find it easier to learn new chunks – as if substance P was somehow stamping in the old chunk. The project will continue this work in several important ways, firstly by finding out whether blocking substance P facilitates forgetting of the old chunk as well as learning the new chunk. In addition, we will examine the role of other striatal neuropeptides in chunking, and also extend our work to look at innate chunks, that is ones that do not have to be learned. Here, grooming will be our focus as this is often executed in a defined series of moves in rats that get played out as sequence.

**What 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 is a basic science project designed to investigate a fundamental property of brain function. Given that striatal neuropeptides are disrupted in Parkinson's disease and that sequence learning, and the expression of habitual behaviours, is affected in Parkinson's disease, we believe that the current work has clear therapeutic implications. The current studies are part of a programme of work which will build on our findings. Future work will be directed at chunking in the parkinsonian brain, and will examine the effect of drugs that mimic neuropeptides on chunking in animal models of Parkinson's disease. The existing work and that to be undertaken under the current licence will establish a functional baseline against which work in parkinsonian animals can be measured. In the longer term, we hope that our work will provide a novel therapeutic approach to one aspect of the pantheon of dysfunctions in Parkinson's disease, and hence find translation into human studies. Although standard pharmacological treatments for Parkinson's disease do assist with sequence learning, there are contexts in which they are less appropriate (for example if those drugs produce side difficult side effects). In addition, having a range of possible drug treatments increases the flexibility for clinicians and allows treatment to be more effectively tailored to the individual.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use a maximum of 300 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 levels of severity? What will happen to the animals at the end?**

The behaviour of animals will be assessed following the administration of drugs that block the actions of neuropeptides. We will look at behaviours that are spontaneously produced by the animals, and also at learned behaviours, such as pressing a lever in an experimental chamber. Drugs will be given to the animals either by injection into their abdomen or directly into the brain through guides that have been implanted using prior surgery under anaesthesia. Where the procedure includes anaesthesia, animals will be carefully monitored for adequate anaesthetic depth during the procedure and for adverse effects during recovery. For procedures performed in conscious animals, additional care will be taken during training and in the testing stage to ensure minimal stress and suffering to the animal. The expected level of severity for the recovery experiments is moderate. If any procedural complications do arise veterinary advice will be sought immediately from the named

veterinary surgeon. In all cases the animals will be killed using an appropriate Schedule 1 method.

## Application of the 3Rs

### Replacement

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

#### Replacement

This is a systems-level neuroscience project that will investigate a fundamental aspect of brain function. At present there is no alternative to using invasive experiments with animals to gain the knowledge required. However, REDACTED who use biological data derived from our work to parameterise their computational models. The progressive refinement of these models will gradually replace the need for biological testing.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Our studies will frequently use repeated measures designs in which the responses of each subject to different experimental conditions are measured repeatedly. Therefore, statistical significance is determined within the data of single subjects. Additional animals will be required to demonstrate between group effects. Sources of variability will be controlled by consistent use of animals according to sex, age and source (supplier vs in house breeding).

## **Refinement**

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

## **Refinement**

The rodent striatum (basal ganglia) has been widely studied and an extensive literature exists on all aspects of its structure and function. This and the fact that the structure is highly conserved in the vertebrate brain means our results will generalise across many species, including humans. Care will be taken during surgical recovery, and during training/testing to ensure minimal suffering to the animal. For surgery, animals will be anaesthetised with an anaesthetic agent appropriate for the species and all animals will receive treatment with analgesics post-operatively. For testing, animals will be habituated to the task and handler where applicable, and during periods of food restriction, animals will be allowed periodic access to food ad libitum.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 155. Pathophysiology of inflammation processes in the lung</b>
<b>Key Words</b>	Lung disorders, Oxidative stress, Exacerbations
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of the project is to identify new and characterise existing processes that are involved in lung disorders.

The key objectives are to identify and characterise the underlying mechanisms that result in different clinical outcomes of disease in animal models, to determine the interactions between with other diseases like type 2 diabetes and heart conditions, to study the effects of very small particles in models of disease and to determine exacerbating effects between 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)?**

The short-term benefits are the identification of new pathways that are involved in lung disorders like asthma and Chronic Obstructive Pulmonary Disorder (COPD). How they interact and relate to other disorders like type 2 diabetes and cardiovascular disorders. Using this information will subsequently generate new ways to intervene with disease progression in different patients. Long-term effects will eventually be new treatments and treatment strategies for chronic lung disease that benefits the patients directly.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the course of the project it is estimated that approximately 5000 mice and 150 rats will be used. Although less complex, organ structure in mice and rats are still very similar compared to human and model the chronic diseases in a comparable way.

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

The animal models proposed in the application are designed to induce a disease state in animals comparable to the human diseases such as asthma and type 2 diabetes. Animals will experience some form of lung disease which may result in shortness of breath due to narrowing of the airways. This might be worsened with an infection or exposure to low oxygen levels. The diabetic or metabolic phenotype will result in weight gain of the animals that might reduce the mobility of the animal. In addition, the increase of urinating frequency is often seen in the diabetic state and may occur. Blood sampling during procedures will only happen in a small fraction of animals and these animals might experience some discomfort. Animals that receive medication or compounds that are under investigation might experience some discomfort from the administration technique in the form of a needle prick or short term (few minutes) anaesthesia. The Procedures will not exceed moderate in severity. At the end of all experiment animals are killed in a humane way appropriate to the type of animal.

## Application of the 3Rs

### Replacement

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

#### Replacement

Most of the previous research has been performed in cells but has limitations to compare to the human condition. In addition, there are no non-animal alternatives available to study inflammatory processes in whole organisms. To be able to mimic the human situation the processes requires an intact blood circulation, the presence of cell-to-cell contact and interactions. These factors are essential for inflammation or processes of disease to reach full effect. We think that cell culture experiments are still essential to study the different components and pathways and will be used.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

In order to use the lowest amount of animals during the procedures, advanced statistical techniques will be used. These techniques will be used to calculate the number of animals that are needed to detect differences between groups. Previous calculations indicate that on average 8 animals are needed in each group. These calculations will repeatedly be performed using generated data. This potentially might reduce the number of animals needed in future experiments.

### Refinement

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

### **Refinement**

The procedures will be performed in mice and rats. These animals still have a similar lung physiology and responses to drugs and treatments comparable to humans.

The animals that are being used in the procedures will be regularly monitored by staff. Particular attention will be given to food and water intake, coat condition and general demeanour. Where there is any concern animals will be carefully assessed and appropriate veterinary advice regarding treatment/maintenance of the animal or take steps to humanely terminate the procedure.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 156. The autonomic nervous system and sudden cardiac death</b>
<b>Key Words</b>	arrhythmia, sudden cardiac death, autonomic nervous system
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>No</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Abnormal electrical activity (arrhythmia) of the heart is a common consequence of cardiovascular disease and a major killer. It is known that the nervous system affects the pumping and electrical behaviour of the heart, for example, during exercise, and that the nervous system can trigger arrhythmia when there is underlying cardiovascular disease (e.g. during a heart attack). However, our understanding the interaction between the nervous system and the heart is incomplete. This project aims to investigate how the nervous system acts to promote arrhythmia, by what mechanism(s) these effects occur, and what can be done to prevent it.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Work under this project aims to understand the complex interplay between the nervous system and the electrical stability of the heart. Studies will focus on clinically relevant conditions associated with sudden cardiac death, including, long QT syndrome and acute myocardial infarction. It is hoped that by improving our understanding of how the nervous system affects the heart, this will lead to advances in clinical practice and patient care.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

650 mice, 200 rats and 150 guinea 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 levels of severity? What will happen to the animals at the end?**

The vast majority of experiments will be performed in isolated tissues, in which animals will be humanely euthanized before tissue isolation. As such, most animals are not expected to experience adverse effects other than those of the anaesthesia protocol (from which they will not recover). A smaller number of animals will undergo a procedure to induce structural remodelling of the heart, comparable to that observed in humans with heart disease and with aging. The chosen model is well characterised and is minimally harmful whilst achieving the aims of the studies. There is a very small risk that animals will experience heart failure, and animals showing such signs will be immediately euthanized to prevent suffering. Some animals may also receive drugs to abate structural remodelling, but expected adverse effects are minimal and animals will be closely monitored throughout the treatment regime. At the end of the treatment regime, animals will be humanely euthanized and tissues taken for study.

## Application of the 3Rs

### Replacement

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

#### Replacement

Cardiac electrical function is complex, involving the interaction of multiple factors that cannot currently be studied without animal models. Our understanding of the processes involved, and their relative importance, limits our ability to use computer modelling, though this is a goal we are working towards.

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.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Experimental data will be continuously analysed and assessed to achieve the aims of the project with the minimum number of animals. All protocols will be refined and conducted by trained individuals, which reduces errors and experiment numbers. Studies will conform to the NC3Rs ARRIVE guidelines

### Refinement

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

## Refinement

Mice are the smallest mammalian species suitable for experimental study of cardiac arrhythmia and where possible experiments will be performed in mice. However, in specific cases, scientific and technical considerations determine that guinea pigs or rats are the more suitable. For instance, human forms of congenital long QT syndrome are commonly caused by mutations in genes that are not expressed in the mouse or rat heart, whereas the guinea pig heart is more similar to that of humans. For studies of acute myocardial infarction (e.g. a heart attack), the rat is better established and more reliable model, whereas the small size of the mouse heart makes such experiments difficult and prone to error. Moreover, the interlinking of the coronary blood vessels in the guinea pig heart makes it unsuitable for this aim.

Our experimental protocols have been developed to limit harm to the animals, being as short as reasonably possible and mainly conducted after terminal anaesthesia. We will continue to make efforts to refine protocols and further reduce the welfare costs. Current best practices (e.g. the use of flexible gavage tubes) will always be followed. Wherever possible, to limit sources of bias, experiments will be conducted and analysed in a randomised and blinded manner.

In addition before conducting each experiment, it is discussed with the NACWO and NVS to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to acquire the scientific endpoints. We will also 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 the next experiment

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 157. RoboChick: an autonomous platform for data-collection in poultry sheds</b>
<b>Key Words</b>	animal welfare, chickens, precision livestock farming, animal behaviour
<b>Expected duration of the project</b>	1 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Poultry meat birds (broilers) are bred for high muscle gain but require very careful husbandry. Monitoring technologies have been developed to automatically collect flock level data to help manage these delicate birds. However, current technologies are limited as they are unable to dynamically record environmental data across a poultry shed and at the height of the birds. The overall aim of this project is to develop and trial a multi-functional robotic system that can autonomously collect data (such as climatic or atmospheric conditions or bird condition) within a poultry shed, to fulfil this need in the industry. At this preliminary stage in the project the aim is to trial the robotic platform in a small flock of broilers whilst it is under manual control to monitor the behavioural responses of the birds and to ensure that the robot does not negatively affect bird 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)?**

Within the intensive chicken sector, tens of thousands of birds are housed together and often across multiple buildings with small numbers of stockmen to monitor them. Monitoring technologies can be used to aid the stockmen on large farms, ensuring that any issues in the flock are detected quickly to maximise bird welfare, health and productivity. As the robotic platform being trialled will move among the animals it is important to ensure that they react appropriately and that their welfare is not compromised. Broiler chickens should benefit through improved health and welfare, and humans through improved bird productivity.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 1500 Ross 308 broiler chickens will be housed together to recreate a small-scale commercial environment. They will be housed at 1 day old and will be kept until they are approximately 39 days of age in line with industry standards.

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

The animals will be raised as they would be on a commercial farm. There are no expected adverse effects beyond industry standards and the animals will be sent to a commercial slaughterhouse at the end of the study.

## Application of the 3Rs

### Replacement

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

### Replacement

The purpose of this study is to observe commercial broiler chicken behaviour in response to the robotic platform. It is therefore essential that a common commercial strain (Ross 308) is used.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The room used for this study is the smallest possible to ensure that the robotic platform can move through it as it would in a commercial house, whilst ensuring that the birds can freely move away from the robot.

### Refinement

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

### Refinement

The Ross 308 broiler chicken is the most popular meat chicken in the UK. It is therefore a good example of the type of bird that this robotic platform will eventually be implemented among. Welfare of the birds is of utmost importance during this feasibility study. The robot will be manually controlled to ensure that it can be stopped if birds do not behave favourably and birds will be treated/culled as

appropriate if they are found to have developed any health issues (as a result of the breed, rather than the robot).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 158. Deciphering the cellular abnormalities that precipitate immune-related immune disorders</b>
<b>Key Words</b>	Type 1 diabetes, thymus, stromal cells, immune cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Type 1 diabetes (T1D) is a condition in which the immune system inappropriately attacks the insulin producing beta cells of the pancreas. The condition is both life limiting and life threatening, there is no cure. The reasons why the immune system decides to target our own beta cells is unknown, but is believed to be linked to a breakdown in the 'quality control' machinery whose job it is to kill off any immune cell that has the ability to recognize our own tissues. Our recent research using animals genetically predisposed to developing T1D in a manner similar to man has provided novel data that links the breakdown of the quality control machinery in the thymus, an immune tissue specialised in purging the body of T cells that may target our own tissues. We showed that impairment in development of the cells that form a functional thymus, as well as an unexpected immunological attack on cells that perform quality control is linked to T1D development. In this program of work, we will capitalize on this new data to determine succinctly:

1. Why the thymic tissue is developmentally abnormal in animals predisposed to developing T1D
2. How does the immune system evolve an inappropriate response to the quality control cells in the thymus

Can 'humanized mice' i.e. animals that have a human immune system in place of a murine one, recapitulate the key features of the thymus as outlined above, when the human immune system forms in animals genetically predisposed to developing T1D.

**What 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 why the immune system inappropriately attacks host tissue (both the thymus and pancreas) will be beneficial to other researchers of diseases where the immune system targets host tissue e.g. rheumatoid arthritis, multiple sclerosis, lupus as it is believed there are commonalities in the mechanisms by which these diseases target our own tissues. In addition, considering increasing abnormalities in the structure and function of the thymus in humans as they age is linked to increased risk of infection and poor prognosis for those receiving bone marrow transplants, our data will benefit scientists in fields distinct from our own. Ultimately, those that will benefit the most will be current and future generation of people genetically predisposed to developing T1D. Our long term goal is to use the knowledge gained from our studies, in collaboration with other scientists and clinicians, to develop robust therapies that will resolve/prevent people developing T1D.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The project will utilise mice only. We expect no more than 12,800 mice to be used over the 5 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 levels of severity? What will happen to the animals at the end?**

The majority of our mice will undergo one procedure-breeding and maintenance-followed by Schedule One for ex-vivo analysis of tissues. The severity level is mild as mice are used prior to diabetes development. Some mice will undergo additional procedures where they may be injected with substances, or irradiated followed by transplantation with stem cells. These procedures are moderate in severity. Animals injected with substances may experience slight, transient pain due to the needle.

## Application of the 3Rs

### Replacement

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

### Replacement

The immune system is complex. Autoimmune diseases always involve a multitude of cells, molecules and pathways whose function can be altered depending on the inflamed status of the environment in which they reside. Many of these changes will inevitably involve the contribution of molecules that are as yet undiscovered. As

such, it is impossible to recapitulate *in vitro* the multicellular, multistep conditions that contribute to disease.

We will strive where applicable to use *in vitro* systems such as cell-culture of cell-lines, murine tissue assays on our stored tissue sources, and human cell clones to address the aims in this application.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We use state of the art technology to allow analysis of multiple parameters for cellular phenotyping enabling us use reduced numbers of animals.

We cryopreserve excess immune cells from extracted tissue and use these cells for future optimisation experimental conditions and/or new *in vitro/ex-vivo* immunological investigations.

We always use the most up to date best practice in experimental design and implementation of techniques. Power calculations are employed to determine the minimum number of mice required per experiment for statistical significance.

We cryopreserve embryos to archive lines that are no longer required for the project to avoid wastage from the need to maintain colonies by continuous breeding.

In addition, as part of collaborations with colleagues we retrieve rodent tissues essential for our studies, but not our collaborators', thereby minimising duplication in breeding of certain rodent strains.

## **Refinement**

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

## **Refinement**

Strong similarities between mouse and human genome/immune system coupled to ease of manipulating models genetically make the mouse an ideal animal model to decipher the complexities of autoimmune diseases. Studies in NOD mice have generated major discoveries on the immunopathology of T1D in man, leading to several key cells/molecules that are potential targets for therapeutic intervention. More recently, the availability of humanised mice as a model system of a human immune system enables investigations into the dysfunctions of the human immune system that destabilises effective control of immune responses to human tissue.

By using a different mouse strain, we were able to avoid transferring cells between mice, reducing an experimental approach from moderate severity to mild severity.

The majority of our research involves mild procedures - Schedule 1 killing of pre-diabetic animals and subsequent analysis of the immune cells.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 159. Wound healing and Ano rectal Fistula</b>
<b>Key Words</b>	fistula,
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Current treatment options for ano-rectal fistulas (an abnormal connection between and outer skin) are inadequate resulting in poor and inconsistent healing rates. Patients experience considerable pain and poor quality of life. Having previously investigated why these fistulas don't heal we have developed a collagen paste. Our goal is to use this paste to heal the fistula by replacing the lost collagen framework within the fistula tract with one supplied by the paste. The paste can also be combined (optional) with antibiotics or the patients own stem cells to speed up the healing process.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Current treatment are either surgical or non-surgical, the former can result in incontinence and the latter in inconsistent healing. The benefit from our approach is that it will not damage the surrounding tissue and therefore even in the unlikely event it doesn't work it will cause no harm unlike surgical treatments. It has been specifically developed to repair the damaged tissue and can be used as vehicle for the delivery of either drugs or cells to promote healing. The scientific knowledge gain would support the concept that in order to promote long term healing the underlying structure of the tissue is crucial and where possible we should be aiming to replace like for like.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Our chosen animal is the pig due to its close resemblance in anatomy and physiology to humans. Over the course of the study (5 years) approx. 50 pigs 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 levels of severity? What will happen to the animals at the end?**

The severity of the study is moderate, possible adverse effects may include pain (moderate). No adverse effects are expected from the surgical procedure itself. In our experience, animals tolerate these procedures very well with no adverse effects to their physiology. There is a very remote risk of bleeding post surgery, infection post surgery or loss of the indwelling setons. All of these incidences will be closely monitored for and treated, if necessary under veterinary instructions and any that became physiologically altering would define a humane endpoint. No adverse effects are expected from the use of immuno-suppression since it have successfully implemented appropriate dosage in previous studies. Since a full depth analysis of the fistula tract and surrounding tissue will be essential at the end of the experimental period all animals will be killed at this point

## Application of the 3Rs

### Replacement

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

#### Replacement

An ano rectal fistula is a painful and debilitating disease ; treatment options include either surgical intervention with the possibility of permanent incontinence or non-surgical treatment with unreliable healing rates. We have developed a novel collagen paste to fill the fistula track and replace the damaged tissue. The paste has been tested in the lab and in lower sentient animals (i.e. rats) to ensure it is safe (i.e. non-toxic) and cell friendly. We are now at the stage where we need to test the paste for its intended clinical application in a model which closely mimics the human with respect to ano rectal anatomy and physiology.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

To date all the necessary experimentation surrounding the development of the paste has been completed and tested in rodents, removing the need to test the paste in large animals. Additionally, a separate study investigating the paste for skin wounds is underway allowing for considerable knowledge to be gained on how the paste performs. This will further reduce the number of animals required in this study. Typically, for comparison of three modes of treatment requiring replicates of 6 of each treatment a maximum of 6 animals would be used as this is calculated to provide robust statistical data during subsequent analysis. From previous experience animals tolerate 3 fistulae very well and this has allowed us to reduce the number of animals by increasing the fistula numbers per animal. Finally up to 3 individual fistula

tracts can be created in each animal with no adverse effect thereby reducing the number animals in each experimental arm.

### **Refinement**

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

### **Refinement**

Our chosen animal is the pig; this animal has a diet similar to humans and hence its anatomy around the rectum and anus closely matches making it an ideal model, including similarities such as e.g. little hair, dermal and epidermal structure, musculature, vasculature and healing properties. Additionally, this model allows for the creation of fistulas of comparable size and potentially similar complexity. Whilst this is the best model we have been able to develop it is recognised that the pig is quadrupedal while man is bipedal and we have accounted for this in the way we analyse our results.

Furthermore, although animals may need to be singly housed to prevent damage to the wound area by cage-mates, our accommodation allows visual, auditory and scent contact with other animals of the same species.

Animals welfare will be closely monitored by the Named animal care and welfare officer who can be supported by and the named veterinary officer if required ; animals will be placed on a loose diet prior to and after the creation of the fistula to help with bowel movement.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 160. Collateralisation of blood vessels</b>
<b>Key Words</b>	Blood vessel, Diabetes, Vascular disease, Endothelial collateral
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

People with diabetes are more likely to suffer from peripheral or coronary vascular disease (ischemia). These diseases occur because blood vessels get blocked. In people without diabetes new blood vessels can grow around the blockage, but diabetics are less able to grow new vessels around areas of blockage. We don't know why this happens in diabetes but there is evidence that is due to changes in the way that white blood cells make growth factors that stimulate vessel growth, and changes in the extracellular matrix proteins that help these vessel grow. After identifying some of the molecules that control this, and potential ways to reverse this in cultured cells, we aim to find out whether this can reverse this in mouse models of ischemia and diabetes. We will give agents (molecules, cells, antibodies) that may improve blood flow to the animals after they have undergone a surgical procedure to limit blood flow to their hindlimb.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Finding out what the changes are in diabetic patients that stop blood vessels growing in ischemia would help us develop new treatments for this, the major cause of amputations, and of heart attacks in diabetics. While we can get some of the data from studies on human cells and cells in culture, to test these ideas we need to show in an animal model where all the factors controlling vessel growth are present. If we can identify ways of increasing vessel growth then this could lead to new treatments for patients resulting in better quality and length of life.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use approximately 8 mice per week over five years (2000 mice).

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

We will use mice that have diabetes, pre-diabetes (metabolic syndrome) as well as non diabetics. Diabetes can be either spontaneously diabetic due to genetic mutation, or we can induce diabetes chemically. This will make the animals hyperuraemic (they urinate a lot), so bedding is frequently changed. We will give them tests that will tell us whether the diabetes is affecting their blood vessels - this includes measuring protein in their urine, and measuring their ability to detect a stimulus that they would try and avoid (e.g. a heat spot or pressure point). Both the urine test and the behaviour test are an indication of diabetic vascular disease. They will all undergo a surgical procedure where one of the arteries supplying the hindpaw is closed off, reducing blood flow to that paw, which makes them lame for a few days. As blood vessels grow round the arterial closure the blood flow to the paw recovers, and they start to walk normally again. We will measure blood flow to the paw five times over the following four weeks using a laser based camera. Most of the animals will be treated with compounds, cells or other agents that we have identified from non-animal experiments to have the potential to restore blood vessel growth and blood flow to the hindlimb. Some will be given placebo type agents or inactive molecules so that we can compare the effects with the active ones. They will have a general anaesthetic for this. Animals will be killed at the end of the experiment.

## Application of the 3Rs

### Replacement

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

### Replacement

Growth of blood vessels occurs in a complex environment depending on blood flow, the tissue through which it is growing and the immune system. These are not fully formed in non protected animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Experiments are designed to use sufficient animals to answer the questions we are posing and no more. We can reduce numbers by using non invasive imaging, so that each blood flow measurement is paired with its previous one, and we compare one paw to the other, non-ischemic paw.

## **Refinement**

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

## **Refinement**

The vascular disease model in mice mimics human disease, both diabetic and ischemia. Blood vessels in mammals grow differently from non mammals due to the differences in the DNA. The mouse is used as it can be genetically or chemically manipulated or bred to be diabetic, or have molecules that will help or hinder blood vessel growth more easily than rats, rabbits or other animals. The minor blockage means there is low likelihood of damage to the paw, and we check the animals carefully for any adverse effects. Animals are given anaesthetics for surgery and blood flow measurement, and are given painkillers after surgery.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 161. Mechanisms Underlying Abnormal Heart Rhythm</b>
<b>Key Words</b>	cardiovascular, arrhythmia, heart disease, sudden cardiac death
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Disorders of the regular beating of the heart known as arrhythmia are an important cause of death in clinical medicine. For example, sudden death due to arrhythmia may account for up to 11% of unexpected deaths. This is dramatically and topically illustrated by the sudden collapse of elite athletes but occurs through all age groups and occupations. Our proposal focuses on how disturbances in heart cells lead to cardiac arrhythmias and how the nervous system, infections or hormones in the body might regulate this. We use mice and rats with and without genetic modification to approach these problems.

**What 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 our studies will lead to increased understanding why heart rhythm disorders happen and potentially new approaches for the treatment of those diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Recent advances have allowed researchers to manipulate the rodent in particular the genes of proteins in heart cells in increasingly sophisticated ways. This allows us to study how genes can cause disease in the living animal with far greater potential for subsequent use than was previously possible. Studies will be conducted using rats and mice including animals that have been bred with genetic alterations that enable

us to study the effects of individual genes of interest. We will monitor the heart beat and pattern and how well the heart muscle contracts. In particular we will use imaging and electrical techniques to understand how loss or too much of these proteins lead to normal and abnormal heart rhythm. In addition, we aim to ask how these arrhythmia causing genes might interact with the commonest cause of sudden arrhythmic death for example blockage of the arteries supplying the heart. It is also becoming clear that common diseases such as infections and diabetes can drive cardiovascular disease and we aim to model this in the rodents. We anticipate studying 2000 mice and 200 rats\year

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

A stepwise approach will be used so that initial studies will be carried out using tissues from animals bred under the licence or will be done under a general anaesthetic from which the animals will not recover. As the lines of enquiry progress, studies may involve surgery to, for example, implant devices that allow blood pressure and other measurements to be made. Some of these transmit radio waves to allow recordings to be taken from the animal following its recovery from the surgery without the animal being aware that the measurements are being made. In some studies the animals use wheels or treadmills so that the effects of exercise. In others surgery will be performed or infection given so that the effects of an immune reaction can be assessed. The protocols which cause the most deleterious effect for the animals involve an operation to place a thread around a coronary artery so that the blood supply to part of the heart is restricted to mimic a heart attack or to apply pressure around a major blood vessel in the abdomen to mimic high blood pressure. All surgery is conducted using general anaesthetics and the same types of measures to prevent infection as are used in human operating theatres. The animals will receive pain killers following the surgery. The animals are also monitored very closely and will be euthanised to prevent unnecessary suffering if they develop signs set out in the licence.

## Application of the 3Rs

### Replacement

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

### Replacement

We use extensively various cell based approaches including cell lines and human induced pluripotent stem cells to investigate cardiac arrhythmia. We are committed to the development of alternative approaches and have recently been awarded a

NC3R's infrastructure grant to develop computer models that will substitute for animal testing in the evaluation of the safety of drugs acting on the heart. We also have extensive links with clinicians looking after patients with these diseases. We are also performing genomic analyses for ECG traits and these will yield new molecules for study. However, the analysis of cardiac rhythm and associated diseases often requires the use of intact animals. Complex physiological processes involving the function of a number of interacting body systems are being examined. Heart function requires the in vivo function of several distinct organ systems including a functioning nervous system, vascular and renal function and respiratory function. As such these cannot be reconstituted fully using in vitro experiments.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The numbers of animals used will be minimised by maximising tissue use from each animal and by designing experiments according to good statistical and scientific principles. Important experimental design features will ensure that the correct physiological conclusions are reached. The structure of our study protocols also allows repetitive imaging and prolonged telemetry that reduces the number of animals that to be used to address the scientific questions.

## **Refinement**

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

### **Refinement**

Using new genetic technologies we can delete genes in only some organs and/or at only some times during the lifetime of the animal. In general this allows us to reduce the severity of overall impact to the animals' health.

The most severe aspects of the proposal are the models of heart attack or limb death achieved by tying off the coronary and femoral arteries and increased blood pressure achieved by constricting the main blood vessel leaving the heart. These are

critical to our investigations as they mimic important human diseases and allow us to see how a particular gene or pathway can change the disease process. We have refined surgical methods enabling us to achieve our scientific goals with outcomes equivalent to those in the literature.

We are developing and using cutting edge technologies that remove the need for surgery during data collection. For example the "ECGenie" which avoids the need to implant telemetry devices and sequential imaging techniques reduce animal numbers as it is possible to watch the progression of pathology in a single animal. Many of the technological refinements also result in a reduction of animal usage.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 162. Production of Genetically Altered Animals</b>
<b>Key Words</b>	Genetically Altered, Breeding
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project licence is to provide genetically altered (GA) mice and rats for scientific groups REDACTED We can do this by importing live animals into the rodent facility that already has a supply of the GA animals.

If live animals are not available then we can import either sperm or eggs from the GA lines that are of interested to the

We will use the imported GA sperm to fertilise eggs from a non-GA animal. This entails using in vitro fertilisation techniques in a dish, fertilising as many eggs as possible and then transplant (surgery) those eggs into the reproductive tract of a non-GA female recipient animal. If successful, the recipient female will be pregnant and produce a litter of GA animals approximately 3 weeks after surgery.

On occasions we will receive fertilised eggs from donor females that have been mated with a GA male mouse. They will be sent between 1 and 4 days old and they will be transferred into the reproductive tract of a non-GA recipient female. The transfer can be either by using surgery (1-2 day old eggs) or by direct insertion of a pipette into the lower reproductive tract (3-4 day old eggs). The recipient will then be monitored for signs of pregnancy and if successful, offspring will be born around 3 weeks after implantation.

We will also create new GA animals by directly injecting the altered gene directly into the egg (1, 3 and 4 day old eggs). This procedure requires the use of a microscope and specific injection equipment. After injection the eggs are checked to see if they are alive after a minimum of 1 hour. During this time they are held in a solution that encourages growth and also kept at around body temperature by using an incubator.

The live eggs are then transferred into the reproductive tract of a non-GA recipient animal.

During the life of this licence we also want to develop our skills in techniques that will help with the success of producing GA animals that may have breeding difficulties, for research groups. Collectively this is known as “Assisted Reproductive Techniques” (ART). Specifically we would like to introduce the following;

Direct sperm injection into an egg. The sperm head is injected into the cytoplasm of the egg to aid fertilisation. If using 1 male you can perform this technique on potentially 20 – 30 eggs generated from 1 donor female. This is called “Intra Cytoplasmic Sperm Injection” (ICSI). This is useful when breeding efficiency of a specific GA line is poor with low litter numbers, poor male performance or low pregnancy rates.

Artificial Insemination (AI), again where low productivity is seen, sperm is introduced into the vaginal opening via a pipette to encourage fertilisation.

Another technique we would like to introduce is Electroporation. This technique involves placing fertilised eggs into a dish containing a solution to maintain viability of the egg. Then the fragment of genetic material is added and a pulse of electricity is applied. This will open pores on the surface of the egg and the genetic material can then enter the egg.

The treated eggs are then surgically implanted as described above to generate the GA offspring.

**What 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 many research grants covering a range of research areas e.g. understanding infection spread and control. This project is to provide research groups with GA animals that can be used as models for animal and human disease. This project licence will indirectly help with publication of research work that can be shared with the public and other groups within the scientific community. The development of new techniques to produce, breed and store GA animals will improve breeding efficiencies and therefore reduce the number of excess animals bred/kept.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The work set out in this project will involve approximately 83,000 mice and 2,500 rats. The project will run 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 levels of severity? What will happen to the animals at the end?**

Protocol 1 – Breeding and Maintenance of GA animals, having a “Mild” classification.  
Protocol 2 – Superovulation – use of reproductive hormones to generate larger than

normal numbers of eggs from each female animal: Adverse effects during the breeding and maintenance of GA animals on the project are expected to be rare. The animal will experience a small amount of pain for an extremely short time (seconds) during any necessary tissue biopsy or a brief pin prick when a blood sample is removed by use of a needle and syringe. The tissue biopsy is when, using a small hole punch, a section of the outer ear is removed and analysed in the research lab, to determine whether or not the animal is carrying the genetic material of interest. Hormones are injected using a needle and syringe (protocol 2). This protocol is classified as “Moderate” because the animals receive approx. 2 injections directly into their abdomen. If necessary where a larger tissue sample is required from the tail, the use of an inhalation anaesthetic will reduce the sensation of pain. Also, during their lifetime, an assessment will be made based on information gathered at another facility (e.g. an import) and/or continuous monitoring of animals bred within our own facility regarding any particular effect that may be caused by the gene alteration. Protocol 1 is classified as “Mild” and no adverse effects are expected. Protocol 2, 4 and 5: Animals held on these protocols will experience surgical procedures. During this time they will be exposed to anaesthetic gas and given an injection of a pain killer to reduce any long acting pain. All animals are expected to make a full recovery from the surgery and in our experience this is the case. Rarely do we see complications after this surgery however if we find that an animal appears to be in pain (e.g., inactive, rapid/laboured breathing, or recumbent). Protocols are classified as “Moderate”

## Application of the 3Rs

### Replacement

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

#### Replacement

The purpose of this project licence is to produce live genetically altered animals. Many of the research projects study the immune system, relationship between host and infection and the interaction between different cell types. There is no non animal test that can answer many of the questions and to do this it requires the complexity of the whole animal.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Breeding of genetically altered animals can lead to a large number of young mice being produced if not controlled properly. There are several ways in which we can reduce the number of animals used on the project.

Ensuring that the staff are experienced and where necessary training updated in breeding techniques. Crossing specific GA animal combinations can produce fewer litters with a larger number of animals carrying the genetic information of interest.

We strive to use the lowest number of animals to achieve the objective of each specific breeding experiment. There is also a group of staff who review each study request to look at study design and in particular, a statistician who will look at the number of mice in each grouping to ensure that the desired outcome is met.

A piece of the animals skin is removed to analyse the genetic profile and determine if the animal has the gene(s) of interest. Routinely this sample is taken at around weaning age (3 weeks) and can take up to a week or in some cases 2-3 weeks to receive the results. For every breeding litter you may generate 2 – 4 cages of mice. We are taking this skin sample when the animals are around 2 weeks of age in order to reduce the animals weaned and placed into additional cages. This method generally reduces the number of cages generated by 1-2 per breeding litter.

During periods where the genetically altered animals are not required, rather than keeping mice alive for long periods, we freeze down sperm and eggs. This allows us to reduce the number of animals on the shelf. The freezing of sperm and eggs does not kill them, and when thawed, they are alive. This allows us to re-introduce the genetically altered animal back into the facility when required.

### **Refinement**

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

### **Refinement**

Mice and rats are the chosen species for this project for several reasons: There are several different mouse models that are of interest to the research groups. Their anatomy, immune system and physiology is similar to man and many scientific questions can be answered. There are several thousand of genetically altered rodents in the world and within a short time we can request and receive a rodent model.

One drawback with this model is that on occasions breeding efficiency can fall. We have animal health monitoring forms where we gather as much information to help improve the overall health of the animal in the hope that breeding performance will also improve.

When creating new genetically altered animals, we inject genetic material into the fertilised egg (mouse only). To increase the number of eggs available we inject a female mouse (egg donor) with hormones that increase the number of eggs

produced. We adjust the volume or concentration of the hormone injected as we can see a difference with the number of eggs produced with different strains of mice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 163. Maintenance of animal models for studying the inner ear</b>
<b>Key Words</b>	hearing, deafness
<b>Expected duration of the project</b>	0 year(s) 1 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The project aims to understand some of the many different causes of deafness, and to develop methods for preventing or reversing hearing loss caused by aging or certain medicines.

There are several reasons for doing this project. First, although mutations in many of our genes are known to cause hereditary deafness, a form of deafness that is passed on from generation to generation, the exact nature of the defects in the ear caused by these mutations are often unknown. Without knowing what is wrong with the ear it is certainly not possible to fix it, or to advise on the best type of treatment. Second, commonly used medicines and loud sounds can also cause hearing loss, and the effects of noise, medicine or aging are often made worse by mutations we carry in our genes. Quite why there are these unfortunate interactions between, for example, medicines and mutations remains unknown, and we need to understand why this is so if we are to stop it from happening. Finally, there is evidence that the hair cells in the inner ear that enable us to hear can, in some types of animals, regenerate if they are lost. Although regeneration does not occur in mammals like man, hair cells can repair themselves if damaged, and it may also be possible to re-awaken the regeneration process that is normally dormant.

The plan is therefore to (i) make animals that will allow us to tell how mutations in different genes cause deafness in humans, (ii) use these animals to understand why

mutations make us more susceptible to deafness caused by medications, noise and ageing. We will also search for compounds that might prevent medications from causing deafness and explore ways of improving the repair process or arousing regeneration.

The inner ear is a very complicated organ. It contains very many different cells types and, as yet, there is no substitute for using animals in this research. Where possible will use cells growing in a dish, but these can only be used for preliminary studies. We will use the minimum number of animals possible, the numbers required to obtain results that are mathematically significant. The project will use ~3000 mice and 10000 zebrafish per year.

The protocols should involve the least suffering possible due to the use of anaesthetics and analgesics. Where a procedure does has the potential to cause suffering we have taken every step possible to reduce the possibility this happens. We use mice because they have genes and ears that are very similar to those of humans. We use fish because the hair cells on their surface that detect water flow are very similar to the cells in our ears that enable us to hear.

The procedures in mice involve introducing genetic mutations into the animals and then exposing them to medications or noise, or they involve expressing genes in the ear that may help regeneration. In fish, we will look for compounds that prevent medicines from killing hair cells. The likely adverse effects include deafness and audiogenic seizures - short epileptic fits caused by noise from which the animals rapidly recover.

**What 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 should advance our understanding of how noise, certain medications and mutations in our genes cause deafness, and may lead to the discovery of drugs or strategies for stopping, reversing or repairing various forms of hearing loss.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We plan to use mice and zebrafish for the experiments proposed. Over five years we expect to use ~15000 mice and 50000 zebrafish, many of which will be larvae.

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

Expected adverse effects include deafness and sensitivity to audiogenic seizures on exposure to noise. The levels of severity range from mild to moderate. At the end of the experiments the animals will be killed using a Home Office approved Schedule 1 method, usually overdose of anaesthetic.

## Application of the 3Rs

### Replacement

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

### Replacement

The objectives of the project include making animal models for human hereditary disease in order to study how mutations in various genes lead to deafness and sensitise animals to auditory seizures and cannot, by definition, be achieved without the use of animals.

For a part of this study we will be using cochlear cultures as a method for studying interactions between mutations and aminoglycoside antibiotics. These cultures are, however, derived from early postnatal animals that have been killed via a Schedule 1 method. Their use, therefore, does not obviate the use of animals, and the relevance of the results obtained with this in vitro system need to be confirmed in adult tissues in vivo. The use of inner ear cell lines derived from the Immortomouse has been considered and rejected as the cells do not produce bona fide sensory hair cells that are mechanotransduction competent.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Appropriate power calculations will be used to ensure the minimum numbers of animal are used to ensure statistical significance. In general, when phenotyping genetically altered animals, a minimum of 3 animals of each genotype will be examined at a number of different stages of embryonic or postnatal development, and the observations will be repeated on animals that have been generated by breeding on 3 independent occasions. For example, to test whether a dominant mutation in gene X increases sensitivity to audiogenic seizure, a minimum of three wild type X+/+ and three X-/+ animals (preferably derived from a single litter and therefore of similar age and genetic background) would be tested on up to three occasions over the course of 5 days [3 times with 48 hour intervals between each test], and this experiment would be repeated on 2 independent occasions with different groups of mice of the same genotype derived (if possible) from the same breeding pairs. When necessary we will consult with expert statisticians REDACTED for more detailed advice.

## Refinement

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

### Refinement

The mouse is thus far the most suitable species for the production of transgenic models of human hereditary hearing loss, as suitable ES cell lines are not available for other mammals. They are the most refined for the experiments proposed as their genome is very similar to that of humans. Most genetically altered lines should only have hearing deficits and thus be mild in severity. Whilst social interactions may be compromised we have thus far not noted any problems with the breeding performance and ability to rear offspring in mouse mutants that are severely deaf across their entire hearing range. Quite large losses in vestibular sensitivity (as measured by a reduction in vestibular evoked potentials) can occur without an obvious, harmful behavioural phenotype like circling behaviour, presumably due to visual compensation. If genetically altered animals are produced that display a harmful phenotype due to severe vestibular dysfunction or other unexpected causes, the use of conditional, cochlear-specific gene targeting would be considered as an alternative means of achieving the objective. Suffering during and after surgery will be minimised by the use of general anaesthesia and peri-operative analgesia, suffering during hearing tests in restrained animals will be minimised by the use of general anaesthesia.

Zebrafish produce a large number of progeny under laboratory conditions that are suitable for drug screening programmes and have sensory hair cells in their lateral line organs that behave in a manner very similar to those in the vertebrate inner ear. It is also relatively easy to produce genetically altered fish and many lines are already available. Fish are also considered to be of lower neurophysiological sensitivity than birds or mammals. Any suffering will be minimised by the appropriate use of anaesthetics and analgesics.

To minimise stress and allow natural behaviours, environmental enrichment will be provided for mice and fish will be maintained whenever possible in mixed sex tanks with ~40 individual per 8 litre tank.

There are no protocols of substantial severity.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 164. Application of ruminant immunology for the development of novel diagnostic strategies and vaccines against bovine tuberculosis.</b>
<b>Key Words</b>	Bovine, Tuberculosis, Cattle, Vaccination, DIVA, Biomarkers
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The purpose of the project is to develop diagnostics tests that could allow cattle to be vaccinated against bovine tuberculosis thus help eliminate the current disease problem in cattle.

The project is for 5 years

Currently bovine Tb is controlled in the UK by a testing and if animals are found to be infected they are slaughtered. This is both ethically and financially costly.

- **Objective 1. Development of diagnostic tests for bovine TB.**

The generation of data to support the licensing of a novel diagnostic skin test that and an accessory diagnostic blood test that if used could allow vaccination of cattle with BCG. The current diagnostic tests cannot distinguish between TB infected cattle and those vaccinated with BCG, hence BCG vaccination is not allowed as a method of controlling TB infection in cattle. This will also include using new technologies such as micro-needles to improve the refinement and accuracy of skin test reagent delivery and subsequent measurement.

- **Objective 2. Development of improved cattle vaccine strategies for bovine TB.** BCG is the best current vaccine candidate for cattle but it is not all animals are fully protected after vaccination and also unlike when used in

humans where the protection is considered life long in cattle protection only lasts generally 1 to 2 years

**What 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 better immunodiagnostic tests in live cattle , including tests that could be used alongside vaccination (vaccination of cattle against tuberculosis is currently illegal and the vaccine currently available interferes with the currently approved diagnostic test). These so called DIVA (Differentiating Infected from Vaccinated Animals) tests that could be used alongside vaccination strategies. The work on the licence will prioritise those tests that can be further evaluated in large field trials and eventually licensed by regulatory bodies, such as the OIE (World Health Organisation for Animals).
- Generation of scientific knowledge and Intellectual Property Rights for the UK government
- Potential to be used in other susceptible domestic animal species that have had cases of TB (e.g. deer, alpacas, llamas, goats, sheep or companion animals (e.g. cats) that could be vaccinated with the same vaccines that will be effective in cattle.
- Bovine TB is an international problem particularly in some of the poorer countries the finding of novel diagnostics that could be used alongside vaccination could allow these countries to implement vaccination with BCG as a control measure.and improve their food supply and public health

**What types and approximate numbers of animals do you expect to use and over what period of time?**

750 cattle 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 levels of severity? What will happen to the animals at the end?**

Expected levels are mild Adverse effects would be due to vaccination, therefore limited to mainly local reactivity at injection sites; On the protocols with a challenge infection phase of the experiment which is necessary to assess how well the vaccine has worked, experiments are time limited and as TB is a slow progression disease, the scientific end point can be reached so that there will be no obvious clinical signs in the cattle.

## Application of the 3Rs

### Replacement

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

### **Replacement**

For the development of TB diagnostic reagents as well as the vaccination strategies, no non-animal alternatives are available due to the need for complete immunological response only possible in the live animal. The use of the target species, in this cattle, is necessary due to the variation between species.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Statistical assessment of sample size will be guiding every experiments and number of animals used per group. This analysis will be based on previously published data, with professional statistical advice to be sought on a case by case basis.

### **Refinement**

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

### **Refinement**

Cattle are the target species of vaccination and associated diagnosis and therefore the appropriate species to be used to achieve our stated objectives. The methods we will apply are standard technology used worldwide in this type of research, On the protocols with a challenge infection phase of the experiment which is necessary to assess how well the vaccine has worked , experiments are time limited and as TB is a slow progression disease, the scientific end point can be reached so that there will be no obvious clinical signs in the cattle.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 165. Culture of sea lice on Atlantic salmon</b>
<b>Key Words</b>	Salmon, lice, aquaculture
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to maintain sea lice using salmon so that they can be used to monitor resistance to existing treatments and develop new approaches. Sea lice are the most significant health problem for salmon farming because treatment involves significant cost and because farmed fish may provide a reservoir of sea lice which provide a threat to wild salmon fisheries. Commercial producers cannot always control sea lice because of resistance to current medicines, and the result is losses and early harvest to avoid harm, reducing production. New treatments are needed, and developing them means growing sea lice on live salmon as hosts.

To develop more effective treatments, such as new drugs, we need to be able to test them on sea lice in the laboratory. To check whether new drugs work on lice with different patterns of drug sensitivity we need to keep a few varieties of these parasites. Unfortunately, we cannot grow them without using salmon, so we need to use fish in the laboratory too.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Permission to keep sea lice in the laboratory means we will be able to supply them into a range of research projects directed at improving salmon health. These may be designed to understand the basic biology of the sea lice themselves and their interactions with salmon, to understand the spread of resistance to current treatments, or to evaluate the effectiveness of new ones. In the longer term new medicines will reduce the suffering of farmed salmon due to sea lice, and increase the supply for human consumption.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use Atlantic salmon because data from this species will be required to gain approval of new drugs for use in salmon farms. We will only use young farmed fish, not wild, that have adapted to their seawater life stage. We expect to use 30,000 fish over the five years.

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

Most fish used will be infected with sea lice at such low numbers there is light or no damage to the outer skin, the epidermis. As infection is due to many factors including the frequency of encounters with sea lice, and the susceptibility of the fish to infection, some fish will be more heavily infected and suffer minor skin damage. We observe fish carefully, and any fish showing deeper skin damage will be killed immediately. All fish will be humanely killed, for example with an overdose of anaesthetic.

## Application of the 3Rs

### Replacement

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

### Replacement

We have searched the scientific literature and consulted with colleagues and have confirmed that it is not yet possible to culture sea lice without growing them on host fish. We will continue to search, and if and when methods using fewer fish and that are suitable become available we will use them.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Sea lice produce large numbers of larvae that can be used to screen out many of the candidate compounds that will not work as medicines, so we only need to test the most promising on live fish.

We recognise that sea lice are harmful to fish, so we will only maintain strains of sea lice that will be useful for research and will only produce enough sea lice to meet the requirements of planned research.

We can control the intensity of infection, meaning we can use fewer fish while limiting the harm experienced by the majority to a mild level.

We may require up to 2,000 fish per year to maintain sea lice and up to 4,000 fish per year for experiments. We have chosen not to re-use fish for maintaining our sea lice colonies in order to minimise the harm done, but this does mean we will use more fish. The experiment that will require the most fish is genetic selection of salmon for resistance to sea lice for a breeding company. There are four separate year groups but a single round of genetic selection for one requires infection of 2,000 fish.

## **Refinement**

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

## **Refinement**

We will only keep sea lice species known to infect salmon, and only strains that will be useful for developing new medicines or investigating resistance to current treatments.

We take into account the size of the fish and keep infections lighter on smaller fish.

We check the level of lice on fish after infection and will observe the fish at least twice daily throughout their use. Any fish found to have an unexpectedly high level of infection or deeper skin damage will be killed by a humane method such as an overdose of anaesthetic.

Anaesthesia will not be performed on an individual fish more often than at weekly intervals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 166. Investigation of the regulation of energy homeostasis, glucose homeostasis and reproductive function and their interactions</b>
<b>Key Words</b>	diabetes, obesity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Obesity and diabetes are two of the greatest health threats facing the world. In addition there is an increasing burden of poor reproductive health. This project seeks to identify mechanisms which regulate energy homeostasis, glucose homeostasis and reproductive function. While the basic mechanisms regulating these processes are known many of the details are not understood and not all of the systems controlling their function have been identified. The aim of this project is to identify mechanisms underlying the regulation of these processes and also identify novel systems which regulate them. The project will identify novel mechanisms and processes which regulate food intake, energy expenditure, blood glucose levels or reproductive 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 ultimate aim is to develop novel therapies to treat obesity diabetes and reproductive problems. Such treatment would have obvious benefits reducing the ill health and premature deaths which result from these conditions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats and mice will be used for this project, with approximately 20 000 used 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 levels of severity? What will happen to the animals at the end?**

Most of the animals will be injected with substances which will alter food intake, energy expenditure blood glucose or reproductive function. We do not expect this in itself to result in adverse effects. Some of the animals will undergo minor surgery which may result in minor pain or discomfort that will be managed by the use of pain killers and anaesthetics. In common with all operations there is a risk of minor infections and these will be treated with appropriate antibiotics under NVS advice. The severity of the experiments is likely to be low to moderate. All animals will be humanely killed at the end of the studies.

## Application of the 3Rs

### Replacement

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

#### Replacement

The processes being investigated are complex and require many interacting organ systems to regulate them. In addition some of the outcome measures are only possible in intact animals, so it is therefore not possible to conduct this work without the use of animals. Cell lines and tissue collected from animals will be used where appropriate throughout this project and where appropriate such work will be conducted before the corresponding work in living animals is undertaken. Organoid cultures derived from both human and rodent tissue will be used where possible.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

To ensure the minimum number of animals are used whilst allowing the results of experiments to be reliable, all experimental designs will be based on the use of special calculations which work out the minimum number of animals to use. In addition, where it reduces numbers and overall suffering, experiments will be conducted so that each animal acts as its own control as this reduces variability and improves the statistical power in studies thus reducing the number of animals required. To further reduce variability where appropriate, for example food intake and body weight studies animals will be separated into groups based on their body weight. Where appropriate when genetically altered mice are to be used, mice in which both copies of the genes are changed will be bred together to reduce the generation of mice without the required alteration to their genes. Experiments will be conducted to enable compliance with ARRIVE guidelines for publications.

## **Refinement**

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

## **Refinement**

Both mice and rats will be used during the course of this project. These species have been chosen as they are widely seen as the most appropriate models for examination of the processes we are interested in since many of the systems that regulate food intake, energy expenditure, glucose levels and reproduction are conserved between rodents and humans. Mice are particularly useful since genetic alterations are easy to perform in them and a large number of relevant models for human disease are available. Rats are particularly useful since targeting individual brain nuclei is easier in these.

Pain will be minimised by the use of appropriate analgesia and anaesthesia. We will use techniques which minimise the suffering and distress of the animals. For example we have recently developed a method of testing how well animals respond to glucose intake conducting the test with glucose administered by voluntary consumption, rather than delivered directly to the stomach with a tube and blood samples are collected via an implanted needle rather than having to use a separately inserted needle for each blood sample. Where possible very small blood samples will be collected.

All animals will be closely monitored post-surgery and any animal showing signs of poor recovery which fails to respond to NVS prescribed treatment or whose condition deteriorates will be humanely killed

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 167. The role and regulation of reactive oxygen species in development and regeneration</b>
<b>Key Words</b>	Early embryonic development, Tissue repair and regeneration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The primary goal in regenerative medicine is to facilitate the replacement of aged, injured and diseased tissues with fully functional counterparts, thus extending the healthy life expectancy of our ageing population. My research group has been investigating the molecular and cellular mechanisms involved in tissue formation, repair and regeneration in *Xenopus* and zebrafish, two animals with high regenerative capacity. We have discovered that appendage regeneration and embryonic development in these animals require sustained production of reactive oxygen species (ROS). ROS are natural by-products of metabolism, which, when produced at high levels, have traditionally been associated with degeneration and aging. Remarkably, our findings have shown that low, but sustained levels of ROS promote regeneration. We propose to extend these findings by addressing the following questions:

How is ROS production regulated during development and appendage regeneration, so that the right levels to promote regeneration are produced?

How does ROS promote regeneration?

We expect that answering these questions will pave the way towards the development of novel therapies, including the identification of novel pro-regenerative drugs, aimed at promoting tissue repair and regeneration in human patients, where regenerative potential is normally limiting.

**What 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 aim is to identify the sources of ROS production and how they are regulated during development following tissue injury. How ROS promote regeneration will provide clues about how ROS production might be manipulated following injury or disease in humans, as a means of promoting regenerative healing in patients. We expect that, from these findings, we will identify one or more possible drugs or drug targets, which may improve tissue repair and regeneration. That can then be explored in pre-clinical and clinical trials for their potential pro-healing/pro-regenerative effects in humans. The immediate beneficiaries of this work will be the fields of regenerative biology and regenerative medicine. However, the ultimate beneficiaries of these findings will be clinicians and eventually, patients who have suffered acute or chronic wounds or are suffering from degenerative diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

All proposed studies are to be performed on embryos and larvae from two frog species, *Xenopus laevis* and *Xenopus tropicalis*, and zebrafish embryos, larvae and adults. . These species have been chosen because they have remarkable abilities to repair and regenerate fully following injury. The approximate number of animals to be used under this licence will be approximately 44,000, of which most will be at the larval stages.

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

The vast majority of procedures proposed in this license will not lead to any expected long-term adverse effects. The most often used protocol in the licence involves a simple injection of hormones, to induce ovulation and/or mating in *Xenopus*. The second most often used protocol under this licence will be the generation and maintenance of genetically modified frogs and fish. The remaining protocols involve transplantation of cells between embryos and larvae of fish and frogs, the treatment of larvae or adult fish and frogs with substances or heat pulses to alter gene expression, and the creation of wounds in larvae or adults in fish or frogs. On very rare occasions when adverse effects occur, the animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

## Replacement

The main objective of this project is to investigate the role and regulation of reactive oxygen species in development and regeneration. Most of the planned studies will be conducted in embryos or on isolated cells and tissues in culture. To study the formation, repair and regeneration of complex tissues and organs, it is necessary to perform work *in vivo*, as it is not possible to recreate fully the complex environment of the developing and regenerating tissues in culture. The complex multi-tissue events that occur during tissue repair and regeneration cannot currently be replicated fully, using tissue culture techniques alone.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Rigorous experimental design considerations will be employed in the conduct of all experiments to ensure that the minimum number of animals is used to reach meaningful conclusions. Overall numbers of animals required are based on initial sample size estimates. These numbers will be updated as more recent and relevant data becomes available.

## Refinement

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

## Refinement

Our recent work has shown that early embryogenesis mimics many of the same mechanisms, in respect to the role of sustained ROS production in tissue

regeneration---- and replicated tissue development. This has allowed us to refine many of our experiments in order to exploit these similarities, and thus, we will focus much of our future work investigating the basic science mechanisms of ROS in cultured early embryos. This has led to both **reduction** and **refinement** in our procedures.

In addition, frog embryos and larvae and fish larvae and adults are particularly well suited to this project because they have remarkable capacities to heal wounds quickly, without leaving scars. Complex tissues are regenerated within days or weeks following injury. This makes these organisms particularly useful in studying both the development of tissues and their repair following injury.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 168. Cellular and network mechanisms of defensive behaviours</b>
<b>Key Words</b>	Brain, Computation, Neuron, Behaviour, Threat
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No

(d) protection of the natural environment in the interests of the health or welfare of man or animals;

No

(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No

(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No

(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to understand how the brain makes decisions in threatening situations and how it coordinates defensive behaviour, such as fight or flight reactions.

Successfully, avoiding or escaping from threat reduces the chance of an animal being harmed or killed but these threats often co-exist with desirables such as food or mates. Balancing these behaviours requires the brain to integrate information about past experiences, sensory stimuli and motivation to decide whether or not to engage defensive behaviour. We record the activity of neurons in the brains of mice exposed to threats (such as overhead shadows which mimic objects) and investigate properties in single neurons and neuron networks that control the conversion of threat information into defensive actions.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The main benefit that will arise from this project is knowledge about how the brain decides to engage in defensive behaviour, and more generally about how the brain processes information. The defensive system is similar in mice and humans and therefore the results of this project will give fundamental insight into the function of the human brain. Gaining knowledge about these processes in the healthy brain is an essential prerequisite for understanding what goes wrong in the diseased human brain, such as in anxiety or post-traumatic stress disorders. This project will also increase our knowledge of mouse animal behaviour, and in particular will identify stressor stimuli and behavioural signs of stress, which can be used to refine future animal experiments. Moreover, during the project we will develop new tools for data

acquisition and analysis; these and the data will be made freely available and will be of interest to scientists in many different disciplines (neuroscience, mathematics, clinicians, AI and machine learning, psychology).

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This work will use less than 5000 laboratory mice over 5 years. Mice exhibit recognisable defensive behaviours in the face of simple threats and a large range of cutting-edge techniques are available for recording and manipulation of the neurons in the mouse brain.

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

In order to record from neuron networks in the brain, mice undergo surgery under deep anaesthesia to implant recording devices, fixed externally to their skull. They are cared for after surgery and receive pain relief until they recover completely and adapt to the devices; there are no pain receptors in the brain so complications may only arise if the device detaches from the skull, in which case it is repaired or the animal is killed. The mice are then exposed to situations in which a threat is present (e.g. a shadow suggestive of a predator or an unfamiliar mouse intruding into their space) and the simultaneous activity in their brain is recorded. In some studies, the function of specific neurons may be knocked out by using chemicals (such as pharmacological drugs or by removing cells) in order to confirm which regions are responsible for certain behaviours. In all these studies, it is critical that the mice exhibit natural behaviours so it is essential that the surgical procedures do not, in themselves, cause adverse effects which interfere. Repeated exposure to threatening stimuli may increase generalised anxiety but the recording sessions will be limited in duration and frequency to ensure no lasting harm. At the end of experiments, or if mice show signs of ill health, distress or suffering, they will be humanely killed. Brain tissue will be collected from animals post mortem in order to study the relationships between behaviour, neuron recordings and anatomy.

## Application of the 3Rs

### Replacement

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

### Replacement

This project aims to understand how neurons in the brain control defensive behaviour, which require studying the intact brain in mice. It is therefore impossible

to avoid the use of animals for addressing these questions, as other approaches such as neuronal cell cultures do not replicate the connectivity structure of the brain, and preclude behavioural measurements. However, computer models will be employed throughout as a replacement for subsets of experiments.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will use several state-of-the-art methods simultaneously, together with sophisticated data analyses, to maximise the amount of data and information collected from each animal. In addition, the statistical power of each experiment will be increased by conducting studies in which functional, anatomical and cellular data are collected from the same animal. Also, in most procedures the experiment and control can be performed in the same animal, which further increases statistical power and reduces the number of animals used.

## **Refinement**

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

### **Refinement**

All experiments will be done in mice.

To minimise harmful effects, we will use recording and stimulation techniques that are well established and with which we have vast expertise.

All interventions in the brain will target specific areas so that effects on other areas and functions are minimised.

Surgical procedures will be done under aseptic conditions with appropriate anaesthesia and analgesia.

Experiments in awake animals will only be performed if the animals are stress-free and experience no visible discomfort.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 169. Developmental and Reproduction Safety Testing of Medicinal Products Using Small Animal Species</b>
<b>Key Words</b>	Regulatory, Safety Assessment, Developmental, Reproduction
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and 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, Alzheimer'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 through 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 types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 year life of this Project Licence, it is estimated that 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 levels 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 are 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

### Replacement

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

### **Replacement**

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.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

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.

### **Refinement**

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

### **Refinement**

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 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, whilst 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).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 170. Molecular Signalling in Neutrophils and Endothelial cells</b>
<b>Key Words</b>	Neutrophils, Endothelial cells, Inflammation, Leakage, Cellular Signalling
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We aim to understand how neutrophils, abundant immune cells, and endothelial cells, the building blocks of blood vessels interact and regulate inflammation. It is already known that these cells talk to one another and this regulates immunity and inflammation. How this 'cross-talk' is regulated on a molecular basis is still unclear. Intracellular 'signalling proteins' including phosphoinositide 3-kinases (PI3Ks) and small GTPases are important regulators of neutrophils and endothelial cells. To address how they regulate cross-talk between the two cell types, we first analyse neutrophils (freshly generated from laboratory mice) and cultured endothelial cells in isolation under conditions that mimic an inflammatory stimulus. The cells carry mutations in or lack signalling proteins (e.g. small GTPases, PI3Ks), or regulators of small GTPases and PI3Ks. We next study the cells in co-culture, to learn how they influence one another. With the results and the literature we build models, which we then test by performing experiments with mice. This is important, because the body contains many additional cell types and less defined conditions, therefore our models in the test tube may be oversimplifications. We give inflammatory stimuli, so that we can assess the effect of altering individual signalling proteins in endothelial cells and neutrophils on inflammation in 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)?**

Inflammation forms an important part of many chronic diseases that represent a particular challenge to health services world-wide. Unfortunately, current therapeutic approaches for inflammation itself are not well targeted and cause a lot of side effects. We know that neutrophils are important 'effector cells' in inflammation, which means that they represent a major cause of injury to the body's tissue that occurs in

inflammation. Endothelial cells are also important contributors to inflammation which regulate 'leakage' (i.e. entry of blood fluid and proteins into the surrounding tissue at sites of inflammation). Leakage is essential for the immune response, but needs to be tightly regulated to avoid damage to the body. The regulation of leakage is only poorly understood, and there are currently no drugs to manipulate it. In the long run, our work will help with the design of improved future therapies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use up to 1200 mice per calendar year over a 5 year period. Many of these are used in breeding programmes or as donors of tissue for use in the test tube. Roughly one third will be used in experiments that employ an inflammatory model.

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

Mice that are used as organ donors may receive a single injection or they may be fed or dosed with inducing agents. This causes only momentary discomfort. Some mice will be used in models of sterile inflammation. Before the actual experiment is carried out, we may again induce a genetic modification as laid out above. In some cases we may irradiate mice and administer bone marrow cells carrying a genetic mutation. The mouse only feels momentary discomfort when it is being injected. Most of the inflammatory models themselves are of very short duration (typically 1-4 hours). The experiments typically involve injections (e.g. in the skin between the shoulder blades of the mouse). Where this is practical, and considered less invasive than the procedure itself, we briefly anaesthetize the mice before administering any reagent (e.g. to drop it down the wind pipe of the mouse). We use two longer models of inflammatory disease, rheumatoid arthritis (RA) and cancer. The RA model we use is the most severe of all of our models. We use a model of RA, in which animals completely recover from the disease within days. RA induction occurs by two injections (on two different days). The mice will exhibit some redness/swelling to their toes/feet for up to six days. At peak (one single day), they avoid running, and change their gate slightly, suggestive of some discomfort. 1-2 days later, although their feet still look pink, the mice run once more (upside down) along the top of the cage, suggesting that their paws are no longer sore. We use mild models of cancer, typically injecting some tumour cells under the skin which causes tumours to grow on the flank of the mouse without causing discomfort. Where we test tumour invasiveness we do not keep the mice long enough for them to become negatively affected by their tumour burden. Sometimes we monitor the inflammation or tumour growth by whole body imaging. This is always done under anaesthesia, therefore causing no pain or distress to the mice. At the end of all models we euthanase the mice. Most of the analysis happens after that, to ensure the least adverse effects for any of the mice.

## Application of the 3Rs

### Replacement

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

### Replacement

The immune system is very complex and cannot be modelled in tissue culture systems yet. There are no cell lines able to convincingly recapitulate neutrophil behaviour properly, such that we need to use animals to obtain primary neutrophils.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We currently explore a model system that makes use of the ability of blood cell precursors to self-renew, 'immortalising' precursor cells whilst amplifying them, and 'differentiating' the final neutrophil only once a sufficiently large numbers have been grown in a culture flask. We hope in time this will permit us to dramatically reduce the number of animals sacrificed merely to purify neutrophils.

Where we need to use animal models, we use statistics to calculate the smallest number of mice to be used in these models for a reliable result. To do this we perform a pilot experiment and then do 'power calculations'. We get help from a statistician with this and also with the analysis of our data where this is required.

### Refinement

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

### Refinement

Immunity and inflammation are very complicated processes that cannot be modelled in cell culture. Whilst different to humans, mice represent the best available animal model at present to recapitulate inflammation in human beings. Mice have a comparable immune system and inflammatory responses to humans, and there is a wealth of genetic mutants that allow us to decipher the importance of individual regulators. This is important since some of these may turn out to be good drug targets. We constantly strive to improve our animal models.

Very often we look at early inflammatory events which means that our experiments are generally of short duration and injuries are titrated to induce the minimum adverse effects that would allow us to achieve our scientific objectives.

We have found an alternative to a commonly used model of rheumatoid arthritis which relies on injection of commercially available antibody cocktails to induce short-lived arthritis. We obtain less variation between individuals and can induce mild disease which dissolves more rapidly than with other commonly used models of the disease. We will continue to make improvements as and when the opportunity arises.

We use standardised monitoring regimes and scoring systems and apply strict humane endpoints in all our models.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 171. How does protective immunity develop, function and persist to bacterial pathogens and vaccines?</b>
<b>Key Words</b>	Vaccination, infection, Salmonella, antibody, T cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Infections account for 25% of deaths worldwide, with the young, old, or immunocompromised for other reasons at greatest risk. Bacterial infections, which kill ~5 million yearly, make a major contribution to this. This staggering number would be even higher were it not for the millions of lives saved yearly by vaccination and natural immunity, which prevent infections from becoming disease. Vaccines provide a powerful tool to help prevent the development of antimicrobial resistance as they prevent the need to use antibiotics in the first place, reducing any selection pressure that favours the development of resistance to these life-saving drugs. This project aims to address gaps in our knowledge that will help us understand how to resist better infectious diseases and their consequences.

To do this we wish to look at how natural immunity develops after infection of mice with *Salmonella* and how we can make better vaccines against this organism. *Salmonella* infections are deadly. Across the globe, there are 200 000 deaths each year from typhoid alone, and from non-typhoidal *Salmonella* (NTS) infections, this number is far greater, with current estimates in the range of 600 000 yearly. The importance of *Salmonella* infections in human health is highlighted by the fact there are three vaccines against typhoid, representative of all the different types licensed for use in humans, although none offers >75% protection. Moreover, there are no licensed vaccines for use in humans for NTS, highlighting limitations in our capacity

to deal with the scourge of disease caused by these pathogens. Combined, these studies will allow us to understand how we can modulate our immune response during infection, to maximize the benefit and minimize the harm we do to ourselves as we respond to the infection (immunopathology), as well as be able to generate new vaccines to prevent disease from these types of infections. We also want to learn how to maximize the magnitude of responses to vaccines and extend the longevity of the anti-vaccine response as this is a significant unmet need in vaccinology. Both of these are important as when we don't control infections appropriately then infections and their consequences can be devastating, even if death is avoided - autoimmunity and sepsis are two examples of this.

Therefore, our work will address the following fundamental questions:

- How do protective immune responses develop after vaccination or during infection?
- How are immune responses maintained after vaccination or infection?
- How can we improve the benefits of vaccination?

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

REDACTED Understanding how we can optimize the immunity induced to this antigen will help bring its development and translation forward. Moreover, our work in mice has indirectly helped in the development of a new treatment that has been used in humans to treat some people who make too much antibody to their infecting organism. Therefore, we believe that understanding how immunity is regulated has great potential to benefit people. This is really clear when we examine why people get sick from infections. In some cases it is because bacteria make a toxin that interferes with how the body works. In many cases though, it is because we "overreact" to encountering the bacterial pathogen and end up harming ourselves as we try to clear the infection. In this case, we end up causing huge amounts of "collateral damage" to ourselves and to our organs, because the immune response doesn't reach a low enough ceiling. This is really clear when we look at some of the devastating consequences of infections such as sepsis, where 30% of sepsis patients die and many others are left with long-term damage. Therefore, we will try to identify the checks and balances that enable protective immunity to work, but with the minimum potential to harm ourselves. Once we understand this, it may be possible to control these processes better.

**What types and approximate numbers of animals do you expect to use and 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: 10000 mice for breeding 3500 mice for infection with Salmonella 6000 mice for vaccine studies

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

The adverse effects experienced by the mouse will obviously depend upon its experiences during the protocols. Below, are the types of activities that could be experienced during the course of an experiment. Injection: The process of immunizing will usually involve needles, which can cause discomfort at the time of injection and for a short time thereafter. The generation of bone marrow chimeras and altering gene expression: To generate mice with defined genetic alterations it will be necessary sometimes to either feed mice agents that alter the expression of some genes. Feeding mice agents that cause gene alteration can result in some weight loss in the mouse but the mouse otherwise appears normal. Sometimes, it may be necessary to achieve an altered genetic make-up in the mouse by irradiating a mouse and reconstituting it with bone marrow cells from another mouse and so make a chimera. In this instance, the irradiation will cause damage to the tissues and increase the risk of the mouse to infection. In the first week after irradiation mice lose some weight before recovering as they reconstitute afterwards. To reduce the risk of infection antibiotics are given prophylactically. Immunization with non-viable antigens: After immunization, there is typically some short-lived weight loss and sometimes an inflammatory response at the site of immunization, which can last for up to a few days before the mouse recovers fully. Immunization with non-viable antigens is not expected to cause any more persisting effects and these transient effects are likely to be of a similar level to those we may experience after a vaccination. Infection with Salmonella: After systemic Salmonella infections the animals are typically unwell transiently for the first 24 hours. They then recover before exhibiting clinical signs for some or most of the period between days 7 and 21 days after infection. This reduced activity most commonly presents as a swollen abdomen, reduced activity and starry coat. After this period, mice recover and are indistinguishable from non-infected mice. This is a moderate level of severity. Our experience suggests that if immunization with an antigen induces protection then mice will show these clinical signs at a much lower frequency and to a much reduced extent. At defined times mice will be killed by a schedule 1 method and organs and blood harvested for further study using microbiological and immunological techniques.

## Application of the 3Rs

### Replacement

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

### Replacement

Experiments in vitro can be powerful tools to understand how immune cells develop. However, in reality the development of many responses against bacterial infections and vaccination is highly co-ordinated, requiring the interplay of multiple immune and non-immune cell types in limited sites within the spleen or lymph nodes. The instructions received at this time dictate what type of immune response is induced. We, and others, have repeatedly shown that these interactions in vivo are not fully replicated in vitro. Nor can we observe how long-lived responses are maintained in vitro or how immune responses work to kill bacteria. This means we need to work in vivo. Mice are an appropriate model for these studies because the key features that underpin human immune responses are maintained in mice and we can look and compare responses at surfaces (like the gut) and within tissues (systemic). This is not possible in vitro nor in lower order animals (they lack an adaptive immune system) and therefore such approaches are unsuitable for this project. We will therefore use the mouse as the least sentient species that would give meaningful information applicable to human immune responses and disease.

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.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

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 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

## **Refinement**

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

## **Refinement**

We will perform experiments using the minimum doses of antigen and bacteria, for the minimum time needed, to observe a biological effect, with this being dictated by the experimental objective. For the majority of experimental approaches and infections, our experimental protocols are well established and published based on experimental data. As the project progresses we will ensure that experiments are continually reassessed to ensure that we continue to balance animal welfare against achieving experimental aims. For instance, after Salmonella infection, we have identified the period when the animals will exhibit the most clinical signs. At this time, we provide additional support such as mashing food and additional measures.

In addition before conducting each experiment, it is discussed with the NACWO and NVS to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to acquire the scientific endpoints. We will also 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 the next experiment

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 172. Brain mechanisms underlying cognition and emotion</b>
<b>Key Words</b>	
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overarching aim of this project is to identify the neural, genetic and neurochemical circuitry in the brain that underlies the cognitive and emotional impairments that are important symptoms of psychiatric disorders such as depression, anxiety and schizophrenia. This includes identifying how genes and physiological stressors impact upon the development and subsequent functioning of this circuitry, how this affects cognitive and emotional processes, and how current therapies (ie antidepressant drugs) interact with this circuitry to treat these symptoms. These are important questions because over 40% of patients suffering from neuropsychiatric disorders are not helped by current therapies for reasons that are unknown, and when the therapies are effective, we don't understand why and thus can't predict which patients will do well on which treatments. This severely limits treatment options and treatment development. It is recognised that this is because we have very little understanding of the different brain mechanisms that can cause these symptoms, and until we understand how the neural, genetic and neurochemical circuitry within the brain contributes to the normal and symptomatic cognitive and emotional processing we will not be able to improve treatment strategies for the sufferers of psychiatric 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)?**

This project will provide a basic understanding of how some frontal brain areas contribute to a variety of cognitive and emotional behavioural impairments (for example, compulsivity, anxiety and loss of sensitivity to rewards) that are common in patients with neuropsychiatric and neurodegenerative disorders. It will provide an understanding of how damage to different brain mechanisms contributes to the different cognitive and emotional processes that cause these impairments, such as problems in switching attention away from negative stimuli or problems in predicting when negative events may occur. By identifying the underlying psychological, neural, genetic and neurochemical causes this will not only help stratify patients but also

improve their chances of getting personalised therapy. For example, if you are anxious because you find it difficult to switch attention away from negative things due to dysfunction in one region of prefrontal cortex, this will require different treatment than if you are anxious because you can't predict when negative things will happen due to dysfunction in a different part of the prefrontal cortex. It is this basic knowledge that is currently lacking. Thus, understanding the different brain circuits that mediate different aspects of such psychiatric symptoms, and combining it with information about how particular therapies interact with such circuits will help us to identify particular symptoms and eventually target existing therapies more effectively.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 340 marmosets 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 levels of severity? What will happen to the animals at the end?**

All animals are housed in either stable male/female pairs or live in their family groups in cages that exceed the UK and EU guidelines and contain an extensive array of environmental enrichment aids. Animals may occasionally be housed without a partner in the event of an argument with their cage mate or if their cage mate is humanely euthanased upon study completion (e.g., in cases where timing of brain assessment is critical). A new partner will be provided at the earliest possible opportunity, depending, e.g., on availability of opposite-sex partners, experimental status, etc. A typical study lasts between 18 months to 2 years. During that time marmosets are likely to receive behavioural testing 5 days a week on a range of cognitive and emotional tests that either last 15 minutes or 40 minutes. The rest of the time they are in their home cage with their partner or family. Over that 18 month to 2 year period they are likely to have between 3-5 general anaesthetics, 2-3 involving a surgical procedure such as brain surgery and implantation of a measuring device, the remaining for restraint purposes only in order to e.g. perform brain scans. Normally, the animals recover well from their surgery or general anaesthesia and are back in their home cage within 2 hours of coming round from the anaesthetic. With all surgical procedures, animals will be fully recovered from one surgical procedure before undergoing another, with a minimum of 2-3 weeks between procedures. At the end of a study the animals are euthanased. In such a study an animal will undergo behavioural testing either in the home cage or in a specialised apparatus. The latter is a purpose built test box including a computer and touchscreen. It allows animals to be presented with positive stimuli (e.g. food rewards and visual and auditory stimuli predictive of food rewards) and mildly negative stimuli (e.g. mildly aversive loud noise (0.3-0.7sec) or darkness and visual and auditory stimuli predictive of these negative stimuli) to study learning, attention and emotion. Animals learn to voluntarily enter a transport box for transfer to the testing apparatus, to which they have been gradually acclimatised to minimise stress. Testing away from

the home cage is limited to 40 min, typically once, but very occasionally twice a day, and is halted if the animal exhibits signs of distress. No adverse effects are associated with behavioural testing, and even when mildly aversive stimuli, such as brief loud noises, are used, animals enter the transport box for testing. Animals undergoing restricted access to water during more intellectually demanding experiments utilising a liquid reward receive 2 hours of unrestricted water 5 days a week in addition to rewards received during testing. Water restriction does not affect the weight of the animals, who often ignore the water when it is returned to their cage, suggesting that they are not very thirsty. To study the brain mechanisms underlying behaviour and cognition, selective surgical procedures may be carried out under anaesthesia. Animals are gently caught from their home cage by an experienced handler and carried to the surgical suite. Premedication with a sedative is achieved via an injection into the muscle which causes only mild, momentary discomfort. A gas anaesthetic is used thereafter to ensure no pain is experienced during the surgical procedure (typically lasting 3-6 hours depending upon the procedure). Through small holes made in the skull, we can infuse substances that permanently or temporarily alter brain function in a discrete region or insert an implant that allows the later injection of substances to the implanted region. The latter is fixed in place using screws attached to the skull and dental adhesives. We may also temporarily implant devices to measure local brain function. Animals are monitored closely throughout the procedure and during recovery, and are usually fully recovered and back in their home cage eating, drinking and behaving normally within 2-3 hours. Long-lasting pain relief is given prior to surgery via an injection under the skin, and for several days after as an oral treatment delivered in marshmallow to minimise the need to catch them. Extra care is taken during the first week after surgery to observe any changes in normal behaviour or appearance. Long term implant sites are cleaned regularly throughout the life of the animal to prevent infection. Surgical procedures (lasting 90-120 mins) are also performed in some animals to implant a small radio transmitter into the abdomen to record physiological measures of emotion (heart rate and blood pressure) in animals that move freely during behavioural testing. Brain imaging (typically lasting 90 mins) may be carried out using anaesthesia to keep the animal still so as to ensure good quality images. Animals receiving certain brain scans may have an intravenous access device implanted under the skin to allow the injection of a radioactive substance without the stress of injecting directly into a vein. Following these surgeries animals typically return to the home cage within two hours.

## Application of the 3Rs

### Replacement

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

## **Replacement**

The aim of the proposed work is to investigate how neural circuits in the brain control cognition and emotion. To do this, functional brain circuits are required. Furthermore to be able to determine the contribution of a particular brain region or circuit to the expression of a certain behaviour it is essential to be able to alter its function. As such interventional experiments cannot be done in humans for ethical reasons, and cell cultures are unable to contribute to a functional, behaving circuit, animal models are indispensable for this work.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

We are very aware of the need to minimise the number of animals that we use in a year while optimising the validity of our scientific results. For this reason, and to keep numbers to approximately 70 per year, we screen all our animals for their suitability for studying particular behaviours and for their genetic background to optimise which animals go into which study. We also use brain scanning to ensure the precise targeting of the location within the brain which are of interest, and plan to investigate the use of imaging as a way of measuring brain structure, connectivity, chemistry, and function, allowing individual animals to act as their own control rather than requiring both control and experimental animals. All new surgical techniques are piloted in rodents first where possible, and any new techniques are tested first in one or two animals to ensure the experiment is optimised. We repair surgical implants, when possible and when there is no risk to the animal, rather than implanting additional experimental animals. We regularly consult with local statisticians to ensure that we are using the optimal group size for the results that we see, to ensure that we use the minimal number of animals while optimising the mathematical power of our analyses.

## **Refinement**

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

## **Refinement**

Primates are used specifically because their brains, in particular, those brain regions most important in regulating our cognitive abilities and emotions, such as the prefrontal cortex, are far more similar in structure and function to that of humans than lower species, e.g. rodents. To illustrate this, the cerebral cortex, that region of the brain with the most sophisticated processing abilities, makes up 80% of the brain

mass in humans and 60-70% in primates, compared to just 26% of the brain mass in rodents. Marmosets are a particularly valuable species to use for the proposed work as their relatively small primate brain makes it possible to target cortical and subcortical structures and to make regionally selective neurochemical interventions with relative ease, with little risk to the animal. Often, the same approaches cannot be used in larger primates, such as the macaque, because the surgical procedure involves too many brain entries, which increases the risk of collateral problems such as damage to major internal blood vessels. Having a breeding colony in the same establishment as the experimental program affords us considerable experimental control over the entire lifetime of the marmoset. This is an important factor, particularly when studying negative emotion and its regulation, since it is known that stress and early life experiences can have an enormous impact on the cognitive and emotional regulatory processes under study. The on site breeding colony means that animals do not have to experience the stress of transport to the laboratory and allows us to separate some of the environmental and genetic influences on behaviour. We constantly review all of our behavioural and surgical techniques to ensure that we refine procedures in order to minimize potential animal suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 173. Roles of the lymphatics in inflammation and immunity</b>
<b>Key Words</b>	Lymphatic trafficking, leucocyte, immunity, inflammation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The human body is under constant threat of invasion by bacteria and viruses both through contact with infected individuals, inhalation and traumatic tissue injury. Our main defence is from white cells of the immune system that constantly patrol our tissues for the presence of invaders, engulfing them on contact and conveying news of their detection to local lymph nodes that trigger the dispatch of protective lymphocytes to the original site of attack. To move between tissues and lymph nodes, patrolling white cells must first enter and navigate the vessels of the lymphatic network that permeate virtually all organs and open into the lymphocyte-rich nodes themselves. This is also the case in inflammation, where white cells recruited to sites of injury are subsequently cleared via lymphatics, allowing the tissue to return to its normal resting status. Understanding the mechanisms behind these processes would allow us to develop therapeutic agents to block inflammation and destructive responses eg in autoimmune diseases.

The aims of this present project are to determine how cells enter and move through the lymphatics in inflammation and infection, how they navigate to lymph nodes to orchestrate immune responses. Knowledge gained in our previous research has identified likely roles for certain sugar-like molecules on the surface of migrating cells which bind to a specific category of “receptors” in lymphatic vessels and hence our current research will focus on the consequences of blocking these interactions, in order that we may gain new insight into the workings of the lymphatic system and how to target it for inflammatory disease 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)?**

As we expect to learn the intricate details of how lymphatic vessels orchestrate the movement of white cells that report injury or infection in our tissues to lymph nodes (the factories of the immune system), our work will yield benefits in a number of different areas related to human health. These include new ways to treat autoimmune diseases, where the immune system mistakenly attacks itself, and to prevent transplant rejection and inflammation. On the other hand they may also teach us how to enhance white cell movement through the lymphatics, so they can deliver vaccines more quickly and efficiently to lymph nodes and hence boost our protective immunity.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Our studies will be confined to mice. We anticipate using approximately 2,000 mice per year over a five-year period (total 11,000).

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

In most cases, mice will be given agents that cause limited skin inflammation or swelling, or that induce white cells to congregate in the body cavity (peritoneum) or the lungs, so that we can look at how they are taken up by lymph vessels. These will be administered along with compounds that block certain pathways and we will observe whether these make the inflammation better or worse so that we can learn more about whether these pathways are really important and how they contribute to inflammation. In the majority of cases mice will not suffer any adverse effects. In the case of mice that will develop peritoneal inflammation, the adverse effects may be moderate and include short-lived fever and abdominal discomfort. In the case of mice that develop lung inflammation the adverse effects may also be moderate and include shortness of breath but these will be closely monitored for seriousness and animals will be humanely killed promptly if found to be suffering. All animals will be humanely killed at the end of each procedure

## Application of the 3Rs

### Replacement

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

### Replacement

Because we are investigating the traffic routes for white cells as they move within the body and navigate to lymph nodes to make immune responses, we have to use living animals for our experiments. The movement of white cells through the lymphatics relies on the flow of lymph through these vessels which in turn relies on the flow of blood through the circulation. All of this happens only in a living breathing

animal while its heart is beating; **we cannot reliably model the overall traffic of white cells through lymphatics using tissue culture models so currently, we cannot use non- animal alternatives but will keep this under review. Lower species, such as fish or amphibians would be unsuitable for use in our work. Although these species have rudimentary lymphatic and immune systems, they are not as closely similar to the human as is the mouse and hence we cannot adequately model human diseases in them. In addition, all the tools we need for our experiments are specific to mice, and it would not be possible in the time-frame of our research to generate the new versions required for work on other species.**

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We only conduct experiments using animals where we have no suitable experimental model as an alternative. Importantly, we can and do use such models to study how individual parts of the “trafficking” process work, and to ask questions about which molecules attract white cells towards lymphatic vessels and which ones allow them to squeeze inside. These model studies add to our animal studies, they allow us to pre-determine the effects of some of the blocking agents we need to test in animals, and hence they help us reduce the number of experiments that we need to carry out on animals.

In general, we use the minimal number of mice needed in any one experiment to obtain statistically significant results, helping ensure that inconclusive studies that would be wasteful on animals are avoided.

## Refinement

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

### Refinement

Our choice of mice as an animal model is based on the following considerations. Firstly, the lymphatic and immune systems of mice have been exhaustively studied in the past so that the anatomy of the lymphatic drainage system is well understood in our experiments, and we can accurately predict the time taken for an immune response to an antigen to be generated. **In addition, the lymphatic system in mice is very similar to that in man, so the results of our experiments should be applicable to the human situation. Furthermore, we and others have made strains of mice in which specific genes of interest and predicted relevance to**

**cell trafficking in lymph have been deliberately deleted, allowing us to assess their involvement in the process, particularly in the context of disease. Likewise, mice in which we have previously engineered versions of these genes to make them instantly visible by microscopy are essential for our work.**

To reduce anxiety during procedures that require anaesthesia, we use inhalable anaesthetic agents because of their more rapid mode of action and more rapid recovery afterwards. Where necessary we also administer analgesics (pain killers) before or after each appropriate procedure. **In addition, after carrying out procedures we provide additional feed to the mice to enhance well-being, and follow expert advice from the NVS on the use of antibiotics. For all injections and drug administrations we also apply aseptic techniques as far as possible to avoid infection and any unnecessary distress that might arise from it.**

**In terms of harms to the animals, we will continue to develop the most humane procedures in our experiments. For example when administering agents to induce lung inflammation, we will use direct delivery *via* a small endotracheal tube, or transcutaneous injection. In both these cases the animal will be anaesthetised, but the recovery should be rapid as no surgical incisions to the trachea or suturing are involved.**

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 174. The function of key proteins in T cell signalling and disease</b>
<b>Key Words</b>	T cells, Signalling, Immunity, GSK-3, PD-1
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The main objective of this application is to study the function of glycogen synthase kinase 3 in the immune system. It has previously been shown that treating mice with drugs which inhibit this kinase can suppress tumour growth or viral spread. However, the mechanism behind this is unknown. Using inhibitors and gene-deficient mice I plan to investigate this further and identify other proteins which may be involved. It is uncertain how specific the drugs used to inhibit GSK-3 are and the use of gene-deficient mice will aid to confirm this specificity. During this project, we expect to identify other proteins that are up- or down-regulated in response to GSK-3 inhibition and this could lead to improved or alternative 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 primary benefit of this research would be to obtain a more complete understanding how GSK-3 functions in the immune response. This could further knowledge in the scientific field and result in the design of new or improved treatments for cancer and viral infections. Many available drugs are not target-specific and can suppress the whole immune system leading to other infections which can be fatal, improving specificity or identifying new proteins which can be targeted specifically is of extreme importance.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice provide the best animal model to study immune function that is very similar to the human immune system and provides a system in which genes can be readily manipulated or deactivated. The minimum numbers of animals will be used that will still provide a statistically valid study (a statistician will be consulted when necessary). The use of pilot studies will help to assess animal numbers and how best to design the main study in order to gain maximum information. We expect to use approximately 12,500 mice over 5 years from the 32,140 requested on the project..

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

The majority of animals will make a full recovery in most protocols; those used for tumour induction will indeed grow tumours up to 15mm in diameter but are not expected to show signs of adverse effects that impact materially on their general well-being. In rare cases, moderate clinical signs such as weight loss, stary coat, hunched posture and poor appetite may be observed. In the majority of studies, mice will be culled by a schedule 1 method.

## Application of the 3Rs

### Replacement

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

### Replacement

The need for animal models has arisen from extensive previous studies in the lab where the use of cell lines produced contradictory results that could only be resolved or confirmed with the use of an animal model.

Further to this, *in vitro* work has given rise to possible candidate genes as potential anti-cancer/viral drug targets and it is essential to validating these genes *in vivo* and to analyse their function in tumour/viral development. Where possible *in vitro* work using cell lines will be performed and only extended into animal studies where absolutely necessary. The aim of this project is to identify key proteins in disease which will be initially sought *in vitro*, but the final aim will be to look at possible treatments using these proteins as targets and therefore will mostly result in the use of animal models for therapeutic purposes.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Statistical analysis, including power calculations, will be used to determine the minimum numbers of mice used while ensuring sufficient data is generated to produce meaningful results.

The use of pilot studies will help to assess animal numbers and how best to design the main study in order to gain maximum information.

To maximise the information from a single animal, we will aim to collect tissue samples from multiple body sites and provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments.

Non-invasive imaging techniques in live animals and analysis of tissue samples collected post-mortem will allow us to maximise data collection during and after experiments and reduce the total number of animals required.

## Refinement

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

## Refinement

Mice provide the best animal model to study immune function that is very similar to the human immune system and provides a system in which genes can be readily manipulated or deactivated. In addition, most antibodies for studying immune cell function are available for murine immune cells.

This project continues our existing program of research and builds on our previous findings. We will use genetically modified mice to determine which proteins are involved in these diseases. Interventions such as treatment with immunological reagents or drugs will be used to test well-defined hypotheses. Outcomes will be measured by determining the progression of disease in treated animals compared to non-treated. Blood sampling and imaging studies will provide essential information, alongside *in vitro* tests at the end of each study which will be performed using tissue samples.

Animals will be housed in groups with suitable environmental enrichment. They will be checked daily and regularly handled. When the animals are on study, the frequency of handling and checking may be increased to ensure that the animals are

not suffering. The animals will have access to food and water. Blood samples may be taken at regular intervals and other samples e.g. tissues at the end of the study. Blood sampling volumes will be kept to the minimum required to obtain information for this study.

When generating transgenic mice in which a harmful phenotype may be displayed, extra care will be taken in monitoring these mice to minimise suffering and where possible inducible constructs will be used, so that the phenotype is only displayed when the gene expression or deletion is induced.

Throughout the protocols a number of optional administration routes have been provided, this is so that the mice may receive the least intrusive method but yet give the optimal effect. I.e. Intranasal infection of some viruses is the least intrusive method and gives rise to optimal levels of infection, however, other viruses require different routes to give optimal levels. The least intrusive methods will be used where possible.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 175. Protecting the inner ear to preserve auditory and vestibular function</b>
<b>Key Words</b>	hearing, deafness, balance disorders
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The inner ear is a complex organ that has two roles. It enables us to hear and provides us with our sense of balance. Loss of either function is common and can be very debilitating. Medicines that are commonly used to prevent life-threatening infections or cancer cause deafness in 20-80% of all treated patients, and ~30% of the ageing population has a significant hearing loss that impinges upon the quality of life. One in every 2000 babies is born deaf due to one of a number of inherited genetic defects, and age-related loss of balance causes falls in the elderly that cost the NHS billions of pounds every year. The aims of the project are (i) to refine existing and discover new compounds that will protect people from deafness and balance disorders caused by the unwanted side effects of certain medicines, and (ii) to determine if age-related changes in the extracellular structures that are unique to the inner ear are a cause of dysfunction that can be prevented.

A combination of approaches using larvae from fish, parts of the mouse inner ear growing in a dish, and live mice will be used to improve the efficacy of potentially protective compounds we and others have already discovered, and to assess their suitability for future use in the clinic. Genetically modified mice and genetically altered fish will be employed to determine how the specialised extracellular structures of the inner ear can be maintained for the lifetime 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 should advance our understanding of how certain medications and mutations in our genes cause deafness and balance disorders, and may lead to the discovery of drugs or strategies for preventing dysfunction of the inner ear.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We plan to use mice and zebrafish for the experiments proposed. Over five years we expect to use ~15000 mice and 20000 zebrafish.

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

The levels of severity range from mild to moderate. Mild adverse effects include killing of newborn mice by decapitation in order to remove their inner ear organs for in vitro experimentation. Decapitation is required because dislocation of the neck of mouse pups younger than 4 days old damages the inner ear tissues, reducing the reliability and repeatability of the in vitro experiments. The procedure is classified as mild because decapitation is expected to result in an almost instantaneous loss of consciousness. Another example of a mild procedure is the testing of balance and swimming behaviour in fish: the tests monitor normal spontaneous or evoked behaviour (eye movements; swimming against a current; startle response to a sound; orientation in response to light). Expected moderate adverse effects include deafness and balance disorders as well as testing of the balance system in mice. These tests include whether the mouse can stay on a raised platform without falling off it (with a mound of sawdust to soften the fall); normal righting responses when a tube in which the mouse is positioned is rotated 180°; observing whether the mouse runs up a slope (normal behaviour called positive geotaxis); forepaw stretching in response to being picked up by the tail and held about a sawdust-covered floor; observation of normal explorative behaviour; observing whether the mouse can stay on a rotating rod; observing whether the mouse can swim in tepid water). Applying compounds that may protect hearing directly to one of the inner ears by a surgical approach, while prescription drugs that have hearing loss as a side effect are applied systemically, is also classified as moderate, and involves the mouse experiencing: recovery from anaesthesia; possibly dehydration due to use of a drug that increases urine production, which will be countered by lactate-saline injections and the availability of wet mash post-operatively; possibly hearing loss. In some cases we will test compounds that may protect hearing, together with drugs that cause hearing loss, over many days. In this case we will monitor twice daily for signs of ill health, and once a symptom is observed, follow the animal closely. If such a sign (hunched posture; hairs standing up; lack of appetite; inactivity) persists for more than 2 hours without improvement we will kill the mouse. Some of these mice will also experience hearing loss and, more rarely, loss of balance function. At the end of the experiments the animals will be killed using an overdose of anaesthetic.

## Application of the 3Rs

### Replacement

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

### **Replacement**

The key aim of our project is to find ways to prevent damage to human hearing and balance due to the unwanted side effects (hearing loss) of important medicines that fight infections (aminoglycoside antibiotics such as gentamicin) or cancer (cisplatin), and due to ageing. We plan to investigate whether certain compounds, when given together with the medicines, can prevent or reduce loss of hearing and balance. To test candidate compounds we use in first instance an 'ear in a dish' taken from newborn mice, as a model for the human inner ear (which serves hearing and balance), but ultimately we need to know whether compounds protect in the whole, adult, organism and do not themselves have side effects. This necessitates the use of animals. In the scientific literature there are examples of models for inner ear cells and tissues: cell lines derived from mice and 'organoids' (organ-like structures) derived from mouse and human stem cells. We have been doing research on these cells and tissues ourselves and found that they do not sufficiently resemble the inner ear: for example they mostly do not respond to sound-like stimulation, they lack uniformity, and they are not as sensitive as real inner ear to gentamicin and cisplatin. We therefore cannot use these cells or tissues as reliable alternatives.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will use statistical power calculations in our design of experiments using animals, to try and ensure that we use the minimum number of animals that will give robust, statistically significant results. Usually, we aim for a power of 90%. For example, when testing compounds in mice for protection against hearing loss due to drug treatment, this means that we accept a 10% chance of incorrectly concluding that the compound is not protective. By using optimally calibrated equipment for measuring hearing sensitivity, this will require 10 mice for each drug tested at this stage. To reduce the number of adult mice used for testing compounds, we screen the compounds first in zebrafish larvae up to 120 hours gestation, followed by testing for protection in 'an ear in a dish'.

### **Refinement**

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

### **Refinement**

We will use two complementary animal models for our studies. Zebrafish have lateral line neuromasts that contain externally visible hair cells similar to the cells in the cochlea or the balance system in the mammalian inner ear. They produce very large numbers of larvae that are suitable for screening compounds that may protect hearing. The mouse inner ear is more similar to that of humans because they are mammals. Many mouse mutations mimic human hearing loss or loss of balance, and they are therefore suitable for testing mechanisms of action and any side effects of potential protective compounds in more detail. We will minimize suffering of the mice during and after surgery to test the effect of candidate protective compounds directly applied to the inner ear by using general anaesthesia supplemented with pain control before, during and after surgery. Hearing tests will also be conducted under general anaesthesia to minimize distress due to the necessary restraint and exposure to unfamiliar, sometimes loud, sounds. Environmental enrichment (for example bedding, cardboard tubes) will be provided in the cages to minimize stress, provide shelter and allow natural exploratory behaviour.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 176. Defective regulation of pancreatic hormone secretion</b>
<b>Key Words</b>	diabetes mellitus, complications, pancreatic islet, hyperglycaemia
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Diabetes is a major metabolic disorder. It results from insufficient release of the body's blood glucose-lowering hormone insulin combined with oversecretion of the body's principal blood glucose increasing hormone glucagon. Insulin and glucagon are released into the blood by small (0.05 cm) islands of tissue ('islets') within the pancreas (a large gland that produces much of the digestive juice). Diabetes affects every cell of the body, which probably explains the wide spectrum of secondary complications (blindness, kidney and heart failure etc.). Why insulin and glucagon secretion become defective and how these defects are linked to secondary complications are not known.

We will pursue this project using a 'bench-to-bedside' strategy starting with *ex vivo* studies on isolated tissues, progressing to studies *in vivo* in experimental animals and finally testing concepts that have emanated from the experimental studies by conducting clinical trials in diabetic patients as well as non-diabetic volunteers.

Our work aims to explain: 1) the regulation of insulin and glucagon secretion under healthy conditions; 2) how it becomes perturbed in diabetes; and 3) the potential link between defective insulin and glucagon secretion and secondary complications associated with diabetes.

Our hypothesis is that diabetes results from the failure of the insulin-producing cells to secrete enough insulin to maintain blood glucose in the normal range and that increased blood glucose levels results abnormal regulation of glucagon secretion as well as impaired heart and kidney function.

Our preliminary data suggest that these defects arise because of high blood glucose leading to an increase in the intracellular sodium concentration in these cells by activation of transporter in the membranes of the affected cells. Importantly, there are pharmacological inhibitors of this transporter already in clinical use, which facilitates translation into the clinic.

**What 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 test a new – and unifying – hypothesis (the 'sodium overload hypothesis') that explains both the insulin and glucagon secretion defects but also part of the spectrum of diabetes-associated secondary complications. The ultimate goal of this research is to identify means that minimise or even prevent the metabolic consequences of diabetes. The potential benefits of the work proposed cannot be overstated: diabetes costs the NHS in excess of £1.5million every hour and it is associated with a significant reduction of life expectancy and quality of life to both the patients themselves and their family/carers.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

5750 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 levels of severity? What will happen to the animals at the end?**

Experiments on animals will be used to explore in greater detail and under controlled experimental conditions observations made clinically in diabetic patients and/or using human pancreatic islets. We expect that the functional consequences of most genetic modifications will be mild and that hyperglycaemia/diabetes will be seen in only 5-10% of the animals. This license is for both breeding genetically modified mice and some functional tests on a limited number of mice to perform detailed metabolic profiling. The level of severity of these tests will not exceed 'moderate'. These experiments will allow us to gain a more complete profile of the metabolic changes associated with diabetes from individual mice and (in the longer term) generate more meaningful data and reduce the number of animals used. Most of our functional tests will be carried out in test tubes with isolated tissues/cells after the mice are culled.

## Application of the 3Rs

### Replacement

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

### Replacement

Many of the experiments will be performed on human pancreatic islets obtained from a clinical islet transplantation programme. However, supply of human islets is limited and some experiments are technically not feasible using human islets. Thus, some of the experiments will be performed on mice that have been genetically modified to mimic the disturbance in diabetic patients.

We are actively engaged in effort to use computer models and are looking into the use of the zebrafish as an alternative experimental model.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

Use of mouse models in which specific cell types have been 'tagged' allows cell identity to be established before the experiments commence. This will increase success rate and thus result in a reduction in number of animals used.

Although we are primarily interested in the pancreatic islets, we make an effort also to harvest other organs known to be affected in diabetes (including the heart and kidneys) when the animals are dead. Kidney and heart failure are common complications associated with diabetes. We have identified a 'metabolic signature' of cells affected by diabetes and by storing the the tissues, we can analyse the consequences of diabetes without breeding additional mice.

### **Refinement**

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

#### **Refinement**

The mouse is the lowest vertebrate with enough aspects of its genetics, anatomy, physiology and embryonic development shared with humans to generate biologically relevant data that ultimately can be extended to improved understanding of diabetes in humans.

Animals will be closely monitored and if any become unwell they will be killed and an examination performed to identify the cause of death and to inform subsequent experiments. If animals exhibit diabetes, measures will be undertaken to minimise the consequences of this, such as changing wet bedding (or using ultra-absorbent bedding), frequent refilling of the water bottles and reducing the numbers of animals housed per cage if appropriate. When introducing new models, the progression of diabetes will be carefully monitored by measurements of sugar in the urine.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 177. Blood Product Safety Testing</b>
<b>Key Words</b>	Pyrogen Testing, Rabbit, Blood Product
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project ensures the ongoing safety of medicinal products used in the clinical care and support of seriously ill patients, on behalf of a not-for-profit organisation.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The provision of safe therapeutic products that are lifesaving or life preserving.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

3750 rabbits 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 levels of severity? What will happen to the animals at the end?**

Animals may become distressed or agitated as a result of restraint. Slight elevation of body temperature may occur following administration of some test products. At the end of the protocol, animals will be euthanised or transferred to other projects for re-use where this is authorised. Severity – Mild

## Application of the 3Rs

### Replacement

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

### Replacement

The inherent variability of the raw material used to manufacture plasma products in the developing world means that, despite suitable quality assurance of processes at the time of collection and initial processing, results from in-vitro assays such as LAL

and MAT cannot be consistently achieved. This finding follows many years of investigation and procedural refinement that continues to be improved. Unfortunately, for these invaluable plasma products, no suitable test with similar sensitivity or sufficient breadth of coverage for undefined contaminants is currently available for all blood products as a direct replacement for the rabbit pyrogen test although attempts to validate alternative tests not using animals is ongoing.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

The requirement for three rabbits per group is set out in the European Pharmacopoeia Monograph. No further reductions are possible without an update of the Pharmacopoeia Monograph.

### **Refinement**

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

#### **Refinement**

The rabbit is the only animal described in the European Pharmacopoeia Monograph for pyrogen testing.

This is a specialist facility in which the breeding and testing programs have been integrated to ensure the highest welfare standards are observed during the efficient performance of the test. Improvements in husbandry practice, including group housing, dietary enrichment and selective breeding have been identified and introduced. A very calm and constant environment has been established for the procedures to be conducted, ensuring the animals are comfortable and relaxed before, during and after the procedure.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 178. Modelling Human Ciliopathies for Novel Therapeutics</b>
<b>Key Words</b>	development, rare disease, cilia, therapeutics, gene editing
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project aims to breed and study mice with altered genes in order to understand how different cilia function in human health and disease. Ciliopathies are a diverse class of congenital human genetic diseases caused by cilia dysfunction, with over 35 recognized syndromes caused by mutations at ~200 different genes. While mammalian cilia are on almost every cell type and highly conserved structures, ciliopathies are highly clinically variable with varying degrees of severity and penetrance between tissues.

Here, we aim to understand disease-relevant mammalian cilia types, in control and mutant mouse models. We will use this improved understanding of ciliary diversity to inform on therapeutic strategies for ciliopathies where possible. In particular we will initially focus on treatment for primary ciliary dyskinesia (PCD), a ciliopathy which primarily affects the motile cilia in the airway, and for which we have a suitable mouse model exhibiting many of the human phenotypes.

By generating mouse models of known or suspected genes implicated in human ciliopathies, the project objectives are:

1. To understand the mechanisms of disease at molecular, cellular and physiological levels by deep phenotyping of these mouse models.
2. To administer compounds to pharmacologically or genetically ameliorate or modulate the disease phenotype, focusing on PCD.

3. To develop novel formulations (viral and non-viral) for delivery of *in vivo* gene editing reagents, focusing on the airways, optimizing for efficiency and safety.

**What 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 studying mouse models of human genetic ciliary disease, we will develop new approaches to understand disease mechanisms as well as developing potential treatments for patients with currently untreatable genetic conditions. We will learn how these gene products work normally and what happens when they go wrong, improving our understanding of how and why ciliopathies develop. By understanding disease mechanisms, we can develop novel therapeutics including gene editing, gene therapy and small molecule modulation. Our progress in this project lays the foundation for performing clinical trials and it is our hope that within the next five years we will commence preclinical and clinical studies for treatment of PCD. Clearly, this research could have an enormous benefit for patients where PCD airway dysfunction is a major cause of patient mortality. PCD is a major medical and economic burden to the nation. In addition, optimising delivery strategies of gene editing could enhance treatment of other genetic lung diseases. We will share our findings with scientists at meetings, in journals and with patient groups and the general media. All new mouse models we generate will be shared with others interested scientists to help their research.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over 5 years, we will use 2000 mice per annum for genetic and phenotypic analysis, including about 200 mice used per annum for *in vivo* gene editing. The majority of these animals will be examined by mild, non-invasive techniques and have no procedures done to them prior to death. We will be using both wild-type and genetically modified mice for these studies.

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

Most of the genetically altered animals generated through this licence will have no symptoms of any disease/disorder, as they will be bred to have one genetically altered copy of a gene and one normal copy, thus having normal biology. Within this licence, homozygous mouse mutants for some ciliopathy candidate genes are expected to have severe embryonic phenotypes (if they completely disrupt cilia structure- midgestation lethal) or a spectrum of postnatal phenotypes. These range from later onset mild phenotypes include retinal degeneration, obesity, infertility and inner ear infections, to early onset moderate ciliary phenotypes including hydrocephaly, kidney and complex cardiac defects (laterality). For each animal line,

we will identify these phenotypes clearly, monitor as they change and determine humane endpoints, as the project progresses. For lines that have mild symptoms, these do not lead to pain or suffering. In the case of lines with moderate adverse effects, we will keep these lines for a minimum time, observing wellbeing frequently and humanely cull before the onset of severe symptoms, as is the case for our PCD mice (hydrocephaly). Ways to minimize the effect of the hydrocephaly, including genetic background modifiers as well as central nervous system (CNS) transgenic rescue, are also used. Expected adverse effects and likely incidence from other protocols: Delivery methods of potential therapeutics: To avoid unnecessary discomfort, animals will be anaesthetised prior to more intrusive delivery (i.e. intratracheal delivery) of potential therapeutics (gene therapy, gene editing or candidate therapeutic compounds). Monitoring for adverse reaction to potential therapeutics: Some potential therapeutic compounds and/or delivery vectors may cause transient inflammation of the lung and airways. Animals which appear to be in particular discomfort will be killed. We will continually reassess the humane endpoint, i.e. when we have the information we need with inflammation and toxicity studies.

## Application of the 3Rs

### Replacement

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

#### Replacement

In order to understand clinical spectrum of human ciliopathies, we need animal models that recapitulate the functional and structural diversity of mammalian cilia. Also, the use of live animals is needed to confirm delivery of molecular therapeutics an organ, like the airways, which contains many different cell types and a complex structure. Live animals produce physiological responses to treatments that cannot be mimicked *in vitro* which may be important for assessing efficacy, safety and toxicity of treatments.

Alternative models involving culture of cell lines or human cells will be used where possible and scientifically justified. For example, human primary epithelial cells cultured at an air-liquid interface (ALI) present many of the features of the human airways and will be used to optimise delivery methods.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Experienced animal house staff with extensive colony management skills mean that we breed only what is needed for experiments and lines that are not needed at that time can be archived (i.e. frozen down as embryo or sperm). This also makes it easy to transfer these models onto other researchers or to share these resources with the larger community by depositing them in distribution centres like European *Mouse* Mutant Archive (EMMA).

Where possible, the number of animals is minimised by performing multiple analyses and assays post mortem for molecular, immunological and histological studies. Unnecessary duplication will be avoided by careful experimental design.

We will use longitudinal imaging techniques on live animals (e.g., bioluminescence, fluorescence), which will help to minimise group numbers for time course studies of gene expression and distribution as imaging technologies allow for longitudinal evaluation within the same animal while providing important information in vivo in real time.

### **Refinement**

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

### **Refinement**

All mice are housed in modern, well-maintained and resourced facilities manned by experienced staff looking out for their health and welfare, using current best practice to ensure that this is maintained.

For generation of ciliopathy models, mice provide an excellent model of mammalian embryonic development. With CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) editing, genetic modification of mice is precise and efficient, allowing quick analysis of a gene/process during development. As we can breed together strains of mice carrying different mutations, we can study complex genetic interactions in biological processes like development and postnatal health. As we cannot always predict the result of any genetic modification, mice will be monitored for adverse effects such as signs of pain, distress, weight loss and if necessary vets will be consulted and humane endpoints adjusted accordingly.

For the translational studies, mice are also the simplest mammals appropriate for respiratory studies and are widely used for such purposes, allowing comparisons to be made with other datasets. Other non-mammalian species are not suitable for

these studies as their airways are very different and therefore less relevant for the eventual translation to patients.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 179. Peripheral nerve interfacing</b>
<b>Key Words</b>	nerve, interfacing, prosthesis, amputation, control
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

In the UK alone there are typically 200 upper limb amputee referrals a year adding to an incumbent population of around 11,000. Many of these are a result of trauma and so these patients are mostly under sixty years of age. In the US, in a nine year period from 1988 to 1996, the average rate of upper limb amputation related hospital discharges was 18,946 per year. While lower limb amputees, even if wheelchair bound, are typically entirely independent, upper limb amputees are far more disabled and frequently dependent to some degree with their activities of daily living, and their potential for employment is much more restricted.

Highly sophisticated robotic prosthetics with a wide range of movements closely mimicking normal upper limb function are commercially available (for an example see [www.touchbionics.com/products/active-prostheses/i-limb-ultra/](http://www.touchbionics.com/products/active-prostheses/i-limb-ultra/)). However patients are unable to make anything like full use of this technology because of the lack of an effective control system. At present, advanced prosthetic upper limbs are controlled via surface electrodes, in contact with the patient's skin. These detect activity in muscles in the amputation stump, which the patient learns to twitch voluntarily. This "myo-electric" technology has moved on little in 40 years. In practice patients can usually only manage to produce two or three different myo-electric control signals and therefore they are required to preselect one or two movements or gestures (e.g. opening and closing the hand, pointing, etc) from the available repertoire that they would like to be able to use for the period until they reprogram the prosthetic to do others. Patients are unable to produce graded force, and sweating during physical exertion can lead to loss of myo-electric pickup and failure of control. Importantly, the control never becomes natural - there is always cognitive effort involved and consequent delay, because the control information is coming from the central nervous system in nerves other than those which formerly controlled the missing limb part. Studies have shown that a large fraction of

amputees do not use their prosthetic hand regularly implying that its function is sufficiently poor that it is often more burdensome than useful.

This project concerns the development of a peripheral nerve interface, i.e. a device for implanting in peripheral nerves to extract information from the nerves in order to control prosthetic limbs. The aim over the next 5 years is to develop our present prototype interface design to a point where it is feasible to plan human implantation trials, which would be the subject of a subsequent project. We plan to work on several aspects of the design, including improvements to the design of the electrodes within it, optimisation of a newly-developed method of retarding scarring around and within the device, integration of circuitry for amplification and signal processing, and the development of algorithms for interpreting the recorded signals to turn them into control information for prosthetic motors.

**What 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 beneficiaries of this work will be patients with limb loss who in the future may be provided with prosthetics whose function much more closely mimics the missing extremity. It is intended that at the end of this project we will have the knowledge and capability necessary to design and build a prototype peripheral nerve interface for human clinical trials in amputees. Such a trial is not a part of the present project but is expected to follow from it.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use a maximum of 300 animals over 5 years (i.e. 60 per year). These will all be adult rats.

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

Approximately one third of the animals used will be having non-recovery procedures to obtain tissue for in vitro experiments. The remainder will undergo a moderate severity protocol which starts with cutting the sciatic nerve on one side and implanting an interface device at the site of the cut. Cutting the nerve causes a temporary decrease in mobility but the animals recover from it quickly and move normally within a few days. There are then further steps either awake or under anaesthesia to make recordings from the interface and/or test its ability to resist scar formation. At the end of the experiment animals are killed, and the tissues will be analysed to ensure the scar suppression treatment is successful.

## Application of the 3Rs

## Replacement

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

### Replacement

We will do as much as possible without using animals, including making extensive use of mathematical modelling and in vitro testing, so that the devices are optimised as far as possible before animal use. There are still two things that we cannot do without animals however: (1) we need animals to test the performance of technology aimed at retarding scarring around the implanted devices (this requires the presence of a normally functioning immune system), and (2) we need implants in nerves connected to the central nervous system so that we may obtain realistic motor output signals and signal patterns from regenerated axons within the implants. These two things cannot be done in lower animals, (a) because we need to be sure that the nerve physiology and immune response is similar to that in humans, and (b) because there are size limitations meaning that physically smaller animals cannot be used (both a minimum size the implant can be made and a minimum size of the nerve for implantation to be practically possible).

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will make extensive use of upstream testing methods including mathematical modelling and in vitro testing, prior to in vivo experimentation, in order that only those things that are more likely to succeed are tested in animals. All data will be stored and electrophysiological data in particular can be reused multiple times for testing computational algorithms, reducing the need for repeated recording sessions.

## Refinement

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

### Refinement

Rats have been chosen because (1) their nerves have similar physiology to human nerves (albeit that the axons are somewhat smaller) and regeneration proceeds in a similar way; (2) they exhibit a foreign body response similar to that seen in humans; (3) their sciatic nerves are a suitable size for implanting; (4) we have a large amount of data from previous experiments using this model for comparison. Harms are

minimised by measures such as the routine use of analgesics and appropriate asepsis during surgery, providing additional food on cage floor, soft, non-tangle bedding etc.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 180. Cardiac Development and Disease in Zebrafish</b>
<b>Key Words</b>	Zebrafish, Congenital heart disease, Cardiomyopathy, Cardiac regeneration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The project investigates how genetic malformations of the heart (congenital heart disease), genetic diseases of muscle cells in the heart (cardiomyopathy), or in the main blood vessels (aortopathy) can be caused by inherited and environmental factors.

**What 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 heart diseases 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 types and approximate numbers of animals do you expect to use and over what period of time?**

This project uses zebrafish, a tropical minnow that has a simple heart that develops in a similar way to the human one. Most studies are done on very young embryos and fewer studies on adult fish. These fish 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. We count all embryos after 5 days of age so we record 80,000 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 levels 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, but by interbreeding these adults, young embryos with genetic abnormalities can be produced and studied. The expected level of severity will be sub-threshold. Where fin clipping is required, in order to identify fish carrying a specific genetic change, the level will be mild. We will observe genetically modified adult fish to ensure they do not become unwell as they become older.

## Application of the 3Rs

### Replacement

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

#### Replacement

These inherited problems of the heart and blood vessels involve many different cell types and how they interact to form these organs. This cannot be done 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, we can avoid using other animals such as mice.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Most of the experiments we want to do can be done using embryos at very early stages, as their heart and blood vessels 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 further.

We will store sperm from genetically modified animals, so that we can maintain the zebrafish line without having to keep breeding animals.

### Refinement

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

## **Refinement**

Zebrafish have simple hearts that are similar to human ones and develop in a similar way. Most of the features of these 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

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 181. Multimodal treatments for cancers.</b>
<b>Key Words</b>	Prostate cancer, light therapy, radiotherapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The primary objective of this research is to investigate the benefit of an untested combination of radiotherapy and a new minimally invasive surgical therapy termed “vascular-targeted photodynamic therapy” (VTP) in the treatment of advanced prostate cancer in pre-clinical mouse models of this condition.

Locally advanced prostate cancer, where the tumour extends beyond the capsule of the prostate, is currently treated with intention to cure using combined hormone therapy and radiotherapy. But, a third of locally advanced prostate cancer patients aren’t cured using this treatment, and have limited subsequent options. Moreover, despite improved radiotherapy techniques delivering precise doses to the tumour and minimising damage to surrounding normal tissues, significant radiotherapy-related side effects occur in many locally advanced prostate cancer survivors. Oligometastatic prostate cancer, where the cancer has secondarily spread to a limited number of distant sites such as bone, is currently incurable. Patients with oligometastatic prostate cancer receive hormone therapy with or without chemotherapy, but their prognosis is poor, and treatment options upon disease progression are palliative.

This research will investigate combining radiotherapy with VTP in the treatment of locally advanced and oligometastatic prostate cancer using mouse flank tumour models. It is hypothesised that a particular combination of delivery of these two treatments might result in benefit with an increased likelihood of tumour control and/or cure. In addition, if success in combining radiotherapy and VTP can be demonstrated, then this important observation might allow for reductions in the radiotherapy dose needed for tumour cure within combination treatment, and may enable the radiotherapy-related side effects to be reduced.

**What 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 this pre-clinical research demonstrates that combining radiotherapy and VTP is both feasible and efficacious in terms of treating mouse flank tumour models of locally advanced and metastatic prostate cancer, then this has potential benefits for patients with these common tumours as this treatment combination could be rapidly taken to the clinic in the form of first-in-man early-phase clinical trials. It is the aim of the applicant to have the first protocols for such clinical studies written by the end of the 5-year duration of the proposed plan of pre-clinical work, with the clinical trial proposal being informed by the pre-clinical results. The over-riding aim of this work would be to investigate whether combined radiotherapy and VTP increases the chances of tumour control and/or cure in both the locally advanced and oligometastatic prostate cancer setting, and if so, could this lead to a reduction in the necessary radiotherapy dose needed to achieve this beneficial effect, thereby in turn reducing the short-, medium- and long-term side effects of treatment.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This proposal will use various strains of mice (pigmented and albino strains), which are standard in the field, in order to investigate the effects of combination treatment in the corresponding appropriate cell lines. As outlined in the protocols it is expected that as many as 1550 mice will be used in this research 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 levels of severity? What will happen to the animals at the end?**

The likely/expected overall level of severity for these experiments is moderate, with most animals receiving either radiotherapy or VTP (rather than both, which will only occur for a minority of animals receiving multi-modality treatment). In the typical experience (>90%) of a mouse undergoing either radiotherapy or VTP, (i.e., the case for the majority of animals in these experiments) a unilateral subcutaneous injection of tumour cells under brief anaesthesia will be performed. Once the tumour reaches 100mm<sup>3</sup> either radiotherapy or VTP will be applied locally to the tumour under recovery anaesthesia. Local effects of treatment might include tissue oedema, inflammation, and formation of a dry “scab” to the skin (in the case of VTP), and these will be monitored carefully, with the animal being killed prior to the limits of the protocol being reached. Tumour growth will be monitored by callipers using brief manual restraint, and by further imaging under recovery anaesthesia. The mice will be killed before the total tumour burden reaches 1000mm<sup>3</sup>, or once the mouse reaches 12 months of age if cured by the treatment. In the typical experience (>90%) of the smaller number of animals undergoing combined radiotherapy and VTP, a unilateral subcutaneous injection of tumour cells under brief anaesthesia will be performed, and once the tumour reaches 100mm<sup>3</sup> sequential radiotherapy and VTP will be applied locally to the tumour under recovery anaesthesia. Local effects of radiotherapy and/or VTP might include tissue oedema, inflammation, and formation of a dry “scab” to the skin, and these will be monitored carefully as outlined in the

Protocols Section, with the animal being killed prior to the limits of the protocol being reached. Tumour growth will be monitored by callipers using brief manual restraint, and by further imaging under recovery anaesthesia. The mice will be killed before the total tumour burden reaches 1000mm<sup>3</sup>, or once the mouse reaches 12 months of age if cured by the treatment.

## Application of the 3Rs

### Replacement

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

#### Replacement

It is necessary to use in vivo experiments to investigate effects of combined radiotherapy and VTP because ex vivo approaches aren't sufficient to predict the outcome if this combination is used in patients. Ex vivo experiments may fail to demonstrate possible deleterious side effects that might occur from combined radiotherapy and VTP. The interactions of tumour cells with the host tumour microenvironment or immune system cannot be fully replicated ex vivo. It is necessary to use mice for these experiments to test the most promising sequential combinations of radiotherapy and VTP, and to evaluate host-tumour interactions, including potential deleterious and beneficial effects. It is also necessary to use mice because both the strategies and techniques for these types of experiment have been established in mice.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The fewest mice will be used that enable the proposed experiments to yield statistical significance and minimise animal suffering according to best practice. Preliminary experiments will optimise radiotherapy and VTP prior to combination therapy, and the samples from these experiments will inform potential molecular mechanisms of treatment efficacy, to maximise the information obtained from early experiments. Power calculations will be used for all major experiments to ensure the end result is scientifically informative.

### Refinement

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

## Refinement

The syngeneic flank tumour models described in the experimental protocols are the most refined available for these experiments. All experiments will be based on best practice with optimisation of radiotherapy and VTP before the main experiment investigating combination therapy. Mice will be anaesthetised before tumour cell implantation, and the tumour burden at the start of treatment will generally be very small and limited to the minimum required for a valid scientific outcome. If normal behaviour, ambulation or food/water intake are compromised, or if the skin breaks down to form a wet ulcer, the mouse will be killed. Analgesia will be used to ensure comfort of the mouse from the local effects of radiotherapy and/or VTP treatment. Radiotherapy and VTP will be image-guided to the tumour under anaesthesia. Imaging modalities will be combined under anaesthesia, and environmental enrichment will be employed during animal husbandry. Where necessary the response of tumours to treatment will be monitored using non-invasive imaging and/or biomarkers. Any surgery, such as incisions of the skin over the tumour as a refinement to transcutaneous VTP, will be performed using aseptic technique.

## NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at [www.gov.uk/research-and-testing-using-animals](http://www.gov.uk/research-and-testing-using-animals)).

Word limit; 1000 words

<b>Project Title</b>	<b>Project 182. Neuronal network dysfunction in rodent models of dementia</b>
<b>Key Words</b>	Dementia, Neurophysiology, biomarkers, cognition
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

One of the most frightening symptoms of dementia, both for patients and carers, is a profound sense of disorientation, meaning that patients can easily get lost, even in familiar places. We do not fully understand the brain mechanism which cause this sense of disorientation, but it is likely that deterioration of parts of the brain connected to a brain region called the hippocampus is important. Several different types of nerve cell in these areas form the internal maps of our environment which help us remember where we are.

In this project we aim to elucidate some of the ways in which electrical signalling in these brain regions goes wrong in dementias such as Alzheimer's disease and fronto-temporal dementia. We will use state of the art recording techniques to examine electrical signals in individual and groups of connected neurones. We will use rodents which have been genetically altered or treated with specific compounds in such a way that they develop characteristic pathological features of dementia.

We specifically hope to discover how the electrical properties of individual neurones and groups of neurones are affected by genetic and or pharmacological treatments which produce these dementia-like symptoms. We will also examine how communication within and between brain areas is affected in these disease models. We will also test the interesting hypothesis that artificially stimulating brain waves can reduce the amount of dementia related proteins in the brain and improve the connections in the networks of nerve cells. Finally, we will test established and experimental medicines in an attempt to reverse these changes in electrical activity.

**What 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 specific benefits we hope to derive from this project fall into 3 broad categories: 1) Greater understanding of the changes to electrical signalling in the brain that occur in dementias, such as Alzheimer's disease; 2) development of disease 'biomarkers': biomarkers are changes to physiology or biochemistry which occur in response to disease can be used as a measure of disease progression. These biomarkers are urgently needed to help develop new medicines to treat dementia since they can be easily translated from animals to humans and can be used to test how well new therapies work; 3) discovery of new ways to potentially treat dementia, such as Alzheimer's disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice, approx. 5700 over 5 years Rats, approx. 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 levels of severity? What will happen to the animals at the end?**

Many of the genetically altered mice will not suffer any adverse effects, since most will be bred and then killed for tissue extraction without undergoing any procedures. In all except two strains of mice the genetic alterations themselves mainly cause cognitive defects, such as the inability to recognise a familiar environment. Despite this these genetic alterations will not produce any other easily observable behavioural changes. However, there are two strains of mice which will be used during the course of this project which do have adverse effects. The first is a type of genetically altered mouse called the J20 line; in this strain approximately 10% of the mice die from unknown causes in midlife. The other is a mouse called the tauP301L strain; this mouse develops severe movement disorders from approximately 6-7 months of age. We will not keep any of the tauP301L mice this strain of mice beyond the age of 7 months. Whilst these mice do have some moderate adverse effects, they are the best way to study the effects of Alzheimer's disease and other dementias. Some of the mice and rats will undergo surgical procedures to implant recording devices and/or infuse specific substances in their brains. A small proportion (<10%) of these animals may experience some adverse effects associated with the surgical procedures, including bleeding, infection and post-operative pain. Any animals which do experience these adverse effects will be either treated or, if that is not effective, they will be humanely killed. Animals which have undergone surgical implantation of electrical recording devices will then be attached, via a cable, to additional equipment to amplify and record the electrical signals in the brain. The animals may experience some level of stress associated with the tethering procedure, although the vast majority (>90%) rapidly become accustomed to this. Some animals will undergo testing in various behavioural tasks designed to assess cognitive performance. Most of these tasks will not cause any harm or lasting stress to the animal, but some will be associated with a certain level of stress. For example, one task, known as the Morris Water maze, involves animals swimming in

a pool of warm water and as such may result in a certain level of stress. Other tasks may involve restricting access to food, in order to motivate them to perform the various tasks, in which food is provided as a reward for correct performance on the task. This will necessarily result in a certain level of hunger. In some experiments, we will use a technique called optogenetics which allows us to stimulate specific parts of the brain using light. This will allow us to test whether brain waves can improve the symptoms of dementia. Finally, some animals under this licence will be treated with clinically approved or experimental medicines in an attempt to improve the symptoms of dementia. Some of these medicines have known side-effects, such as diarrhoea, whilst for others we do not have any information about possible poisonous effects. However, since all of these medicines are designed to affect the central nervous system, it is possible that some of these medicines may produce symptoms caused by interfering with the brain. At the end of each study the animals will be killed and their brains will be taken for experimental analysis.

## Application of the 3Rs

### Replacement

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

#### Replacement

Mammals such as mice and rats have comparable complex brain anatomy and physiology to humans, which cannot be accurately modelled using other non-human alternatives such as flies, worms and computer models. We constantly review the scientific literature for appropriate non-animal alternatives for our research purposes.

Furthermore, the most accurate models of dementias such as Alzheimer's are genetically altered mice or rodents which have been surgically infused with proteins excessively present in dementia.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Wherever possible, genetically modified mice will be imported onto the authority of this licence from external breeders, rather than breeding a separate colony ourselves. This reduces the numbers of animals generated and wasted from the breeding process.

We will carefully control the conditions under which the animals are maintained in an attempt to reduce animal-to-animal variability. For example, all animals used in a

particular study will be bred and housed under the same conditions, therefore reducing environmental variability and reducing the overall statistical variability in data sets. This inevitably leads to a reduction in the number of animals required for any given study.

We publish all of our work in scientific journals that support the ARRIVE guidelines and we make regular use of the NC3Rs online research design assistant (<http://www.nc3rs.org.uk/experimental-design-assistant-eda>) when designing our animal studies.

## **Refinement**

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

### **Refinement**

Mice and rats can be genetically altered to produce specific pathological features of dementia. These genetically altered models represent the current gold-standard for examining the physiological effects of specific dementia-inducing pathologies.

An additional, complementary approach to understanding dementia like pathology is to infuse dementia-related proteins such as  $\beta$ -amyloid or tau into the brains of rodents and allow them to spread in much the same way as they do in the disease. This is a refinement of an older version of this model, where animals are studied a short time after infusion, thus missing the clinically relevant spread of disease pathology.

The in vivo recording techniques outlined here provide the best compromise between obtaining high resolution and high quality data and animal welfare. Future developments in wireless technology will be monitored and employed when it becomes sufficiently useful.

When testing new drugs, we will carry out small pilot studies in a few animals, to see if the drugs cause any harm to the animals. We will only carry out the full study if the mice in this pilot study appear to tolerate the drug.

The behavioural tasks used in this project are generally minimally or non-aversive and, where possible, animals will be motivated to perform these task using positive food rewards only, rather than aversive stimuli.

Some of the genetically alter mice that we will use have been previously reported to be aggressive, leading to them being housed in cages by themselves, which leads to a certain level of ongoing stress. However, we have founded in our previous work that with careful monitoring this is not usually necessary, so we have improved the welfare of these mice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 183. Investigation of oxygenase-regulated signalling pathways.</b>
<b>Key Words</b>	Oxygen, Cancer, Vascular disease, Transplantation, Therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

An adequate supply of oxygen is critical for life. Problems with oxygen supply are central to many diseases. The body has evolved pathways for controlling gene expression to try to ensure oxygen supply and demand are matched in all its tissues. These pathways include both 'on' switches and importantly 'off' switches too. Our group's work using cellular, biochemical and animals (ranging from worms and flies to mice) over the last thirty years has been pivotal to understanding the function of many of the critical components of these pathways. It is believed that manipulating these processes may improve outcome in many diseases (e.g. stroke, heart attack, cancer, pulmonary hypertension) and on exposure to altitude, but we now need to test this. We propose to do this first in mice which share many of the complex aspects of this system with humans and have sufficiently similar organs and physiology to provide adequate models of these diseases. Based on work in cells we have now developed ways of artificially manipulating the activity of individual components of these pathways in the whole animal. The techniques we have developed allow us to make these adjustments in ways that are limited to particular times and cells and are reversible. In this project we aim learn which components contribute to individual aspects of the body's response to low oxygen levels (we routinely use a stimulus equivalent to ascent to 5000metres (which is less than ascending Mount Kilimanjaro) and what changes to make when and where to get the best outcomes in different disease models (including experimental models of cancer,

wound healing, skin transplantation and diseases affecting the heart and blood vessels). We have already published data showing that relatively course changes we can make with agents that are currently available can alter acclimatisation to altitude and improve short-term outcomes following blockage of arteries supplying the brain or a limb so we intend to build on these results to improve things further and hopefully establish ways of getting long-term benefits.

We will mainly use mice. We estimate that over the five year course of the experiments proposed in this project we will need to breed up to 24,000 mice to produce the ~2,600 mice with the very precise characteristics we propose to test in our more detailed experiments and a similar number of control animals which will be produced by the same breeding programme. We will also use a small number of rats for some experiments where they provide technical advantages. We currently intend to use no more than 50 rats over the five year course of this project.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

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 to modify disease models and what side effects we might anticipate from such treatments. The pharmaceutical industry is already working on 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 and transplantation. Hopefully our results will stimulate the pharmaceutical industry to produce products that have the relevant beneficial effects.

**What types and approximate numbers of animals do you expect to use and 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 24,000 mice to produce the ~2,600 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 levels 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 15% 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 5000m, 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, receiving a defined wound or skin graft to examine effects on healing or receiving a series of inoculations to stimulate an immune response. 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 5000m. 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

### Replacement

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

### Replacement

The approaches we will be taking have been tested and shown to have the intended outcomes 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.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

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.

## **Refinement**

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

### **Refinement**

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 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.
- 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 ischaemia model chosen provides an adequate ischaemic stimulus with a lower risk of distal necrosis than models previously described.

- 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.
- 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.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 184. Regulation of V(D)J Recombination, Antibody Generation and Transcription</b>
<b>Key Words</b>	Antibody genes, Leukaemia, Recombination
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Lymphoid cancers are the fourth most common cancer with over 15,000 new cases each year in the UK alone. A frequent cause of these cancers is mistakes in the production of antibody genes. These genes are produced by selecting one part of the gene from a large pool of gene segments which is joined to a second gene segment from a separate large pool. The vast number of different combinations of gene segments generates millions of different antibody genes. However, because the reaction involves the breakage and rejoining of DNA, the wrong pieces of DNA can become joined, leading to the activation of cancer-causing genes. Hence, it is important to understand what regulates the normal reaction to then understand how the wrong pieces of DNA become joined.

The project firstly aims to investigate the individual steps during the cutting and rejoining reaction since mistakes at a number of points can lead to leukaemias and lymphomas.

In a separate reaction, by-products of the cutting and rejoining reactions become reinserted into the genome. This can also lead to cancer by the activation of cancer-causing genes adjacent to the sites of re-integration. Importantly, part of the protein, RAG2, inhibits this re-integration reaction. A second objective is to characterise the inhibitory region of RAG2 with the longer term goal of mimicking this inhibition to develop inhibitors of the dangerous re-integration reaction.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Forty per cent of leukaemias and lymphomas appear to have arisen from mistakes in the production of antibody genes. Thus, better understanding of how the cutting and joining reaction is regulated under normal circumstances will allow us to determine how mistakes lead to selection of the wrong pieces of DNA, giving an insight into how leukaemias and lymphomas are triggered. In addition, mapping the region of RAG2 that inhibits the re-integration reaction will facilitate longer term studies to develop inhibitors of this leukaemogenic reaction.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice were chosen for Objectives 1 and 2 since they are the only animal into which short pieces of extra DNA can be reliably introduced to perform the genetic experiments we propose. In addition, the cells that produce antibody genes have been extensively characterised in mice, meaning that they can be reliably isolated for study, allowing us to build on extensive previous knowledge. These experiments will likely use between 300 and 600 mice per year 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 levels of severity? What will happen to the animals at the end?**

Objectives 1 and 2 involve the permanent introduction of short pieces of DNA into the genome of mice. These sequences already occur naturally in mice and the experiments only involve the introduction of extra copies of, or removal of, tiny lengths of DNA. Very similar experiments to those proposed are already underway and the mice have not been observed to suffer any adverse effects whatsoever. The mice will be bred and housed under optimal conditions until they are humanely killed prior to their use in experiments. The expected level of severity is mild since the procedures primarily involve breeding genetically modified mice where the genetic modification has not been shown to have any adverse effects.

## Application of the 3Rs

### Replacement

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

### Replacement

It is necessary to use some animals in this work since tissue culture systems do not exist that faithfully recapitulate all the steps in the regulation of the production of antibody genes: The production of these genes take place in a number of sequential

cell stages and in vitro systems do not exist that properly progress from one cell type to the next. Also, it is only possible to achieve the genetic modifications we need for some of these experiments by breeding differently genetically modified mice.

Although we need to use mice for some experiments, we are trying to replace the use of animals in other experiments. Specifically, we have generated genetically modified mice where a protein is over-expressed in one cell type. This activates the cutting and joining reactions of one antibody locus. We have made a cell line from these animals which will enable us to replace mice in many, but not all, of these experiments.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We will minimise the numbers of animals used by expanding cells from the animals in vitro prior to use in experiments and/or by using highly sensitive assays to detect the changes we are examining.

### **Refinement**

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

#### **Refinement**

The only animal with which Objectives 1 and 2 can be routinely performed is mouse since this is the species where the technologies for insertion and deletion of small pieces of DNA are well established. Moreover, a large number of studies have characterised the production of antibody genes in the mouse. This allows us to build on existing knowledge and use existing reagents. In particular, our studies make use of a number of existing genetically modified mice. A further advantage of mice is that the cell stages where antibody genes are assembled are well characterised, thus allowing reproducible isolation of the required cells.

None of the proposed procedures are likely to cause adverse effects to the animals; they are a continuation of existing experiments where the animals have not shown any signs of distress.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 185. Mechanisms and strategies for brain injury and repair</b>
<b>Key Words</b>	Neurological disease, neurodegenerative disease, stem cells, neuroprotection, neuroregeneration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This work investigates mechanisms and strategies for limiting damage and improving repair in common CNS diseases such as stroke and Alzheimer's disease. We will use several strategies to limit damage and promote repair including targeting fundamental processes known to cause damage or induce repair and improving the success of stem 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)?**

Due to the ageing population, disorders of the brain, including dementia and stroke, cost the UK an estimated £112 billion. Stroke is the leading cause of adult neurological disability and dementia is now the most common cause of death. Stroke and dementia are the main causes of the decreased independence and life quality in older people. Despite this, no cure exists for dementia and there is a paucity of effective therapies for stroke, with only mechanical or pharmacological thrombolytic therapy only being available to a minority of stroke patients. Therefore the present programme of work aims to address this unmet clinical need by investigating ways of improving the brain's limited capacity to repair itself and by improving the way that stem cells work in promoting brain repair. The results generated will be disseminated

in order to help inform on clinical trial design and help direct future strategies for improving brain repair.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats and mice have similar cerebral architecture to humans (circle of Willis) and are the least neuroscientist animals possible that can be used for this work. In addition mice that have been genetically altered to mimic particular disease states will be used where appropriate. The total number of animals expected to be used during the period of this licence is 200.

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

The welfare of the animal will be closely monitored and any animal deviating from the expected normal pattern of behaviour in animals undergoing this procedure will be withdrawn from the study and humanely killed. After ischaemia, animals (up to 100%) will display postural and behavioural abnormalities (e.g. piloerection, ptosis in one or both eyes, hyperexcitability, hunched posture, hyperactivity or lethargy) similar to a stroke in a human. In rare case animals exhibiting severe behavioural abnormalities will be humanely killed. Possible adverse effects include: Death due to anaesthetic accident: <1%; risk of infection rare (less than 1%); body weight loss (continuation determined by the ability of animals to eat and drink); possible paralysis of hindlimb due to vessel cannulation the animals are closely monitored during this period with clear endpoints for humane killing to avoid animal distress. Though induction of ischaemia has a substantial severity limit, from experiences of previous licences, it is expected that only a minority (~20%) will reach this severity limit. The low number of animals that will reach substantial limit is because the experiments are not long enough end point (e.g. BBB studies), the animals have good recovery and maintain good well-being, or the animals are humanely killed before substantial level is reached. At the end of the procedures, animals will be terminated by Schedule 1 procedure or licenced kill.

## Application of the 3Rs

### Replacement

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

### Replacement

The brain has a complex cellular structure involving different cell types and different cellular compartments and brain damage and repair involves interplay between these cell types, components and compartments.

For example, stroke is a blood–flow related phenomenon that causes a complex cascade of events in compromised tissue and can not be fully replicated in non-animal studies.

Non-animal studies have their limitations for modelling such brain disorders and do not allow behavioural and cognitive assessments of outcome. In addition there is an additional step of translation from non-animal to animal to human. Animal models of stroke produce reproducible lesions under controlled environments with reliable means of quantifying brain damage and provide the best means for determining insight into key mediators of damage and repair. However, where ever possible, non–animal studies are used. For example we optimise cell systems using non-animal studies prior to animal use and also are able to gain information on cell function from non-animal studies and are integrated with the animal studies.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We aim to maximise signal to noise ratio in our animal experiments by controlling variability within groups, improving power of the experiments and reducing numbers used. This is done by performing pilot studies to improve study design and success, controlling conditions under which animals are kept, acquiring statistical input in all studies. In addition we have developed protocols that allow assessment of several outcome measures in the same animals.

In addition by using the refinements below, this helps us reduce the number of animals required. Finally we have developed in vitro systems in the lab that allow us to reduce the number of animals used and we were recently awarded 3Rs funding for developing an in vitro system to look at migration of inflammatory cells that better represents what happens in stroke.

### **Refinement**

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

#### **Refinement**

Rats and mice are the best choice for this programme of work as they have similar cerebral architecture to humans (circle of Willis) and are the least neuroscient animals possible that can be used for this work. In addition genetically altered mice exist that mimic particular disease states relevant to this programme of work. The model to be used involves inducing a lesion in the brain to allow us to investigate brain damage and repair.

This work has been designed so that any distress or discomfort that might be caused to the animal are kept to a minimum. All techniques outlined in this application have been refined as much as possible to achieve the least suffering of the animals yet to achieve meaningful satisfactory scientific results which will benefit the progress of stroke research. We are continuously monitoring, review and refining our protocols and continually update our protocols based on the current literature and guidelines (Ord et al, 2012; Trotman-Lucas et al, 2017; IMPROVE guidelines). I have worked closely with NC3Rs to produce guidelines to improve and refine stroke models (Percie du Sert et al, 2017) and am a co-organiser of a UK stroke symposium which brings stroke researchers in UK together to improve best practice. All animals are monitored closely post-operatively as required. Vet advice is sought as required and we have good knowledge of the indicators of pain and distress or disturbances of feeding and drinking and clear endpoints are defined for termination of the experiment.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 186. Role of innate immune cells in initiation of inflammatory disease</b>
<b>Key Words</b>	Macrophages, Inflammation, pattern recognition receptors
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The tissue resident innate cells called macrophages initiate inflammatory response. Surprisingly they use the same receptors, called pattern recognition receptors and the same downstream signalling pathways to detect and respond to pathogens and non-infectious activators (such as exogenous irritants or endogenous molecules released at the site of tissue injury). This is why most current therapies, which target such receptors or signalling pathways in patients with chronic inflammatory diseases, act as immunosuppressants and inhibit pathological inflammation but also inhibit antimicrobial immune defences leaving patients immunocompromised. Understanding how innate cells such as macrophages direct inflammatory response that is best tailored to its inducer will allow us to design new therapies against inflammatory diseases without compromising the patient's antimicrobial defences.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The key short-term benefit of this proposal is to generate new knowledge about how inflammatory responses are initiated. Therefore, in first instance, scientific community will have the most benefit of the work proposed in this application. Because we also propose to validate our key findings using mouse model of inflammatory bowel disease (IBD) and arthritis, the long-term benefit of this work is potential translation of the new knowledge into the therapy development. These disease models have been chosen as most relevant for our objectives because innate inflammatory pathways that our group studies (i.e. FAM26 proteins, NLRP3 inflammasome pathway, TLRs, CLRs, IFNs) have been linked to those diseases.

through human genome wide association studies and through previous published work. We have generated mice deficient in these key molecules, which will allow us to assess their contribution to the disease development and potential use as therapeutic targets in vivo. British society of gastroenterology estimates that "About 240,000 people in the UK have IBD, approximately 400 patients per 100,000 population. Between 50% and 70% of patients with Crohn's Disease will undergo surgery within 5 years of diagnosis. In Ulcerative Colitis lifetime surgery rates are about 20-30%". Because we will also confirm key findings in an air-pouch and peritoneal model of inflammation and in the model of antigen induced arthritis, another long-term benefit of this work is the translation of the key findings to other inflammatory diseases as well.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project license requires the use of wild type and genetically altered mice with defined immune system defects. Total number of mice required for breeding, crossing different strains, and procedures is 14,000 mice over a period of 5 years.

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

The maximum severity of the protocols in this project is 'moderate'. Some animals will be used for breeding and maintenance of genetically modified lines, and are not expected to experience clinical adverse effects. In vivo infection and immunisation experiments involve the use of infectious agents (*Listeria monocytogenes* and *Candida albicans*) or non-infectious activators such as model antigens, and analysis of immune responses in controlled conditions in localized cavities (such as air pouch and peritoneum) or in secondary lymphoid organs. Infectious are non-lethal but mice typically develop symptoms such as reduced grooming and motility, reduced food and water intake and weight loss. Experiments are terminated if mice reach humane end points defined by the protocol. In vivo colitis experiments involve the use of irritants (DSS) or intestinal pathogens (*Helicobacter hepaticus* or *Citrobacter rodentium*). In these mice will typically develop temporary weight loss, intestinal inflammation and diarrhoea that is either self-limiting and resolves spontaneously or experiments are terminated if mice reach humane end points defined by the protocol (e.g. rectal prolapse, bleeding or weight loss of 15 %) In vivo arthritis experiments involve induction of arthritis using model antigen and adjuvants. Mice will experience swelling of the joints, reduced movement and pain, which will be controlled by the provision of food pellets and water gel packs placed at floor level, and/or supplemental bedding and by the administration of adequate analgesia. Mice may also experience diarrhoea during irradiation and reconstitution of the bone marrow. These will be monitored and treated with antibiotics to prevent opportunistic infections. The mice will be humanely killed at the end of the experiment.

## Application of the 3Rs

### Replacement

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

#### Replacement

To identify inflammatory mediators we will wherever possible use in vitro experiments or ex vivo models to replace, or complement in vivo studies.

But to validate and understand how selected inflammatory mediators from in vitro work affect inflammatory disease progression, particularly in chronic inflammation where multiple organs and cell types are involved, where cell-cell communication and the 3D tissue architecture are important, studies require the use of whole organisms and has not been to date replicated in vitro. These studies also require the use of animal models of inflammation and the use of genetically altered animals, such as animals lacking key inflammatory sensors and mediators.

Mice are the lowest vertebrate groups on which well-established models of inflammatory diseases of interest have been developed. Furthermore, they show high homology to human immune system. Lower organisms do not have an adaptive immune system, and many do not have joints and therefore cannot be used for our studies. Finally, there are more available reagents (e.g. monoclonal antibodies, genetically modified strains) in mice than in other species required for this project. Mice also offer unique genetic modifications generated by others or us, which are essential for the work proposed.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Every effort will be made to reduce the number of mice.

When performing new assays, we will use pilot experiments to determine intra and inter group variations. From there, main statistical principles governing the design of an animal experiment will guide (1) defining an experimental unit; (2) controlling variation; (3) designing experimental layout and (4) calculating the sample size.

Immune cells isolated from secondary lymphoid organs or organs isolated from a genetically modified animal may be used as a source of immune cells for in vivo and in vitro experiments. Cells and tissues may be shared by multiple researchers or frozen for future analysis.

Where possible littermate controls will be used to further increase the sensitivity of our read-outs, untreated control groups may also be shared between treatment groups.

### **Randomisation and blinding.**

To minimize the bias in in vitro experiments we will randomize the sample position on the plate and will blind the genotype of the mice until the end of the experiment. To minimize cage effect and the effect of micro flora on in vivo experiments we will cohoused WT and KO mice and randomly assign mice to treatment conditions. Because males and females are cohoused separately we will also be able to evaluate the effect of sex on the phenotype. For histology scoring we will blind slides and have two independent people from the laboratory of our collaborator (Prof Powrie) score them. For complex experiments, we may use the *Experimental Design Assistant tool from NC3R*.

### **Refinement**

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

### **Refinement**

Mice are the lowest vertebrate groups on which well-established models of inflammatory diseases of interest have been developed. And there are more available reagents for mice (e.g. monoclonal antibodies, genetically modified strains needed for this project) than other species. Lower organisms do not have an adaptive immune system, and therefore cannot be used for our studies.

We have chosen models which closely mimic features of human disease in the treated animals, and that have already been refined in the literature to minimize the number of immunisations and the severity level of adjuvant. We are also collaborating with experts in the field to acquire expertise in the refined methods for animal models of arthritis, air pouch and inflammatory bowel disease.

We will make every effort to minimize the number of procedures per animal. We will pay careful attention to animal husbandry and provide environmental enrichment and co-housing to avoid social isolation. When possible, we will use genetically modified mice models that are conditional rather than complete knockouts to delete or express genes in specific cell- and tissue-type rather than in whole organism to minimize potential harm.

For all dosing, the smallest needle diameter which allows rapid injection of the substance will be used, the smallest possible volume of substance will be administered and aseptic technique will be used. When possible, we will use the

least painful injection method. For instance, we have switched from footpad to subcutaneous injection under the skin next to the hock, which is less painful for the mice as they do not have to walk on the injected, swollen area. Where required (e.g. in arthritis experiments), analgesia will be administered from the expected time of disease onset.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 187. Consequences and mechanisms of respiratory epithelial priming prior to and following respiratory syncytial virus infection.</b>
<b>Key Words</b>	Respiratory Viruses, Bronchiolitis, Asthma, Immunology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Respiratory virus infections can cause a severe lung disease in infants called bronchiolitis, which can predispose to asthma, and they can trigger severe asthma attacks. There is no prevention or effective disease-modifying treatment available for bronchiolitis or virus-induced asthma attacks. Excessive inflammation and high amounts of virus (viral load) are thought to underlie both of these conditions. The project aims to understand the immune responses in the lining of the lung and nose (respiratory mucosa) that control (or enhance) viral load and inflammation, both prior to and after a respiratory viral infection.

**What 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 better understanding the immune mechanisms of the respiratory mucosa that lead to or control viral and asthmatic inflammation or control viral load, and their longevity will be a major scientific advance which will enable the development of compounds that can prime the respiratory mucosa against viral and asthmatic inflammation. In the future such new compounds may become powerful treatments to prevent or reduce the severity of bronchiolitis and asthma attacks and may even prevent the development of asthma.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use up to 7,000 mice over 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 levels of severity? What will happen to the animals at the end?**

Most planned procedures will have no or only short lasting mild adverse effects. The wellbeing of mice will be closely monitored during experiments for clinical signs of distress which include reduced activity, reduced feeding, weight loss, hunched posture, ruffled fur and pallor. If any of these signs are severe or do not resolve within expected short time frames after procedures, the animal will be humanely killed. Many of the animals will be used in breeding programmes of GA (genetically altered) mice. Mice will be infected with RSV by applying droplets to the nose or the trachea under light anaesthesia. RSV infection usually does not result in disease, but in some cases can cause transient weight loss from which mice recover after 2-3 days. In the asthma model, mice will be sensitised to an allergen and receive allergen challenge to the airways by aerosol inhalation or application of droplets to the nose or the trachea under light anaesthesia. Allergen sensitisation and challenges usually do not cause disease. Changes in lung function (without clinical disease) can be detected after RSV infection and in the asthma model. Lung function will be assessed in a chamber, in which the mouse can freely move, using minimal changes in chamber air pressure caused by breathing. Agents provoking short lasting deterioration of lung function will be aerosolised into the chamber, resulting in short lasting discomfort, as experienced by patients during similar lung function measurements. In some mice lung function will also be measured invasively under deep surgical anaesthesia without recovery. At the end of each experiments the animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

The mouse models outlined above are an integral part of my research programme, which uses laboratory based experimentation and clinical studies in humans wherever possible. We will assess lung secretions from infants ventilated due to viral bronchiolitis for immune cells. In parallel, we will study effects of respiratory viral infections on human immune and epithelial cells generated *in-vitro*.

However, there is currently no laboratory based system available that allows us to study the complexity of immune interactions, within and between different organs, and the lung function changes in inflammatory lung disease induced by respiratory viruses and allergen sensitisation. We therefore have to use animal models of disease.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

All experiments are designed to use the minimum number of animals to give statistically significant results or to obtain sufficient numbers of immune cells for ex-vivo analysis. To that end all groups and controls of an experiment will be run in parallel, all organs of interest will be used simultaneously from each individual and animals will be identified individually. Where appropriate we will use statistical tests to calculate the number of animals we will require based on how variable we expect the results of our studies to be and how big a difference we are looking for between groups. Where such calculations are not possible, e.g. in experiments to generate primed immune cells or organs for histology, we will use our previous experience with similar experiment and published data to determine minimal numbers of mice required.

## **Refinement**

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

### **Refinement**

Although the disease processes are not completely identical to those in man, mice offer the best available model system in which a variety of relevant immunological, genetic and molecular tools are available to study viral and allergic inflammatory lung disease.

All work will be carried out by experienced trained researchers or under close supervision. Wellbeing of mice will be regularly monitored. Following any procedure mice will be monitored continuously until they have fully recovered. Any painful procedure will only be performed under appropriate anaesthesia. If required supportive treatment will be provided (e.g warming, oxygen application) and humane endpoint have been defined at which mice will be killed to avoid distress.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 188. Developing therapeutic drugs for cancer</b>
<b>Key Words</b>	Cancer, therapy, immune system
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to develop new and improved medicines for the treatment of 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)?**

Our project plan involves all aspects of cancer drug discovery and development including developing new cancer models (experimental systems that mimic aspects of the human disease), testing how effective potential new drugs are and determining the best way to combine new drugs with existing treatments. We have a track record of successfully developing new cancer therapies that go on to receive approval from regulatory agencies to be prescribed to cancer patients. Under this licence we expect to continue to advance new cancer therapies to the clinic.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use up to 41,500 mice and 700 rats over the course of 5 years. Typically, this may support assessment of up to 30 new drugs and targets for cancer therapy. These numbers were calculated based on our use over the last few years, and an expectation that we will continue to perform similar studies at a similar pace.

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

Animals are kept in high-quality facilities, free from pathogens and with access to food, water, and environmental enrichments. In all the facilities used, the animal care staff are highly trained in mouse welfare and will ensure the animal suffering is minimised. Animals are group housed except in exceptional circumstances such as when aggressive behaviour risks animal welfare or when cage mates have been removed for experimental reasons. In nearly all cases, mice will be between 6-12 weeks of age when they enter into studies, however we plan to use a small number

of older mice up to 18 months of age in order to understand the effects of age on tumours and on our experimental therapies. Most of the animals will be used for studies that measure the impact of experimental drugs on tumour growth rates. The vast majority of tumours will result from injection of tumour cells under the skin which then grow into a tumour at the site of injection. However, in some instances we will introduce tumours into specific organs through specialised injection routes. For example, direct injection into breast tissue mimics breast cancer, while injection into the blood stream leads to tumour growth in the lungs. Producing tumour growth in other internal organs requires surgery, such as to implant tumours into the pancreas or the intestines. This involves making a small surgical incision into the abdomen plus injection of tumour cells directly into the organ followed by closure of the wound. Tumours that grow just under the skin are easy to observe and to measure their size using callipers. Tumours that grow internally are more difficult to observe therefore other methods are employed such as the use of non-invasive imaging techniques and/or clinical scoring systems to carefully monitor the well-being of each animal. In some cases, it is not necessary to use mice bearing tumours. For example, tumours are often not needed to determine whether a drug is tolerated by the mice or to measure how much of a drug enters the bloodstream. In addition, we sometimes can use animals that do not carry tumours when we wish to understand the impact of our drugs on specific aspects of the immune system. In these instances we are able to use experimental systems that allow us to ask very specific questions in the absence of a tumour. In all of these studies, the likeliest sources of adverse effects are from the size and condition of the tumour, from surgical procedures, and from the drug treatment. All animals bearing tumours will be classified as experiencing moderate severity unless their tumours remain below 500mm<sup>3</sup> and are also in good condition. We will humanely euthanize any animals that have developed advanced cancer to minimise unnecessary suffering. Animals undergoing surgical procedures will be also classified as moderate and pain relief or anaesthetics will be provided when necessary. On some studies, we will apply advanced non-invasive imaging techniques that allow us to track growth of tumour cells or to follow distribution of a drug throughout the body . To do so animals will be anaesthetised for the duration of the imaging session. Blood samples may also be collected during some studies to measure levels of drug substance or other indicators of drug effect over time. Blood samples are usually of a small volume and are taken from a vein in the tail or at the end of the study if larger volumes are needed. Drug substances are most commonly injected into the peritoneal cavity, intravenously, or directly into the tumour. Occasionally drugs may be administered orally. Treatment of animals with cancer therapies may also lead to unwanted effects similar to those experienced by patients. Most of these effects will be of short duration and mild but some animals may experience moderate effects. We expect that approximately 30% of animals will be classified as moderate, and the remaining 70% will be mild or lower severity. When animals are used that do not carry tumours, adverse effects similar to those

mentioned above may result from treatment with cancer therapies. At the end of procedures, all animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

#### Replacement

Although we do many experiments using cells, molecular biology, and computer modelling, it is still necessary to use some animals for research so that we can more accurately assess the interaction of cancer cells with other cells and organs within the body. Isolated cells and organs do not reproduce the complex nature of in vivo biology. Animal models also allow us to understand cancer in the organ of origin or as it spreads throughout the body; this is important as when cancer spreads it is often fatal for the patient. An important aspect of our work is to understand how the immune system can be harnessed to attack tumours, and it is not possible to fully recreate these complex interactions outside of a living animal. In addition, regulatory agencies often require animal studies prior to approval for clinical trials.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We acknowledge the importance of reducing the number of animals used and use statistical methods to ensure the correct number of mice are used to achieve our scientific objectives, which reduces the need to need for studies to be repeated in the future. Other measures such as random assignment of animals to treatment groups also increase the robustness of studies. We also always perform small pilot studies for new molecules or models to refine our systems before embarking on more complex experiments. We have implemented an innovative study design for tolerability studies to reduce animal numbers. REDACTEDThis characterisation has been important for reducing animal use since it ensures we can select the most appropriate model for each experimental question, thereby reducing the overall number of models used.

### Refinement

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

## Refinement

Rodents are the lowest species of mammal that allow us to adequately model the complexity of human cancer and immune system biology. The biology of rats and mice is well understood which enables rapid advancement of novel areas of research compared with other species. Many research tools also exist for rodents which enable experiments to be done in these animals that cannot be performed in other species such as zebrafish.

The most common tumour model that we use involves injection of tumour cells under the skin resulting in tumour growth at the site of injection. This is the simplest rodent tumour model available and the easiest to monitor therefore carries the least welfare risks. This model is preferred except in cases where we need to understand more complex questions such as the spread of cancer from one site to another, the influence that specific cell types and organs have on tumour growth, and the responses of tumours to our therapies in these varied settings.

We are committed to refining our procedures to minimize harm to the animals and have a track record of doing so. We ensure small-scale pilot or tolerability studies are carried out for new models or therapies. We carefully monitor tumour burden including the use of whole body imaging techniques when possible, and we also use tumour-free mice in some cases when tumours are not essential. We have implemented innovative study designs to reduce animal numbers and enhanced health checks to minimize suffering. When unexpected severe events have occurred, we have investigated through post-mortem examinations.

We will continue to refine our work to minimise harm to the animals. We plan to investigate alternative methods for measuring tumours and for collecting tumour tissue which should reduce variability and hopefully reduce the numbers of mice used per study.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 189. Generation, Production, Rederivation and Cryopreservation of genetically altered animals</b>
<b>Key Words</b>	Transgenesis, Genetic, Rederivation, Cryopreservation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aims of this project are:

- To produce new lines of mice and rats with changes in particular genes. These animals will then be bred and used in important scientific projects at our institution, at other institutions throughout the UK and elsewhere.
- Embryo or sperm freezing of existing lines of valuable animals, so that these lines can be preserved.

Rederivation of valuable lines of animals to get rid of infectious 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)?**

Use of genetically altered animal models is essential to understand key genes and processes in human health and disease. We provide a service for creating these models. Our group provide all areas of the research community with models, whilst reducing and refining animal use through a central database of available lines. Our expertise ensures that new lines are produced efficiently with minimal wastage. Freezing of lines no longer in use avoids unnecessary breeding and wastage of animals and provides a backup, safeguarding against disasters such as disease outbreaks. Removing infectious diseases from animals ensures that health and welfare of animals is maintained, and ensures that high quality animals are used in research, improving the quality of science. Microbiologically defined animals reduce the number of animals required for each study, while the absence of disease is a refinement. Animals free from infectious diseases are healthier. Other benefits

include improvements in techniques for the creation of these models, which will be communicated to the wider community.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

For the length of this project licence ( 5 years) we anticipate using 63,250 adult mice, 3,000 adult rats, 220,000 mouse embryos and 30,000 rat embryos. Mice and rats 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 levels of severity? What will happen to the animals at the end?**

Some animals will have a single injection with no ill effects expected, and then be humanely killed (protocol 1 - severity limit: mild). Some animals will undergo surgical procedures (vasectomy, embryo transfer), these will be done using best practice techniques under anaesthesia and animals will be given pain killers as required (protocol 3,4 - severity limits: moderate). Animals are expected to recover fully from the procedures within a few days. Many animals will simply undergo natural mating. Some animals may develop adverse effects as a result of the changes in their genes (protocol 5 - severity limit: mild ; protocol 6 - severity limit moderate) – all new lines will be closely monitored for any such effects and animals will be humanely killed if these adverse effects are significant (if the level of severity reach moderate/severe for a protocol with a mild severity limit, or a level of severity reaching severe for a protocol with a moderate severity limit).

## Application of the 3Rs

### Replacement

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

### Replacement

Many research projects use in-vitro methods alongside animals to complement the work. However animal work is essential because many of the effects of genetic changes and biological processes under investigation involve complex physiological pathways that cannot be reproduced in vitro. Mice/Rats will be used because many transgenic and knockout mutant mouse strains have been created as disease models.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Unnecessary production of new models will be avoided by searching databases before new lines are created.

The number of animals needed varies for each strain being used. Predicted animal numbers are based on our past experience. We have considerable expertise in these procedures and have optimised the number of animals needed. Our success rates are regularly reviewed.

Use of new technologies reduces the number of animals needed to produce a new strain.

Breeding of animals will be controlled to minimise the risk of over breeding. Animals will only be bred if a user requirement has been established, and the breeding programme will be reviewed to optimally meet anticipated demand. Spare animals will be made available for use on other scientific projects or for in vitro studies.

Freezing sperm and embryos reduces wastage.

### **Refinement**

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

### **Refinement**

The methods used in this project are standard and have been optimised for this species. Rodents are the least sentient species of mammal in which the technology needed to generate new models works reproducibly. Published guidelines for best practice will be followed and new lines will be monitored closely for any ill effects.

Best practise in the procedures ,husbandry and refinement in consultation with the veterinary surgeon and animal care staff will be followed wherever possible. Specific areas where refinement may be considered include alternative samples to invasive tissue biopsy for genotyping, phenotypic assessment and special husbandry requirements.

We will find out in advance if possible the likely adverse effects and optimum breeding strategy for each line before it is acquired or created, and will ensure that all animals are maintained at a high health status.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 190. Mechanisms of brain damage following stroke and neurovascular coupling in health &amp; disease</b>
<b>Key Words</b>	stroke, cerebral blood flow, neurovascular coupling, Magnetic resonance imaging
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project will use rodents to investigate brain damage caused by stroke and to test new therapies aimed at limiting the extent of brain damage and improving outcome. Stroke is the third leading cause of death in the UK and a leading cause of disability. Current treatment options are limited to a drug that acts to break down the blood clot (thrombolysis) or a newer treatment that involves a catheter that can be inserted into the blood vessel to mechanically remove the clot (thrombectomy). Both of these treatments have a very narrow time window in which they can be given (less than 4.5 or 6 hours after stroke onset) therefore, there is a need for new drugs that can be given to decrease brain damage and/or prolong the time window for current treatments.

Specific protocols are included in the licence that will allow us to understand the mechanisms of damage to the brain and brain blood vessels following stroke in order to identify new targets for development of treatments. It is important to understand how the brain responds after stroke in rodent models that have pre-existing risk factors (i.e high blood pressure, high blood sugar) since these risk factors are often present in stroke patients. In addition, we will investigate new treatments in normal rodents and rodent models of stroke where we will measure the amount of brain damage caused and if the treatment can reduce this damage and importantly can it reduce disability through specific behavioural tests.

Brain blood vessels are important in supplying blood supply to the brain necessary for delivering energy to brain cells. Brain cells and blood vessels are capable of communicating with each other in order to maintain a constant supply of blood flow dependent on the energy use of the brain. In certain circumstances this communication can break down resulting in inadequate supply to regions of the brain

and there is evidence that this may be a factor in conditions such as vascular dementia and alzheimers disease. Specific protocols are included in the licence to understand the mechanisms underlying this communication between the brain cells and blood vessels and the effect of risk factors such as high blood pressure and blood 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)?**

The benefits that will arise from this project licence are related to increasing our understanding of the mechanisms of brain damage following stroke and the effect of risk factors on stroke outcome. This will allow new targets to be identified that may reduce the burden caused by stroke in terms of death and importantly disability. Increasing our understanding of the mechanisms of communication between brain cells and blood vessels will also allow us to identify new targets that can increase blood flow following stroke and may also be of importance for other conditions such as vascular dementia.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

It is estimated that 2350 rats and 1100 mice will be used over the duration of the licence (5 years).

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

Surgical procedures to induce stroke in rodents will involve exposure of the carotid blood vessels in the neck or drilling a small window in the skull to expose the blood vessel on the brain surface. Stroke protocols will either be non-recovery from anaesthesia or if recovery then classed as a severe procedure. The main adverse effects are mortality either during surgery or in the first 1-3 days following stroke however this is minimised with the use of experienced surgeons, reducing the duration of stroke as well as appropriate post-operative care (i.e pain killers, fluids, soft food). Imaging techniques for measuring blood flow in the brain will require exposure of the skull surface and in some instances a cranial window drilled to expose the surface of the brain. For rodents that are allowed to recover from anaesthesia some pain at the surgical site will be experienced with local pain killers administered. Rodents throughout the different protocols will have general anaesthesia carried out, injection of drugs, MR imaging and behavioural testing. Post-operative care will be given to minimise suffering. Rodents will be allowed to recover from anaesthesia (approx. 50% of animals) in specific protocols in order to investigate longer term recovery from stroke (i.e the effect of drug on brain damage and behaviour) and for imaging at multiple different time points. For non-recovery anaesthesia (approx. 50% of animals) we will investigate acute changes in the brain

with imaging techniques. At the end of the protocols animals will be killed for removal of brain tissue and other tissues.

## Application of the 3Rs

### Replacement

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

#### Replacement

In order to understand the complex interaction between brain cells and blood vessels both under normal circumstances and following stroke it is essential to use the whole animal. In addition, the therapeutic potential of treatments aimed at limiting brain damage and behaviour necessitate the use of animals. Where possible we will use cell based experiments as an alternative to using animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The use of non-invasive imaging (MRI) in the current licence allows for repeated scanning to be carried out in the same animal over time and for multiple different physiological and biochemical processes to be imaged. This significantly reduces the number of animals needed as well as increasing the power of our experiments by using the same over time.

### Refinement

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

#### Refinement

Rodents are used based on the similar blood vessel anatomy to humans as well as the mechanisms of damage following stroke. In addition, the availability of genetic models as well as incorporation of risk factors such as high blood pressure and glucose increase the validity of these models for human disease thereby increasing the chances of translation of treatments to the clinic . We have been part of a working group with the National Centre for the 3 R's (NC3R's) to improve the welfare of animals following stroke (IMPROVE guidelines) and we will continue to adhere to these guidelines as a minimum standard.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 191. Development of framework to assess pain &amp; welfare of pigs with clinical or subclinical lameness</b>
<b>Key Words</b>	Pig, welfare, lameness, behaviour
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

**Aim:** to improve pig health and welfare, and so address global concerns about pain and suffering in pigs. This will be achieved through two objectives to ultimately develop a comprehensive assessment framework to assess lameness, pain and welfare in pigs.

Objective 1 will be carried out under licence at university premises, whilst Objective 2 will be carried out at commercial premises in Norway, using existing animals without the need for a project licence. However, for completeness, Objective 2 is briefly described since it is key to achieving the experimental aim.

**Objectives:**

1: Framework validation – to develop and validate a Welfare Assessment Framework (WAF). Using experimentally-induced painful lameness in pigs, we will establish how joint pain causes alterations in a number of measures of pain/welfare. Under general anaesthetic, a substance will be injected into a joint which will bring about changes in the tissues and result in pain and lameness (similar to that induced by naturally occurring lameness) which we will monitor using a range of measures.

2: Application of framework - we will evaluate a working version of the WAF on pigs in a situation of known osteochondrosis (OC) prevalence. OC is a major cause of lameness in growing pigs. We will use existing X-ray images taken for evaluation of body composition as part of normal husbandry on this test farm. We will use these data from selected pigs to examine the relationships between OC severity and aspects of lameness.

**What 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 three potential benefits: 1) knowledge: a greater understanding of the pain and welfare impairment associated with lameness in commercially-managed pigs; 2) technology: development of an automated system to detect lameness in pigs; 3) dissemination: knock-on benefits of this knowledge and technology for various stakeholders as follows: Researchers will benefit from a greater understanding of the effect of lameness on animal welfare through access to the results which we will publish in academic journals and at conferences. Pig farmers will benefit from access to knowledge and technology leading to reduced lameness on farm and hence lower costs of production. Farmers will also benefit from greater job satisfaction through having better tools to monitor lameness and consequently improved welfare of their animals. Society will ultimately benefit from improved pig health and welfare, leading to more sustainable food production and greater food security.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Pigs – 120 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 levels of severity? What will happen to the animals at the end?**

With the exception of inducing lameness, very few adverse effects are expected. Complications from anaesthesia are rare, whilst the use of good standards of hygiene means that adverse reaction to injection of substances into the joints or blood sampling are uncommon. Induction of lameness will cause a mild to moderate level of pain in affected animals, but this will be minimised by use of humane housing (e.g. greater space for each pig to move around the pen) and husbandry (e.g. soft matting or extra bedding on the floor of the pen). At the end of an 8 week period, animals will either be euthanised or, if appropriate, returned to the commercial herd on university premises.

## Application of the 3Rs

### Replacement

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

### Replacement

The behavioural and physical responses that we plan to measure in pigs cannot be reproduced in non-animal models. The project will potentially benefit pig welfare as

well as exploring the novel use of a model of joint disease that is also relevant for human medicine. Whilst rodents are commonly used to mimic certain lameness conditions in humans, pigs have a number of advantages over rodents as they have greater anatomical and physiological similarity to humans.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We have used specialist computer software to statistically estimate the minimum number of animals required to generate scientifically valid results.

### **Refinement**

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

#### **Refinement**

The use of pigs is necessary to confirm that our methodology can reliably be used to assess pig welfare on a commercial farm. Pigs at university premises will receive greater levels of care than is typical of commercial farms, thus giving them the best quality of life possible. Preventive measures to minimise any negative impact on animal welfare include provision of soft bedding or matting in home pens to ease movement, especially when animals are changing from one lying position to another to make themselves comfortable. Pain relief will be provided at the point of injecting substances into the joint, and will also be available to any pig showing adverse effects thereafter. All animals will be closely checked on a daily basis, and defined symptoms which would trigger immediate action (e.g. moving the animal to a hospital pen and/or provision of pain relief) or euthanasia agreed in advance.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 192. Diagnosis and therapy of toxin-mediated diseases and healthcare-associated infections</b>
<b>Key Words</b>	C. difficile, E. coli, Vaccines, Therapeutics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overarching aim of the work is to develop new treatments to combat healthcare acquired infections caused by *Clostridium difficile* and Gram negatives, such as *E. coli*, to reduce the rising morbidity and mortality associated with these infections.

*Clostridium difficile* infection (CDI) is a major problem as a healthcare-associated infection with >10,000 cases reported in UK per annum. This bacterium mainly infects elderly patients on broad spectrum antibiotics causing a range of symptoms from mild diarrhoea to severe, recurrent diarrhoea which is often life-threatening. Severe and recurrent forms of CDI are not particularly well served by current treatment options and there is a need for alternative therapies to be developed. A significant proportion of the work to be undertaken in the current work programme is concerned with the late stage development of therapies targeted at the treatment of severe and recurrent CDI.

Gram negative bacterial species represent a significant burden to the NHS as a healthcare-associated infection and their rapidly growing resistance to frontline antibiotics is severely reducing the options available for effective treatment. Extraintestinal pathogenic *Escherichia coli* are a leading cause of bacteraemia in the UK with >40,000 cases reported in 2016. With few new antibiotics in development, there is now an urgent need to investigate alternative therapeutic options for the treatment of Gram negative infections. Current work is focussed on assessing various bacterial protein factors (e.g. toxins, bacterial surface components) as potential targets for intervention strategies, such as vaccines.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Research and development into antibody-based therapeutics for CDI is focussed on providing affordable and effective alternatives to antibiotics for treating severe and recurrent cases which are currently poorly served by available therapies. The proposed work programme will gain the necessary information to obtain a product license in order to take these products to early phase clinical trials. For Gram negative healthcare-associated infections, new therapeutic strategies are urgently required to deal with both the causes and symptoms of such infections. The proposed research programme is focussed on early stage research which has the goal of identifying key protein targets which could potentially underpin either vaccine or immunotherapy-based intervention strategies

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Hamsters: 2000 (400/year) Mice: 1700 (340/year)

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

Animals will be manually handled, with or without anaesthesia, in order to inject or give them vaccines or therapies against infectious diseases. This process may cause short term distress to animals. Procedures which are designed to raise antibodies in animals, cause only short-term stress and are categorised as mild. Animals infected with *C. difficile* may develop diarrhoea and a tender abdomen. Animals infected with Gram negative bacteria, such as *E. coli*, may show ruffled fur and signs of weakness. For both cases, animals which become immobile or lose excessive weight will be humanely euthanised. For *C. difficile* and *E. coli* infections, regular health monitoring (every four hours) will detect these signs, but in some cases, animals may die before such signs are evident. For this reason, protocols which induce these infections are categorised as severe.

## Application of the 3Rs

### Replacement

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

### **Replacement**

For both infectious disease areas under investigation by the current Project Licence, the pathogenesis of the infection is extremely complex and cannot currently be modelled using *in vitro* systems. Regrettably, therefore the protective efficacy of any intervention can only be validated using the appropriate animal model.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

For vaccine development studies, maximum use will be made of *in vitro* assays in order to characterise antigen immune responses and to allow any candidates that are unlikely to offer protection *in vivo* to be rejected before this stage. For all animal experiments, group sizes will be kept to a minimum using statistical analysis such that the minimum number of animal is used to provide useful data.

### **Refinement**

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

### **Refinement**

The hamster model is considered one of the gold standard models for assessing *C. difficile* infection and potential therapeutics and the mouse sepsis model is widely used in the study of bacterial infection and sepsis. For both models, rigorous scoring systems and humane endpoints have been established. These combined with frequent, round-the-clock monitoring will limit any suffering to the animals and allow early intervention of euthanasia before death occurs as part of the disease process.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 193.      Supplementation and monitoring of minerals in ruminants</b>
<b>Key Words</b>	supplementation, mineral, grazing, ruminant, trace elements
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The project aims to develop new supplements and optimise strategies to fully utilise current supplements to improve the mineral status of grazing sheep, cattle and goats. Grazing ruminants are completely dependent on the mineral composition of the grass/plant in the pasture. In most grazing situations animals are not fed like housed animals but have to forage their own diet from the pasture. If the grazing does not supply optimum minerals for optimal health and/or production then supplements are likely to be required. Indirect supplements include free access powders, blocks and licks all of which are subject to variable intakes and rely on nutritional wisdom (which tends to only occur at deficiency levels resulting in abnormal appetites called pica). Direct to animal supplements include injections (not many available), drenches (orally delivered like a spoon full of medicine) and boluses (large tablet like, which are administered orally and will remain within the rumen (large fermentation stomach) and slowly dissolve, releasing minerals over a long time period (months rather than weeks or days)). Drenches have previously been shown to have a variable response, this depends on which element is being supplemented. Cobalt drenches have been found to be effective for a very short time, if at all, and hence boluses can be better for this mineral, whereas selenium via drench can give a longer response and supply the animal's selenium requirements.

Ruminants as grazing animals are reliant on the pasture they consume, deeper rooting grasses such as festuloliums as well as being more drought resistant and flood tolerant potentially have a better mineral profile. Trees are also a good source of fodder in drought situations and the growing of trees may alter grass underneath through soil structure and shade improving mineral composition of this grazing area. High magnesium grasses are being bred and these will need to be evaluated in the

animal to show that a high magnesium grass can prevent low magnesium status in the animal. Ruminants can selectively graze so to fully evaluate these novel grasses and tree fodder approaches grazing trials need to show animal mineral status 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)?**

The benefits from the projects in addition to the advancement of science via published papers and presentations to conferences will be to the animals themselves (sheep, cattle, goats) and the farmers who keep them as well as the wider agricultural and veterinary industries. The benefits are better characterisation of how current supplements work, an improvement in how these can be used as well as the development of new supplements and increased resilience to weather extremes utilising different grasses and trees.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Supplement and the grazing (grass, tree fodder) trials will monitor the treatments (pasture/supplement) over a time period likely to be between 1 and 6 months and will likely have 3-6 (max 12) sample occasions per animal. Power calculations for monitoring of status via blood indicate that 8-10 animals per treatment group would be required. We would expect that up to 2260 sheep and goats and up to 620 cattle to be used over the 5 years of this project, both adult and ruminating young-stock (weaned calves/lambs).

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

The overall impact on the animal is very low. The severity of the proposed work is mild, mainly blood, rumen fluid and faecal samples to monitor mineral status and supplement dissolution and availability. To check supplements and avoid false negative results then grazing should be of low mineral status and there is some potential of natural mineral imbalances but not clinical deficiency being allowed to remain over the course of an experiment either from ineffective supplements/supplement strategies or within control animals. Small bleeds or haematomas/bruising are the most likely blood sampling adverse effects (5%) but this will be controlled utilising good techniques with vacuum removed before needle withdrawal, pressure applied to the sampling site and any blood cleaned off to prevent fly strike. Animals will be returned to commercial farm stock or sent to commercial slaughter where rumen dwelling supplements can be recovered and weighed and tissue samples taken to add additional status measurements. These studies will be carried out on farms to utilise natural grazing and agroforestry as it is not possible to make an adequate pasture deficient over a short timescale and therefore using natural situations are best. Supplements are best evaluated in

marginal situations and in adequate situations, often false negative results can be obtained and a working supplement can be perceived to be not working as animals do not require any supplementation of that element.

## Application of the 3Rs

### Replacement

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

#### Replacement

This work is using the animal to determine the response of the animal / that species, i.e. sheep work will directly benefit sheep. Additionally, there is some cross species benefit but species differences will mean these should not be taken for granted. Some early supplement development will use in vitro (lab) based evaluation of dissolution rates but due to the complexity of rumen function this will need to be checked in real ruminants (sheep, cattle, goats). Ex vivo analysis of tree fodder, grass and grazing will be carried out, but the animal is required as ruminants can be selective when grazing.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

REDACTEDData will be analysed using ANOVA, mean difference analysis or by using threshold analysis (ie the number of animals achieving 'target' status compared to those who don't). Mean differences are not always appropriate when animals achieve 'adequate' status.

### Refinement

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

#### Refinement

The animals used, are the animals for which responses are required, ie sheep for sheep, and as such are the most appropriate species that could be used. When using animals on farm (under POLE), we try to match to routine farm handling times so that we are not adding additional handling episodes to that animal. On farm specialised handling equipment will be used to ensure safety and enhanced welfare handling. Animals are checked post blood sampling for any adverse effects and if there is any post sampling mortality animals will be post-mortem examined to

determine cause of death. This information will be relayed back to the farm to aid future management.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 194. Avian Ecotoxicology studies</b>
<b>Key Words</b>	Avian, environmental, risk assessment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Governments require and the public expects that substances which may enter the natural environment are safe or their hazards well understood. This necessarily involves the conduct of whole-animal toxicity tests such as those included in this project, which form part of a framework of studies designed to investigate potential effects on all components of the ecosystem. Birds represent a major group of terrestrial vertebrates which may be at risk from exposure to environmental pollutants, and so that risk must be adequately characterised and evaluated in order to protect wild bird populations from unacceptable adverse 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)?**

The principal benefit of the project is the provision of safety data to facilitate risk assessment and sound regulatory decisions on the development/approval of new substances to which wild bird populations will potentially be exposed. By this process, the safety profiles of products under development can be optimised, and unsafe products identified and eliminated, thus contributing to the protection of the environment.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The test species to be used are generally determined by international regulatory requirements and are selected as being representative of wild populations; quail are most often used to assess potential toxicity to galliforms, and mallard ducks to represent waterfowl. For certain study types and/or to evaluate specific types of test substance/formulation it is necessary to use marker species for other groups, e.g. zebra finch (for passerines) or pigeons (for columbiforms). The approximate estimated maximum numbers of birds that will be used over the five-year period of the licence are: quail & other galliforms 17,400; duck & other waterfowl 7,000; pigeon 300; passerines (perching birds) 1100.

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

Since ingestion is the most significant route of exposure to environmental pollutants in wild birds, most birds in this project are dosed with the substance under evaluation by direct oral administration (liquid or capsule administration) or by dietary incorporation. Inhalation exposure can also be important (e.g. crop spray drift) and in a small proportion of studies birds may be exposed in whole body inhalation chambers or, in the case of microbial agents (microbes used in crop protection strategies), treated by direct administration into the upper respiratory tract. A small proportion of studies may also require blood samples to be taken or that birds are confined in small cages for short periods to allow the collection of excreta. No significant adverse effects as a result of any of the applied dosing or sampling techniques are expected (other than transient mild discomfort, e.g. as a result of needle puncture for blood sampling). Most birds used in this project will experience no, or only mild, adverse effects attributable to exposure to test substances. However, some birds may experience moderate effects (for example inappetence and weight loss), and in the case of acute/subacute studies, severe signs of toxicity and/or mortality are to be expected in a significant proportion of birds. Clearly defined humane end-points based on clinical signs are applied and birds humanely killed to prevent unnecessary suffering. Birds captured in field studies for blood sampling are routinely released back to the wild on completion of sampling subject to satisfactory examination that demonstrates it is appropriate to do so. In a few cases birds used in laboratory studies which have not experienced significant adverse effects may be kept alive and may be re-used in further procedures where appropriate (and legally permitted). However, the majority of birds used in this project will be humanely killed at the end of the study for post mortem investigations and tissue collection purposes.

## **Application of the 3Rs**

### **Replacement**

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

### **Replacement**

In order to evaluate adequately the effects on wild bird populations of exposure to a given substance in the environment, it is necessary to conduct whole-animal safety/toxicity tests; currently, no scientifically, ethically or legally acceptable non-animal alternatives that would achieve this objective are available. This is because cells in test tubes cannot replicate the complex interactions that take place within a whole living animal and as we cannot model substance exposure in the laboratory and we need to use animals to provide these complex data.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

All available information is reviewed to ensure each specific test is justified and is optimally designed using appropriate treatment levels. Numbers of birds used in each study are generally linked directly to those in published regulatory guidelines; where non-standard test protocols are required, statistical design principles and relevant experience from previous studies will be used to limit group sizes to the minimum commensurate with meeting study objectives.

In addition, the tiered/sequential approach generally adopted for development/safety evaluation programmes (see also below) enables the results of initial testing (usually single dose/acute exposure tests) to be used to minimise the subsequent tests in the programme, either through provision of a basis for appropriate dose selection, or in some cases to determine whether a subsequent study is undertaken at all (either through regulatory/risk assessment triggers or because it may demonstrate unacceptable toxicity and halt the testing programme).

## **Refinement**

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

### **Refinement**

To prevent unnecessary pain/suffering and to refine studies/data requirements in any given development programme, a tiered approach to testing is adopted; this enables the results of initial testing (usually single dose/acute exposure tests) to be used to refine the subsequent tests in the programme by enabling appropriate dose selection, or through identification of specific effects on which subsequent tests can be focussed.

Birds in all studies are observed regularly to monitor condition, behaviour and clinical health. Bodyweight and food consumption, both useful indicators of wellbeing and health, are also monitored. Appropriate humane end-points are applied to minimise adverse effects while achieving study objectives; severe effects are not allowed to persist, and the affected birds are promptly humanely killed where necessary to prevent further suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 195. Neuroplasticity and Cognition in Health and Disease</b>
<b>Key Words</b>	Learning, emotion, synaptic plasticity, limbic system, animal models
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Plasticity describes the changes in the nervous system that result from experience. For example, this could involve changes in the strength of the synaptic connections (chemical junctions) between nerve cells in the brain. Plasticity is thought to play an important role in learning and memory, and deficits in plasticity (too much or too little) are thought to lie at the heart of various neuropsychiatric and neurodegenerative disorders (e.g. schizophrenia, depression, Alzheimer's Disease). The aim of this project is to understand how different kinds of plasticity, in different neural circuits within the brain, support different kinds of learning and memory, and choice behaviour. In particular, we want to understand how different neurochemicals (including primary neurotransmitters such as glutamate, and neuromodulators such as dopamine and serotonin) and their receptors support different aspects of cognition and why this goes wrong 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)?**

Given the fundamental role that plasticity is likely to play in learning and memory, and adaptive behaviour more generally, then not surprisingly it has been a major target for trying to develop new treatments for numerous brain disorders. However, there has been limited success so far. This likely reflects our failure to understand fully the role of different forms of plasticity in cognition. The outcome of this project will hopefully be a better understanding of the role of different kinds of plasticity in different kinds of learning and adaptive behaviour. It is essential to better understand the functional significance of plasticity in different parts of the brain if we are to understand and treat these different diseases. Ultimately this could lead to the

development of new drugs or treatment strategies, or to a better utilisation of existing treatment options.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rat (<5900 in experiments; 1,000 breeding) Mouse (<8700 in experiments; 14,000 breeding) during 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 levels of severity? What will happen to the animals at the end?**

The experiments will involve testing the ability of rats and mice to perform behavioural tasks in which they have to learn and remember crucial information such as where a food reward is or how to escape from a pool of water. We also assess anxiety, for example, by asking the animal whether it wants to explore a new place or remain in a safe location, or using mild footshock. We will examine the effects of brain lesions, drug treatments, genetic mutations, optogenetic/pharmacogenetic manipulations and sleep deprivation on these behaviours, and record signals of brain activity while the animals perform. The animals will readily learn what to do to get a tasty food reward or how to climb out of the water. Recording of brain signals involves cranial implantation of microelectrodes and could involve single housing of the animals. The expected adverse effects would include brief periods of mild distress during some of the behavioural tests (e.g. after a mild footshock). There may also be transient pain and discomfort after brain surgeries. Our extensive experience of these kinds of experiments is that they are all of moderate severity. Animals will be humanely killed at the end of the experiments.

## Application of the 3Rs

### Replacement

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

### Replacement

In order to show that a particular bit of the brain, or a particular chemical neurotransmitter or receptor, is important for the brain to work properly, it is necessary to remove or silence that bit of brain, or remove or block the neurotransmitter from working. This is not ethical (or practical) in humans. Computer simulations of the brain actually rely on the information that we will provide and so cannot replace the work that we do.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

We will minimize the numbers of animals used by making both the behavioural tests and the experimental manipulations (e.g. lesions, genetic modifications) as accurate and sensitive as possible

## **Refinement**

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

## **Refinement**

We work on rats and mice because they are the lowest vertebrate group which reasonably resembles humans.

Operations on the brain are done very carefully and in state-of-the-art surgical theatres, and the animals are given pain killers after the operations until they have fully recovered. Soon after the operations you would not be able to tell the difference between treated animals and controls in terms of the way they behave in their home cages. It is only with the sophisticated tests of learning and memory that you can begin to tell them apart.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 196. Rodent Regulatory Genotoxicity</b>
<b>Key Words</b>	
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

<b>Yes</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this rodent regulatory genotoxicity project is to evaluate the potential of pharmaceutical (human or veterinary/animal health) or non-pharmaceutical (agrochemicals, food additives and industrial chemicals) compounds to cause genetic damage in rodents, principally the rat and mouse. Genotoxicity is a term used to describe the propensity of a compound to damage the genetic information within the cell causing mutations which may lead to cancer in the future. Thus, genotoxicity studies are part of an overall work package as part of the safety evaluation process.

Initially if insufficient data is available for the project a preliminary test is performed to determine a top dose level and additional suitable dose levels for the main test. The highest dose of a test substance that can be administered either on one occasion or repeatedly to an animal, but which does not cause a degree of pain, suffering or lasting harm ('limiting' signs or effects) that would prevent the completion of the treatment period, thereby compromising the scientific objective of the study, is usually referred to as the 'maximum tolerated dose' (MTD).

Studies are undertaken with the intention of producing data that will be used to gain regulatory approval to initiate or support ongoing clinical trial programs and/or obtain a product licence to market the substance.

The project can also generate biological samples to enable or support identified programmes of work and allow for the development of new or refined methodologies/technologies prior to integration onto tests for determining genetic damage.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The data produced in this project allows the support of ongoing clinical trial programs and aids in the ability obtain a product licence to market the substance. The

development of new medicines, veterinary/animal health products, food additives, agrochemicals and industrial chemicals is necessary for the continued success of efforts to combat disease, maintain food supplies and achieve improvements in the quality of life. It is a fundamental expectation that such substances should not pose an unacceptable risk to the health and well-being of the human population or target animal populations, or to the environment. This project contributes directly to that expectation, and facilitates the development of products that will have minimal adverse impact. The principal benefit is that the work performed will facilitate sound regulatory decisions for the purposes of gaining authorisation for clinical trials or product registration, which otherwise could not be done with as high a degree of assurance. These studies also contribute to minimising the number of animals used, as an adverse response identified in these studies may halt the development of the test substance.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats – 3250 Mice - 2250

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

Animals will be exposed to test substances (as detailed earlier in the NTS) via routes including inhalation, application to the skin, orally, in food or water and injection either into the skin, muscle or bloodstream. This may require anaesthesia on a number of occasions. Administration/infusions of substances can be performed using delivery devices such as catheters in blood vessels. Animals may be blood sampled to confirm exposure, or restrained to enable exposure. Animals will be humanely killed at the end of the study and tissues harvested for examination. Administration of test substances may result, usually at the highest dose level in mild to moderate signs of toxicity. The rats and mice used in these studies may show effects at all dose levels, but they are expected to be transient. Experience shows that (~60%) of animals are not expected to show any clinical signs of suffering. A percentage (~20%) may show transient subtle to mild clinical signs such as reduced weight gain or weight loss, subdued behaviour and fur ruffling. Moderate signs (hunched posture and abnormal breathing) of adverse effects may be seen in some animals (~20%), usually in the higher dose groups. Despite the close monitoring some animals may sometimes inadvertently experience severe toxicological adverse effects such as repeated convulsions, persistent laboured breathing or indeed be found dead. Lethality and/or severe effects are not the desired outcome and animals will be closely monitored and promptly humanely killed at predetermined humane endpoints to minimise the likelihood of unexpected death as far as possible. Most of the dosing techniques, manipulations or investigations do not cause any lasting adverse effects, but a small number of animals may show temporary moderate transitory distress due to, for example, withdrawal of blood.

## Application of the 3Rs

### Replacement

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

#### Replacement

There is currently no regulatory acceptable alternative to the use of animals in these studies. It is mandatory for animal genotoxicity studies, usually involving rodent and non-rodent species, to be undertaken before regulatory approval is given prior to allowing a new drug to be tested in human or veterinary trials or for an agrochemical, food additive, or industrial chemical, medical device/article to be marketed and used safely.

We will however remain vigilant to the possibility of the development and emerging use of any non animal regulatory acceptable alternatives should they become available in future.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The guidance usually indicates the design and number of animals included in a study therefore, there is lesser scope in regulatory toxicology for reduction than in other fields of work. Attention is paid to good study design to use minimum number of animals in the most refined way to achieve the aims of the study.

Some study design decisions may have to be made based on data from bacteria or cell based assays or from preliminary data in rodents, from which a progressive approach to the accumulation of information is adopted. This orderly sequence of data collection reduces the number of animals used and restricts the procedures to which they are subjected. For studies that are being performed at a later stage, where studies in the rodent (and other species) may have already been performed, decisions on study design can usually be made with a higher degree of confidence leading to lower animal use. The involvement of Scientists and Statisticians will be obtained at an early stage as required, so that advice can be given on implementation of the 3Rs and study plans developed which minimise severity of procedures applied as far as possible.

### Refinement

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

## **Refinement**

Rodents (either rats or mice) are used because their use is mandated by regulatory bodies who carry out the relevant risk assessments/safety evaluations for the studies in this project. There is considerable experience and background data for the species and studies in this project and the most refined methods will be used.

Studies are performed in a stepwise manner, starting with preliminary studies using small numbers of animals where there is limited information a so called 'pilot study'. This gives the highest prospect of refining and optimising the programme eg by optimising specific doses of substances given to achieve the desired scientific endpoints in the main study and also in consequence minimising the pain, suffering, distress or lasting harm for the animals used on study.

All animals are regularly monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early pre-determined humane endpoints are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test item, or humane killing of affected animals).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 197. Neuronal coding in the hippocampal system</b>
<b>Key Words</b>	Hippocampal formation, Memory, Spatial, Anxiety
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No

(d) protection of the natural environment in the interests of the health or welfare of man or animals;

No

(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No

(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No

(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The objectives are to discover neuronal processes in health and disease states that occur in the hippocampal formation and regions the hippocampal formation is connected to. The most well-known function that the hippocampal formation supports is spatial and autobiographical memory. The earliest stage of Alzheimer's disease usually involves neurodegeneration in the hippocampal formation, which results in memory problems.

In order to understand what goes wrong in Alzheimer's disease (AD), and other challenges like autistic spectrum disorders (ASD) and , we need to discover how neuronal processes typically operate. Some laboratories target their enquiries at levels of genes and proteins ('gene x is needed for spatial memory'), others at a whole-brain level ('region x is needed for spatial memory, regions y & z are not'). Here, synchronising video recordings of behaviour with activity from groups of single neurons, we conduct our investigations at an intermediate, 'systems' level, that can potentially integrate information about receptors on neuronal membranes, and about neurons in different brain regions interacting with each other, and link these neuronal variables to behaviour. Further, we can record from rodents genetically-modified specifically to model certain disease states to discover mechanisms underlying problems with memory or anxiety.

Example questions addressed:

- 1) What properties do hippocampal neurons have that help to code for spatial memory?
- 2) Which neuronal properties go awry first in disease models?

3) What common effects do neurochemically-different anxiolytic drugs have upon the hippocampus, and do these common effects reflect the central mechanism of anxiety reduction?

**What 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 first requirement for better therapies and better diagnostic tests is knowledge. Science put people on the moon in the 1960s because the underlying physics knowledge already existed. Science hasn't solved long-standing neurological diseases because the knowledge needed doesn't exist yet. The likely benefits are advances in knowledge of the links between cellular operations and behaviour (both cognitive and emotional). Example benefits: 1) Knowledge about viewpoint-independent spatial coding in the hippocampus leading to tests that more accurately diagnose the very earliest stages of Alzheimer's disease. 2) Knowledge of impairments in interacting neuronal groups in models of AD and ASD. 3) Better understanding of how to design an anxiolytic drug.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to 125 rats per year, Up to 90 mice per year

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

Severity is mild to moderate. The animals are kept in large cages. They may be single housed, and this is mitigated by stress-reducing procedures such as species-appropriate frequent handling and tickling and exposure to complex environments. Animals undergo surgery under anaesthesia with implantation of electrodes into the brain through small windows in the skull, and sometimes administration of neural-affecting substances including drugs, and neuroanatomical tracers, during and after surgery. We then seal everything with dental cement. During and after surgery, all animals receive pain-killing drugs and post-operative care. Adverse effects such as infection associated with the dental-cemented implant or drug administration can sometimes occur; further post-operative care will then be applied, and the animal may be humanely killed if appropriate before the end of the procedure. In rare cases, as with Alzheimer-model rodents, the procedure may last up to two years, in order to track the development of the disorder, and the effects of any therapy, over time. All animals will be killed by a humane method, and the brains removed and subsequently analysed after death.

## Application of the 3Rs

## Replacement

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

### Replacement

Our knowledge of the brain's physiological mechanisms is too poor to be able to dispense with experiments. We collaborate with researchers who create computational models of aspects of brain functioning, but these are far, far too simple to substitute for experimental knowledge.

Despite increasingly rapid progress, neuroscience is a young science. We can't hope to understand the most complex thing in the universe (the human brain) without using very complex models of it (animal brains). Because of the importance of the hippocampal system for memory, there has been much work using *in vitro* slices of hippocampal tissue, and computational models. These give us limited information about certain basic firing properties of the neurons, and offer concrete ideas on how the connections between neurons might change with experience. We share data with experts who model hippocampal function computationally, and often test predictions from these models, but even the most valuable computational models are extraordinarily simple in comparison to the complexity of the system under study.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We seek both to maximise the amount of meaningful data obtained from each individual animal, and to refine our techniques so as to reduce discomfort/suffering. Our technique of *simultaneous, stable, chronic multi-site* recording/archiving of neuronal ensembles (lots of neurons), EEG ('brain waves'), and ethological/spatial behaviour greatly improves the suffering-to-information ratio:

1) If brain region A in rat X and region B in rat Y respond differently to the same stimuli, it's possible that this occurs because rat X responds differently to rat Y,

necessitating high numbers of rats. *Recording simultaneously from two or more regions controls for inter-animal behavioural variability, thus reducing the number of rodents required.* Similarly, we are using as much *within-animal* pharmacological comparison (drug vs vehicle) as possible.

2) We can record over long periods (days and months), obtaining considerable data from each animal. The discomfort of the animals typically *decreases* with time elapsed since the surgical operation. *Arguably, the benefit of the extra information we obtain comes at a very low cost.*

3) The *richness* of the information we obtain from each single trial. We obtain EEG data (brain waves: i.e. lower-frequency, large-scale oscillatory activity that may relate to how different brain regions talk to each other), neuronal firing at a millisecond-to-millisecond scale and for up to, say, a hundred neurons, spatial data (e.g. where is the animal? ethological data (e.g. is it freezing, rearing, urinating?). All this information is stored. *The richness of these stored datasets often eliminates the need for pilot work, thus further minimising animal numbers.*

## **Refinement**

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

## **Refinement**

We use rats/mice because they have quite advanced spatial memory, and their brains' circuitry and physiology are well established. It has become important to work on mice models because of the exciting progress in transgenic technologies.

Importantly, the discoveries of several types of spatial-mapping neurons were first made in rats, and then later discovered in monkeys and humans. The text outlining

the rationale for the award of the Nobel Prize in Physiology/Medicine of 2014, for the discoveries of spatial-mapping neurons in the hippocampal formation, explicitly spelled out the potential of these discoveries for translational work on Alzheimer's disease in humans. Overall, it seems very likely that many insights from rodent work apply to humans, and benefit human health.

The main potentially adverse effects of our procedures are post-operative pain and infection, which are minimized with pain-relieving drugs and antibiotics. The ratio of suffering to significant information obtained is minimised not only by constant and progressive efforts to reduce suffering but also by maximising the amount of data we obtain from a single rat/mouse.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 198. Oncology Pharmacology Studies</b>
<b>Key Words</b>	Oncology, Disease models, Efficacy, Pharmacodynamic, Pharmacokinetics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Cancer is a complex disease and despite an increase in the number of new drugs available to treat patients, not every patient will respond to existing treatments and be cured.

It is therefore important to continue to support the discovery and development of new anti-cancer drugs.

This service licence will be used to determine whether potential new anti-cancer drugs can stop tumours from growing and seek to understand how the drug works.

This service licence will also be used to develop new cancer models for use in future research into new 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)?**

Increased knowledge of cancer biology is leading to the discovery and development of new drugs. Some of this success has resulted in improved cancer survival rates seen in recent years (Cancer survival rates have doubled in the last 40 years). However benefits are still limited and are better for some cancer types than others. In addition, the side effects of many existing drugs have a significant impact to the patient's quality of life. There is therefore a clear need for more effective drugs that

can be used either alone or in combination with existing or other new drugs. The work carried out under this licence will provide benefits:-

- In the short term by supporting cancer drug discovery
- In the medium term by sharing cancer research data which will increase the understanding of cancer biology
- In the longer term by supporting the clinical development of new drugs for the treatment of cancer.

It is expected that a number of drugs research programmes supported by this service licence will be successful in developing new cancer drugs.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Only rats and mice will be used on this project. Approx 93% of the total usage will be mice and ~7% will be rats. The total number of animals used over the duration of the licence will be approximately 19,850

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

REDACTEDAnimals will be housed in groups. Tumour growth in animals will be started by injecting tumour cells or pieces either:

- Under the skin
- In breast tissue

Injections require the use of needles and in some cases minor surgery.

- The use of needles will cause brief discomfort or pain.
- Where minor surgery is required, animals are expected to recover quickly and will be given painkillers during and after the surgery and post-operative care just like people recovering in hospital.

The majority of animals will be given the experimental drug every day, using the same drug delivery method as used in patients. In the majority of cases this will involve the use of needles only, which will cause

- Only brief pain or discomfort.
- In a small number of cases, brief local irritation of the skin where the drug has been injected

A small number of animals will have minor surgery to implant a device under the skin which can slowly release the test substance. Animals are expected to recover quickly and will receive painkillers during and after the surgery and post-operative care, just like people recovering in hospital. Animals will be regularly monitored for weight loss and general condition. Animals may become unwell as a result of the test drug. Signs that the animals are starting to become unwell can include:

- Weight loss
- Deteriorating coat condition
- Reduced movement
- Reduced social interaction

Animals will be humanely killed if these signs of being unwell persist. Blood samples will be collected during the experiment, which will cause brief discomfort or pain. The majority of animals will undergo experimental procedures which are classified as moderate severity. At the end of the study it is necessary for the animals to be humanely killed and tissues taken for analysis after death.

## Application of the 3Rs

### Replacement

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

### **Replacement**

Non-animal alternatives are used in the identification and selection of potential new drugs before testing in animals.

However, non-animal testing cannot predict how experimental drugs stop tumours from growing.

Cancer development is a complex process. It involves lots of different cell types including the immune system, which cannot be recreated in non-animal alternatives or non-protected species.

Therefore protected animals are needed for the studies proposed in this licence.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

To obtain good quality data and to use the minimum number of animals, statistical expertise will be applied to work undertaken on this licence.

The following guidelines will be used to minimise the number of animals required:

- The number of animals required in each group will be determined using previous experimental data, historical databases, pilot studies or published data
- Appropriate statistical tests will be used.
- Studies will be designed to enable at least an 80% chance of finding a meaningful result.
- Wherever possible, multiple test drugs or doses of test drug will be compared against one control to reduce the number of studies performed.

### **Refinement**

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

## **Refinement**

The overall plan of the work is to support the discovery and development of novel cancer drugs to benefit human health in the treatment of cancer.

Using non-mammalian species is not possible because they lack relevant tissue physiology and therefore cannot replicate human physiology

Only rats and mice, including strains which lack an immune system, are used on this licence.

Mice will be used in the majority of studies unless there is a scientifically relevant reason that mice cannot be used.

The most appropriate species and strain of mice and/or rats will be chosen based on previous data and choice of tumour model.

To study human tumour growth in rats or mice, strains lacking an immune system are required. The least immune-deficient strain required to promote good, reproducible tumour growth will be used.

Best practice and use of the most refined methods will be applied to all experiments

Animals are observed by trained staff, with referral to the Named Animal Care and Welfare Officer, Named Veterinary Surgeon and Project Licence Holder as necessary.

All animals will be regularly monitored for weight loss and general condition.

For anaesthetic protocols the optimal anaesthetic regime relevant for the species/strain will be developed and used in conjunction with the Named Veterinary Surgeon.

Where necessary, painkillers will be used under the guidance of the Named Veterinary Surgeon.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 199. Safety of Biopharmaceutical Medicinal Products</b>
<b>Key Words</b>	safety, biopharmaceutical
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The objective of the project is to ensure the safety of biopharmaceutical products through the use of animals to:

- assay for potential contaminating agents
- produce antisera for virus seed neutralisation and serological assays
- validation of in-process steps for reduction / removal of TSEs derived from material of human or bovine origin
- characterisation of cell lines for vaccine production.

Biotechnology has long been used to produce medicines for human or animal use in the form of vaccines and monoclonal antibodies and other new technologies such as gene therapy, xenotransplantation and transgenics are in the early stages of development. However, with such products there is a risk of contamination with microbiological organisms which may be endogenous or latent in the animal of origin, or which may be introduced from animal raw material or other sources during the production process.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The benefits of the project are evident in that the testing will help to ensure that biopharmaceutical medicines for human or animal use are free from contamination and other safety risks such as tumorigenic potential.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The species and approximate numbers expected to be used over the 5 year licence period are as follows: Mouse = 117800 Nude mouse = 1500 Hamster = 4650 Rat = 670 Nude rat = 500 Guinea pig = 3150 Chick = 600 Chick embryo = 60000 The number of biopharmaceutical products which are undergoing testing and have already been licenced is increasing as the technology advances, therefore there is expected to be increasing demand for the regulatory testing provided by this facility. The number of animals is predicted across the 5 year period is based on current demand in the facility and the regulatory requirements which currently insist on in vivo testing methods.

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

The animals are kept within a dedicated animal facility; they are group housed with a variety of environmental enrichment to promote natural behavior. The animals are grouped upon entry to the facility and allowed time for environmental acclimatisation prior to the start of procedures. The majority of assays are required by regulatory authorities such as the EMEA and the FDA in order to determine that products intended for human or veterinary use are safe and free from contamination. The combination of animals or avians used, the numbers of animals required and the routes of inoculation are specified in individual regulations and therefore change depending on the type of product being tested. The number of animals used is the minimum required to comply with individual regulations. Although the protocols are designed to detect infectious agents which produce morbidity or mortality in the test animals, in practice the incidence of positive results is extremely low. Unless otherwise specified, there are no expected adverse effects as a result of the procedures described. Rodents (adult mice, suckling mice, hamsters, guinea pigs and rats) may be inoculated once with test article via one, or a combination, of the following routes: intraperitoneally, intranasally, intramuscularly, per os (orally) and intracerebrally (under inhaled general anaesthetic). On occasion, some tenderness or localised swelling may be found around the injection sites. The animals are observed daily for any ill effects and after the end of the observation period (ranging from 7 to 42 days) are euthanised. A small subset of adult mice may be used for LCMV challenge, where they will undergo general anaesthetic and have an intracerebral inoculation of live LCM virus, at least 2 weeks after inoculation with test article. The mice are observed at increased frequency in order to catch onset of clinical symptoms as early as possible. These mice are euthanised as soon as the onset of clinical symptoms is confirmed. Chickens may be inoculated up to 2 times, at least 2 weeks apart, via intramuscular or eye drop route. Embryonated chicken eggs are injected once with test material into either the chorio-allantoic membrane, the yolk sac, allantoic cavity or the amniotic fluid. The eggs are incubated and

observed for viability and any abnormal changes to the embryonic development. The eggs are euthanised at the end of the observation period. For testing of certain cell lines used to generate biopharmaceuticals there is sometimes a requirement to check that the cells used will not cause tumour formation. For these products long-term (up to 240 days) animal studies may be required to show that the cell lines do not have tumour forming potential. Tumorigenicity and Oncogenicity assays can be performed in adult and newborn athymic nude mice, newborn hamsters, newborn rats or athymic nude rats. For tumorigenicity assays the animals are given a general anaesthetic (via inhalation) and then injected with test material either subcutaneously or intramuscularly. A positive control group will be injected with a cell line which will cause tumour formation. The animals will be observed on a daily basis and the site of the injection will be checked for the presence of any tumours. If tumours are found then they will be measured routinely to determine the progressive growth of any tumours. Animals will be euthanised at the end of the observation period. Animals which develop tumours will be euthanised earlier than the scheduled end of the observation period if the tumours grow rapidly or if they exceed a particular size. Due to the long term nature of the studies, animals may suffer from age related illness (eg arthritis) and show loss of condition, these animals may also be euthanised prior to the end of the observation period if the symptoms cannot be alleviated. Oncogenicity testing will only be performed in circumstances where a cell line has been found to cause nodules or tumours in the tumorigenicity testing. The procedures for the oncogenicity testing are the same as the tumourgenicity testing, with the exception that an immunosuppressant may be administered via subcutaneous injection up to 4 times over a 2 week period. Regulatory authorities, including the FDA and EMEA, require evaluation of the risks posed by potential contamination of biopharmaceutical products with TSE's (transmissible spongiform encephalopathy). Animal assays are required for these tests to produce positive controls for in vitro assays, as well as to assess the TSE clearance processes employed during the manufacture of biopharmaceuticals. These studies can be performed in mice or hamsters, which will be given a general anaesthetic (via inhalation) then inoculated intracerebrally and/or intraperitoneally with scrapie and BSE agents. After inoculation the animals will be observed for the appropriate periods (usually up to 350 days). Adverse effects of TSE disease are expected; however a scoring system has been developed to define mild, moderate and severe symptoms. Animals will be assessed against the scoring system to ensure that animals are euthanised prior to developing severe symptoms of the disease. As these studies are long term, animals which exhibit age related illness will be euthanised prior to the end of the observation period if their symptoms cannot be alleviated. Adverse effects are most likely to result from the presence of an extraneous agent in the test material or in some cases from toxicity of the test material. In addition, where challenge and positive controls are used, effects of these are expected and therefore will be closely monitored against humane end-points detailed in the licence.

## Application of the 3Rs

### Replacement

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

### Replacement

In conjunction with *in vivo* studies, *in vitro* testing is performed as an important part of the submission package.

Where possible, *in vitro* testing will be performed, however, current legislation for the safety of biopharmaceuticals also requires *in vivo* testing.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Consultation with regulatory authorities and clients will determine the number of animals to be used on a case by case basis for pre-studies and susceptibility studies. Any background information from *in vitro* and *in vivo* data shall be reviewed. Since studies are compendial the option for reduction is limited.

Where possible (i.e. negative control groups) animals will be shared as common to several studies.

### Refinement

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

### Refinement

Each study protocol shall be reviewed on a case by case basis, and particular attention made to inoculation routes, dose volumes and an assessment made of likely adverse effects. The study protocol shall also include daily clinical assessments of all animals. Advice from the named veterinary surgeon and named animal care and welfare officer may be requested prior to and during the study.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 200. Investigating immune dysfunction in sterile liver injury</b>
<b>Key Words</b>	Liver injury, sepsis, infection, cirrhosis, immune dysfunction
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Liver injury, caused by alcohol, dietary factors and drugs is a common clinical problem. All these agents cause damage to the liver tissue and provoke the body's immune system into a response. This response can be beneficial, helping to heal the liver, but also may exacerbate the injury and cause worsening liver problems. Patients with severe forms of liver damage also show defects in their immune system that make them particularly susceptible to getting infections.

This project aims to look at how the immune system contributes to ill health in patients with liver disease, by:

1. worsening and/or prolonging liver damage or
2. failing to fight infection

**What 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 help advance our understanding of how the immune system reacts to different forms of liver injury. It will increase our knowledge of the way immune (white blood) cells contribute to liver damage and why the white blood cells of patients with liver injury are less good at fighting infection. In the longer-term this improved understanding could lead to improvements in care for patient's with sterile liver injury, including immune based treatments that reduce liver damage or enhance patients' ability to fight infection.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This licence will use approximately 1600 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 levels of severity? What will happen to the animals at the end?**

Mice will be exposed to agents that typically cause liver damage in humans, such as alcohol, high fat diet, and drugs including paracetamol and cancer immunotherapy. Mice may experience transient discomfort during the administration of these agents and in some cases, where severe liver injury is induced the mice may experience fatigue, drowsiness or loss of consciousness for short periods before being humanely killed. In the chronic liver injury models there may be some lasting ill effects, such as weight loss, malaise and abdominal discomfort. Some mice with liver injury may be given infections and will experience symptoms of generalised illness for brief periods. Mice may have imaging procedures performed under general anaesthetic. Though everything will be done to prevent it, there is the possibility of mice dying spontaneously during the procedures due to severe but unpredictable toxic effects of the substances administered. Because of the possibility of mice experiencing significant ill-effects of the substances administered during these experiments, 3 protocols on the licence are graded as 'severe'. The named animal welfare officers (NACWO) and veterinary surgeon (NVS) will be consulted if there are any concerns regarding animal welfare. The Laboratory Animal Science Association (LASA) best practice guidelines will be followed. Mice will be humanely killed at the earliest opportunity to reduce the duration of any suffering.

## Application of the 3Rs

### Replacement

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

### Replacement

This project aims to look at the complicated interactions between liver disease and the immune system. It supports research we do in human liver disease patients and laboratory based techniques that do not require live animals. We use human or in vitro systems wherever possible, however some experimental manipulations and sampling are not possible in humans for ethical reasons, which is why we use mice. Ultimately we want our findings to be relevant and useful for human disease, so we need to use a mammalian animal for our studies.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Through careful experimental planning we will limit the number of experimental and control animals to those necessary to answer the study questions. Experimental design and reporting will be guided by the ARRIVE guidelines. Strict control of housing, diet, administration of substances, animal age and sex will reduce variation and allow smaller numbers of animals to be used.

### **Refinement**

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

### **Refinement**

We have chosen mice as they are the lowest mammalian animal that provides a very useful, relevant model of human liver disease. This project uses well established models of liver damage, which have been refined to provide the best reflection of human disease, while minimising suffering for the experimental animals.

Mice undergoing experimental work will be very closely monitored and will be humanely killed if they show signs of unexpected or excess suffering. Anaesthesia and techniques to reduce harm will be used wherever possible to limit the side effects of experiments.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 201. Investigating Kv7 channel regulation of blood pressure</b>
<b>Key Words</b>	blood pressure, vascular
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Hypertension is a chronic increase in blood pressure that significantly increases the risk of coronary artery disease, stroke, heart failure and renal failure. This results in blood vessels which are narrower than normal, and so blood flow is restricted. This increases blood pressure which makes the heart work harder, and means that some areas of the body might not receive enough blood. One of the key ways that controls blood vessels opening or narrowing is the activity of potassium channels. The objective of this project is to determine the function and role of a particular family of potassium channels (Kv7) on blood pressure in healthy animals, and in animals which have high blood pressure.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Increasing our understanding of mechanisms which control blood vessels, and how these change in disease is incredibly important for the development of new treatments and therapeutic targets.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice – 200 Rats – 200 5 years

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

Blood pressure measurements are non invasive and not likely to present adverse effects. Administration of drugs will provide mild, momentary discomfort. The testing of novel drugs is unlikely to produce significant adverse effects but animals will be

closely monitored for distress or discomfort. Surgery under anesthetic to implant osmotic pumps will avoid repeated injections, and is likely to only cause temporary discomfort. Pain relief will be provided.

## Application of the 3Rs

### Replacement

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

#### Replacement

Blood pressure is an active physiological process which can not be adequately modelled in other preparations. However where possible we use ex vivo or in vitro experiments to minimise in vivo experiments.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Experiments are designed in such a way to minimise the number of animals needed. Ex vivo and in vitro experiments precede in vivo experiments and only studies which are successful are continued into animal studies. Numerous tissues are used or stored for future use to maximise output from each experiment.

### Refinement

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

#### Refinement

Mice and rats will be used as the best studied models of hypertension have been established using these animals.

Mini osmotic pumps implanted under general anesthesia will be used to avoid multiple injections of agents. Pain relief will be used as necessary.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 202. Quantifying the potential of skin swabbing as a refinement for DNA sampling of laboratory fish</b>
<b>Key Words</b>	Zebrafish, Stickleback, skin swab, fin clip
<b>Expected duration of the project</b>	3 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this research is to improve the health and welfare of fish that are subjected to DNA collection in the laboratory. Genetic research on model fish species requires DNA to be collected from live animals. This is typically achieved by fin clipping, a procedure that can alter health, welfare, behaviour and physiology. We will extend our previous research by validating skin swabbing as a non-invasive technique to sample DNA from two of the most frequently used model species, zebrafish and sticklebacks. We will: 1) determine whether swabbing is less stressful for fish than fin clipping; 2) investigate whether treatment with an analgesic reduces any pain caused by swabbing; and 3) examine the effects of immersing swabbed fish in commercial water supplements on health and welfare. This research will refine DNA sampling, with clear benefits for fish kept in the laboratory such as reduced pain and 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)?**

Fish used in laboratory research will benefit if we find that skin swabbing is less painful than fin clipping. Scientists who work with fish in the lab will benefit from the establishment of a potentially less invasive technique to collect DNA. As well as promoting the long-term health of fish, this research may speed up DNA sampling, improve the quality of data collected following collection and reduce the number of animals used in research if skin swabbing causes less variation in experimental results. Scientists in the aquaculture industry could benefit if skin swabbing leads to better growth rates and an improvement of overall health following DNA sampling. To investigate this we will measure the long-term impact of skin swabbing and fin clipping upon health indices. Members of the general public are very interested in

promoting animal welfare and reducing the number of animals used in research. This core aim of this project is to promote fish health and welfare. We will disseminate the results of our research to increase awareness of the 3Rs principles and explain the importance of using animals in controlled experiments that include health and welfare components in their design. Ultimately we aim to replace fin clipping with skin swabbing as the standard technique to collect DNA from aquatic species.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use 1024 fish of each species during a two year period, making a total of 2048 fish. Of these animals, 600 zebrafish and 600 sticklebacks will undergo non-invasive behavioural testing.

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

During this project we will maintain breeding stocks of both zebrafish and sticklebacks as well as measuring behaviour at adult (3 month) stages. Both breeding and measuring adult behaviour are mildly stressful procedures. It involves placing animals in a novel arena and filming their behaviour. All animals will be killed using a Schedule 1 procedure at the end of the project.

## Application of the 3Rs

### Replacement

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

### Replacement

The focus of this research is to improve fish welfare. It is therefore not possible to replace the animals used in this research with an alternative model such as cell culture or an in silico study.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The experiments to be carried out in this research have been designed based upon previous research in our laboratory and using the NC3R's EDA tool. They are suitably powered to enable us to make conclusions based upon the results that we collect. Fish that are genotyped by DNA sampling are typically used in subsequent experiments. While this project presents little direct opportunity to reduce the number of animals in research, data arising from the project may lead to a decrease in the

number used in the future. For example, if we discover that swabbing induces less variable (as well as less severe) stress responses among fish, these may permit fewer animals to be used in subsequent experimental studies.

### **Refinement**

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

### **Refinement**

Adult fish (including both sticklebacks and zebrafish) will be housed in the best possible conditions in our aquarium. This aquarium has constantly circulating water which is regularly monitored for quality. Fish are maintained at low stocking density in environment-enriched tanks. Fish will be minimally handled during the project. Pilot experiments have already been used to calculate the number of fish needed in each experiment.

DNA collection by swabbing in the absence of anaesthetic may have less of an impact upon fish health and welfare than fin clipping. We will investigate this by measuring physiological markers of stress, behaviour and general health in both zebrafish and sticklebacks. We hypothesise that swabbing will cause fewer changes to cortisol release, weaker activation of stress marker genes, and less anxiety-like behaviour.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 203. Fish Passage Through an Entrainment Simulation Unit</b>
<b>Key Words</b>	Anguilla anguilla, Entrainment, Barotrauma, Mechanical damage, Heat shock, Shear stress
<b>Expected duration of the project</b>	2 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
Yes	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The population of European eel (*Anguilla anguilla*) in freshwater have declined by as much as 95% across their home range in the past 30 years. This has led the IUCN to list the eel as a Critically Endangered species and for the UK Government to list eel as a species of conservation concern requiring conservation action.

The eel's life history, migratory pathways and swimming efficiency, particularly in the juvenile stages, make them vulnerable to entrapment and entrainment at cooling water intakes and has been cited by specialist organisations as a potential major cause of eel mortality in some rivers.

The survival of individual species and lifestages through a cooling water system have been studied previously using a laboratory-based cooling water system simulator rig known as EMU (Entrainment Mimic Unit). Initial research covered a limited range of species that did not include eel. The trials however concluded that predicted survival rates cannot be reliably extrapolated between species and lifestages.

A subsequent EMU study included elvers, however the range of passage conditions assessed within the trial were site and plant specific. The trial conditions (e.g. screen types and apertures, pressure and temperature profiles) to which elvers were exposed within this study are not considered to adequately represent the mechanical screening, stressors and pressure profiles used in proposed new stations and may be over-optimistic when considering the more fragile earlier, glass eel, lifestage.

The aim of the current study is to determine the tolerance (survival) of the glass eel and elver lifestage of the European eel to passage through a simulated modern

power plant once-through cooling water system. The approach proposed in the current study considers separately and sequentially the elements of intake screen passage, pressure flux, condenser system shear stress and temperature rise.

Outputs from the Project will inform models used to calculate losses arising from cooling water entrainment at a population level for a new power station, directly informing design mitigation and improving the accuracy of predictions made at the planning and Environmental Impact stage.

**What 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 on the tolerance of the glass eel and elver lifestage of the European eel to passage through a simulated power plant cooling water system. Outputs from the Project will inform models used to calculate losses arising from cooling water entrainment at a population level for a new power station, directly informing design mitigation and improving the accuracy of predictions made at the planning and Environmental Impact stage. The project is being run in direct communication with the power station engineering design team to ensure the cooling water design is optimised for fish survival. Medium to long term the project will help to inform the development of best practice in cooling water system design. This in turn will improve definitions of best available technology for dealing with fish entrainment for existing and new stations.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to 3000 glass eel and elver will be used over a period of approximately one month.

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

Glass eel and elver may be subject to mechanical injury, abrasion, barotrauma, shear stress and thermal shock. The likelihood of injury however is expected to be low based on field observations (power station monitoring programs) and observed behaviour within the natural environment. The majority (>95%) of fish exposed to a regulated procedure are not expected to suffer more than transient distress with no lasting harm and are expected to make a rapid (1 hour) and unremarkable recovery. The severity category is considered to be mild to moderate. At the end of the observational period fish will be released to the wild. In the event that any fish are deemed unfit to release to the wild by a suitably qualified or other competent person (under direction from a veterinary surgeon) they will be humanely killed using a Schedule 1 method appropriate to the species and lifestage.

## Application of the 3Rs

## **Replacement**

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

### **Replacement**

Earlier EMU trials have shown that predicted survival rates cannot be reliably extrapolated between species and lifestages.

No surrogate species is considered suitable for the replacement of European Eel.

Live fish are required as the tolerance of glass eel and elver to the range of stresses that they would be exposed to during passage through a new fleet of power station cooling water systems has yet to be determined.

There is no theoretical basis for computer simulation analysis owing to the lack of published data in this field. Inanimate tracers and cadavers cannot be used in all aspects of the experiments but will be used initially when assessing barotrauma and shear stress to minimise risk of harm to live subjects.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The sample population of glass eel and elver subject to assessment will not be of a uniform composition. Sex, length, weight and health history will vary, and may affect behaviour and swimming ability.

It is important that a sample of sufficient size is tested to represent these characteristics within a population. There are no prior data on likely variance of response in glass eel and size, preventing determination of sample size via power analysis.

## **Refinement**

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

### **Refinement**

Species selection: Eel are catadromous, which is to say that they migrate into freshwater and estuarine environments as juveniles before migrating back downstream as adults in to a marine environment to spawn. They are identified as being threatened (Critically Endangered - IUCN), requiring conservation action and are listed as a species of conservation concern under the UK Biodiversity Action

Plan. Their life history, migratory pathways and swimming efficiency in the juvenile stages make them vulnerable to entrapment and entrainment at cooling water intakes.

Unnecessary harm to fish will be avoided by the initial use of cadavers where appropriate. Where two or more cadavers used in Protocol 1b and three or more cadavers used in Protocol 1c exhibit significant mechanical damage, that stage of the experiment will be completed using cadavers.

Glass eel and elver will be continuously observed for the first hour following treatment and then visually assessed at least once every 24 hours for up to 72 hours following treatment. A PIL holder will be present to perform the observations required within the first 24 hours following treatment. Fish observed to be suffering from damage resulting from a regulated procedure will be humanely killed using a Schedule 1 method appropriate to the species and lifestage.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 204. Parasite life cycles</b>
<b>Key Words</b>	Cryptosporidium, diarrhoea, drug discovery, parasitology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Diarrhoeal disease is a major cause of illness and mortality in young children worldwide. Approximately 10% of the deaths of children under the age of five are due to diarrheal disease. A recent global epidemiological study revealed that *Cryptosporidium* is the second leading cause of diarrheal illness in young children. Cryptosporidiosis can also cause chronic, life-threatening diarrhoea in immune-compromised adults, ie AIDS patients. For young children and immuno-compromised adults there are no efficacious drugs.

*Cryptosporidium* is an intestinal parasite for which, as yet, there are no good methods for culture in the laboratory. Therefore, we have to propagate the organisms in mice in order to generate sufficient numbers for these studies.

We have developed genetic tools that allow us to genetically modify *Cryptosporidium* and produce transgenic parasites. We will generate genetically modified versions of the parasite as tools to investigate how the parasite infects humans (and other animals) and to discover new medicines. As there are currently no effective drug treatments available for cryptosporidiosis, this work is critical to provide new candidates that can be advanced for the clinic. We will evaluate how genes contribute to growth and transmission. This insight will guide development of new medicines or methods to reduce exposure to this parasite in the environment.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Cryptosporidiosis is a major global enteric infection in both animals and people, usually arising from contaminated drinking water. In children and in people with poor immune systems, infection can lead to very serious consequences (chronic diarrhoea, dehydration, malnutrition, death, etc). There are no effective drugs to treatment cryptosporidiosis, the need to discover new medicines is significant. We

need to understand the molecular details of the infectious cycle in order to identify and evaluate targets for new medicines. Because cryptosporidiosis is a disease of poor sanitation and of poverty, there is little commercial effort to identify and develop treatments for cryptosporidiosis. Therefore, it is important to advance this work in the absence of commercial interests.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Cryptosporidium can infect mice, but only if the mice are partially immuno-compromised. Genetically altered mice with this property will therefore be used to maintain the various strains of the parasite needed for our studies. Over the five years of the licence, we expect to need about 4000 animals for this purpose. We shall use about 1000 mice for testing whether novel agents, identified in our research, might be potential treatments for cryptosporidiosis.

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

In most cases, mice will be infected orally. The course of infection is well-understood and the animals will be euthanized humanely before they show any signs of serious disease (most animals will show no visible signs at all). The parasites will be harvested from faecal pellets, which will be collected from the cage floor. When developing a new strain of genetically modified parasites we will first have to implant them direct into the small intestine, through a small incision carried out under surgical anaesthesia, as the particular life-stage of the organism would not otherwise survive oral delivery. This is a minimally invasive procedure and the mice recover quickly (within minutes). Thereafter, the line can be passaged orally.

## Application of the 3Rs

### Replacement

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

### Replacement

There is no continuous culture system for *Cryptosporidium*, so animals must be used for this purpose. All systems claiming to support continuous culture fail to robustly produce usable numbers of organisms for further analysis and are not an adequate surrogate for evaluating medicines. Because there are currently no means of freezing or preserving *Cryptosporidium*, parasites must be maintained in infected animals until the project is finished. Therefore, to propagate genetically modified parasites, we require animal models. Mice are one of the best-established models for *Cryptosporidium* infections and study.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The majority of the animals will be used for the production of genetically altered parasites for detailed laboratory investigations. We will therefore plan the animal work very carefully according to the anticipated demand from the laboratory research programme. When conducting tests of a substance's potential as a new medicine, we will use a statistically robust experimental design. New laboratory culture systems are unlikely to replace all animals, but if they are developed they will be evaluated for their ability to reduce animal numbers.

## Refinement

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

### Refinement

We utilize mice that are genetically modified to be partially immuno-compromised. These mice breed normally and do not have any welfare costs associated with the genetic modification. Cryptosporidiosis patients are themselves immuno-compromised (young children with undeveloped immune systems, or adults immune-compromised due to advanced HIV or immune suppressive therapies). Mice will not be further genetically modified as a part of this project; these genetic modifications in mice are necessary only to facilitate propagation of *Cryptosporidium parvum*. Parasites are derived from faecal pellets, collection of which is a completely non-invasive process. This model has predictable and reproducible outcomes and is the leading standard for studying *Cryptosporidium* and for developing new treatments for cryptosporidiosis. As the field refines the animal models used, we will incorporate these refinements.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 205. Clarification of endogenous and pathological roles of new chromatin replication factors</b>
<b>Key Words</b>	Genome stability, epigenetics, DNA replication, Cell cycle, Cell proliferation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

All the cells of our bodies contain the same DNA, but this is 'expressed' differently in, for example, nerve cells, liver cells and muscle cells. A great part of these differences are specified by biochemical modifications that do not change the DNA sequence, but do alter the activities of the genes that the sequence encodes. I am interested in how the genetic material (the DNA sequence) *and* these modifications are preserved intact as they are copied during cell division. My laboratory have identified a group of proteins that associated specifically with newly copied DNA and are believed to be involved in this process of ensuring accuracy. Several genes for these proteins, are known to be mutated in human diseases and syndromes, further signalling the importance of understanding how they function normally.

**What 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 better how factors associated with newly copied DNA ensure its complete integrity, we expect to learn more about how this process can go wrong, not only in rare diseases, but in ageing and cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice. We shall use about 3000 mice over a five-year period, mostly for breeding.

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

We shall introduce specific alterations in target genes and study their consequences. We shall be primarily interested in mouse embryos. We do not know exactly what the

consequences will be of making these changes in mice though, by analogy with human disease, there may be some developmental changes, e.g., dwarfism. We hope that some of the genetic alterations can be “inducible”, i.e. they will only take effect after administering a particular substance to the mice.

## Application of the 3Rs

### Replacement

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

#### Replacement

Development is a highly complex process and cannot be replicated in simpler systems. The gene of primary interest is very similar in mammals, making the mouse a powerful system in which to study its properties.

I will use mES cells KO for Fam111A to explore the molecular function of Fam111A. However, given that mutations in Fam111A provoke a severe developmental defects in human, the use of animal model is required to understand the function of Fam111A in pathological context.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We shall work primarily with mouse embryos, and cell lines derived from them at various stages of early development which reduces the number of protected animals which are used.

We shall manage our breeding programmes carefully, so as to produce just the required number of embryos for our studies. We will use power calculations to design experiments in which these effects can be more precisely determined and their biological significance evaluated.

### Refinement

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

#### Refinement

By working primarily with embryos and using an optimised breeding strategy, we intend to reduce the risk to animal welfare to the minimum. We have no immediate scientific need to keep animals after birth which have discernible welfare problems.

However, we do not yet know whether these can be completely avoided in our breeding programme. As noted above, we hope that in some instances mice will not experience any effects of genetic alteration; the change will effectively be “silent” until it is induced in cell cultures that will be derived from mouse embryos.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 206. Regulation of T cell Immunity and Autoimmunity</b>
<b>Key Words</b>	Diabetes, Autoimmune disease, Immune regulation, T-cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our immune system is fundamental for protection from infectious disease; without it we would quickly succumb to infection. An important feature of the immune system is its ability to discriminate between foreign threats such as bacteria and viruses and our host tissues. Diseases like Type 1 Diabetes occur when this process breaks down and the immune system attacks host tissues such as the Insulin producing islet cells in the pancreas. Insulin production controls the uptake of sugar from the blood and so must be replaced by multiple daily injections in people with Type 1 Diabetes. Excess sugar in the blood leads to serious long term complications such as nerve and small blood vessel damage.

This project aims to understand how the immune system decides whether to make a response. We know that a protein called CTLA-4 is involved in this decision-making, but we do not know how this works. We have recently identified humans with mutations in the CTLA-4 gene, many of whom are sick as a result of problems with their immune system, highlighting the importance of this pathway. This project will use mouse models to better understand how CTLA-4 operates to keep us healthy.

To specifically study the immune response associated with diabetes, we have developed a mouse model in which we have altered the immune system so that it attacks the pancreas. These mice develop diabetes in a manner that is similar to diabetes in humans. For example, the same types of immune cells appear to enter the pancreas in these mice and in diabetes patients. In addition, antibodies specific

for pancreas proteins can be found in the blood of both the mice and humans during diabetes development. Because we have an animal model to study diabetes onset, we can manipulate it to ask which genetic pathways are important for causing or preventing 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 identifying which cells and processes lead to the onset or prevention of diabetes in the mouse model we hope to be able to accurately predict the processes involved in human diabetes onset. This will generate information that may be of use to pharmaceutical companies in the development of new medicines. Many of the pathways we are studying are relevant to other diseases as well as diabetes meaning that our findings will be of widespread use. We work closely with world-class clinicians allowing us to quickly validate the hypotheses we generate using human patient samples. It is our aim to ultimately develop ways in which to manipulate these key control pathways so as to prevent the onset of autoimmunity and to reverse established disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice in this study since we have generated or accumulated several relevant strains that allow us to address precise research questions in a controlled manner. We expect to use approximately 17,000 mice during the entire study period (5 years).

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

The vast majority of the mice used during this study will not experience adverse effects. However many mice will receive injections in the tail vein, or at other sites. Furthermore, some strains of mice will develop autoimmune diabetes or systemic autoimmunity, and some mice will undergo a surgical procedure (lasting approximately 30 minutes) that mimics a procedure performed in humans (pancreatic islet transplant). These mice are predicted to experience some discomfort and we will work to minimise this by monitoring the animals closely, and by making appropriate pain relief an essential part of the procedure, ending the experiment at ethical timepoints (for example no later than 3 weeks after the classification of a mouse as diabetic), and only using the minimum number of animals required for our experiments to be meaningful. At the end of the project, mice will either be killed or kept alive at the designated establishment.

## Application of the 3Rs

### Replacement

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

### **Replacement**

This project aims to identify novel cells and mechanisms that regulate the development of harmful immune response. In order to do this it is imperative that we are able to access the cells at the point at which the immune system makes the crucial decision of whether or not to initiate a response. For a pancreatic disease, such as Type 1 Diabetes, this is in the pancreatic draining lymph node. This cannot be accessed in human patients. In order to test the involvement of particular pathways of interest it is also essential to be able to block or genetically manipulate them and assess how this changes the overall immune response.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

To minimise the use of animals in experiments that do not turn out to be informative, pilot experiments are performed. This ensures that large group sizes are not used in experiments that are unlikely to yield useful information.

Animal numbers will be minimised by keeping records of the number of cells typically obtained from donor mice bearing different genotypes. This allows accurate planning of the number of donor mice of each type required in order to provide cells for a given number of recipients.

### **Refinement**

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

### **Refinement**

To study T cell responses to self proteins it is necessary to target the immune response against a self tissue in a manner that has the potential to cause autoimmune disease. By choosing to target the pancreatic islets, we have ensured that we can test the degree of pancreas destruction without killing the animal. This is because as the insulin-producing pancreatic islets become destroyed, the animal loses its ability to control its blood glucose levels. By monitoring blood glucose levels (using the same glucometer used by Type 1 Diabetes patients) we can therefore

obtain accurate kinetic data from a single animal showing pancreas destruction over time. Such an accurate measure of disease progression enables close monitoring of disease progression and thus prevents unnecessary suffering to the animal. We have taken a number of steps to refine the design of experiments. For example, we use markers that allow us to track multiple cell populations within a single animal. In addition, we have changed the formulation of one of the products we inject the mice with in order to reduce discomfort and have also improved the way in which we take blood samples.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 207. Myeloid cells in lung injury and repair</b>
<b>Key Words</b>	immune cells, mouse, lung fibrosis, infection
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project examines how a group of immune cells called ‘myeloid cells’ contribute to the process of injury and scarring in the lungs. We are focusing on two diseases - lung fibrosis or scarring, and influenza which cause a large numbers of death each year in humans.

Lung fibrosis in the form of idiopathic pulmonary fibrosis or IPF is a devastating disease with a median survival of 3-5 years from diagnosis, a prognosis worse than many cancers. Around 5000 people are diagnosed with IPF in UK each year, and there is no cure for the disease. Treatment only slows the progression of the disease. Patients also suffer periods of accelerated fibrosis called AE-IPF which herald a worse outcome – 80% of patients who suffer an AE-IPF episodes die within 3 months of the episode. This project examines the role of myeloid cells in the causation of IPF, specifically, which subtype of myeloid cells is involved in progression and halt of lung fibrosis. We will also investigate whether lung infection (using influenza virus) can cause AE-IPF and how. From our previous studies, we know that influenza infection has a major impact on myeloid cells, and we want to test the possibility that a rush of myeloid cells to the lungs, caused by infection could be a cause of accelerated fibrosis in the lungs.

We are also working on the role of myeloid cells in influenza virus infection, with the aim of generating better vaccines. Influenza still has the potential to cause huge pandemics. Of all the common viral infections, influenza is unmatched in its ability to spread, mutate, reassort, cross species barriers and inflict injury and death upon its host. Influenza viruses lead to regular winter epidemics and intermittently to widespread pandemics related to the development of new strains of the virus. Although vaccines are available for influenza, every year nearly 0.5M people still die world wide from influenza. Amongst various reasons for this high death rate, two are (i) limited understanding of how the immune system remembers its encounter with any one flu strain and (ii) the inability to control lung injury associated with severe influenza. In this project, we question if high levels of myeloid cells help the immune

system remembers its encounter with a flu strain and improves the recall of immune response in its subsequent encounter; and how injurious myeloid cells can be controlled in the lungs.

**What 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 lung fibrosis project, if we are able to delineate when the various subtypes of myeloid cells come into play, and how we can identify the pro-fibrotic subtype, then we have a platform to start working on a treatment to block these pro-fibrotic macrophages in the human disease. Potentially, this may mean another strategy to slow down the progress of lung fibrosis. Importantly, in AEIPF, identification of a treatment or strategy to prevent or halt acceleration of the fibrosis will mean a greatly improved survival outcome for these patients. In influenza, the primary potential benefit of this work relates to new knowledge in the area of immunology and immune memory to influenza. Our findings may allow us to develop new ways of boosting immune responses to more effectively combat influenza viruses. Being able to better activate immune responses may also advance vaccination strategies

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice and we estimate that we will use not more than 25,000 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 levels of severity? What will happen to the animals at the end?**

The mice may suffer some discomfort during the injury and inflammation phase of our lung scarring model and during influenza virus infection. This will be minimised by pain relief, and local environmental enrichment, and high level of attention and care to the animals. We have a level of discomfort threshold judged by visual monitoring of animals and a scoring system, and also by weight loss. The level of severity is defined as “moderate” and weight loss will never be above 20% of their starting weight. If in any case there were a risk of exceeding that level the animals in question would be humanely culled.

## Application of the 3Rs

### Replacement

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

## **Replacement**

We do use human cells, but the lung is very complex and we cannot reconstitute all the components required in a petri dish. In addition, we are unable to sample lungs from severely ill patients due to risks posed by the invasive nature of such a procedure. Finally, only in a model where we know when a disease process is commences can we determine the sequential change in physiology from injury to inflammation and then repair and fibrosis (scarring). This can only be done in animals and allow us to determine how the myeloid cells change in these different phases of infection and scarring and when specific myeloid cell subtypes come into play in the lungs. We are unable to use less sentient animals because we need to have the ability to model the lungs rather than disease processes only, since the complex lung environment greatly impacts on the differentiation and behaviour of myeloid cells. For example, zebra fish do not have lungs.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

We will use statistical models to determine the minimum number of required animals. We will also design experiments in such a way that many data points can be collected from the same animal. Finally, we will use a breeding strategy that keeps the number of mice to a minimum.

## **Refinement**

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

## **Refinement**

We chose mice because their immune system and lungs are sufficiently similar to humans for us to draw conclusions on processes like scarring and lung injury. We have chosen those models of virus infection, and lung injury, repair and fibrosis that are most refined and cause the least possible harm. All interventions for example administration of bleomycin to the lungs is done in the most refined way to achieve the most consistent results eg administration without surgical procedure and under general anaesthesia. , and the generation of chimeric bone marrow mice involves irradiation in split doses rather than single doses. to the lungs rather than via the intra-tracheal method. We will fully monitor all animals involved in the study and continuously seek to identify new methods for refinement. Specific humane endpoints will be applied.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 208. The social behaviour circuit in zebrafish</b>
<b>Key Words</b>	Zebrafish, social, behaviour, circuit, vision
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Humans are fundamentally social animals. We spend the majority of our time with other people. Our ability to communicate with them, is unparalleled. However, even the most complex social skill requires a basic behaviour, “social preference”, which is the ability to recognise, find it rewarding and approach other members of our species. This essential “social preference” is hard-wired into our brain. For example, new-borns immediately prefer to look at faces. If this social preference is lost, then our entire social development is affected, as testified by several neurological disorders (for instance autism).

This research projects aims to understand how social preference is built into the brain, such that we can better understand how it might be impaired. This is difficult to study in humans because the brain circuits involved are established before we are born. Zebrafish, however, are small transparent fish that develop ex utero allowing to follow the development from a single cell into a social organism within just a few weeks. Therefore, they provide a unique opportunity to watch the development of the neural underlying social preference behaviour, and to identify what goes wrong in developmental diseases like autism.

**What 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 aim of the project is to answer basic biological questions such as what are the zebrafish circuits that process social visual information, how they develop, how they process social information together, and how they can be impaired during development. By establishing the zebrafish as animal model to study social behaviour in larvae and juvenile fish for the first time, it will be possible to 1) identify environmental and genetic factors that can cause impairment of the anatomical and functional circuit; 2) screen for drugs that could reduce or rescue alterations of social

behaviour, 3) test how drugs that are already in use in humans can cause social impairments during development.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The projects will look at the development of zebrafish from larvae to juvenile. We expect to use about 70,000 zebrafish for experiments and maintaining the line over the course of 5 years. The vast majority will be used for breeding and maintaining our genetic lines.

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

The majority of the experimental procedures will only cause mild or undetectable adverse effects on zebrafish. During the experiment we will routinely monitor the state of health of the fish by imaging specific brain areas. In some experiments we will remove some of the elements of the social neuronal circuit in order to verify their causal key role in processing visual responses. These experiments will be necessary to prove the direct involvement of a specific brain area to a social function or behavioural output. At the end of these experiments larvae or juvenile zebrafish will be euthanized using an overdose of anaesthetic. This is a procedure approved by the Home Office.

## Application of the 3Rs

### Replacement

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

### Replacement

The social brain network comprises multiple structures that are scattered throughout the brain. In order to fully understand how these areas are established, and how they process social information during the whole development we will need to monitor brain activity in a living animal that is presented with social information

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will reduce the animal number to the minimum by:

- 1) always maintaining only the minimum number of fish per line to maintain the colony.
- 2) making statistical power calculations that will give us an idea of the minimum number of fish per experiment that can provide significant results
- 3) Using powerful imaging methods that allow acquiring better quality and larger amount of data with fewer fish.

### **Refinement**

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

### **Refinement**

Zebrafish are the best animal model for this project for the following reasons:

- 1) They are vertebrate and therefore their brain structure is similar to humans
- 2) They are transparent and therefore allow non-invasive imaging of brain activity throughout the whole brain and with single cell resolution. This method is harmless and allows us to monitor many more cells simultaneously and reduce number of experiments.
- 3) Their development occurs *ex utero*, so we can follow the development of the whole brain from fertilization.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 209. Molecular switches in inflammation</b>
<b>Key Words</b>	Arthritis, Macrophage, Neutrophil, Inflammation, Molecular switch.
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The consequences of severe inflammatory conditions include a wide variety of diseases with huge social impact ranging from autoimmune diseases to asthma, heart conditions, Alzheimer's and cancer. We are interested in a specific type of immune cell which plays a key role in inflammation and are essential components of our defence against these diseases. We are also interested in identifying master molecular switches of these immune cells and we believe that, by turning this switch on or off, we can dampen down inflammation present during autoimmune disease or, alternatively, boost the immune system in people with a compromised defence against disease. Our strategy is to understand how these master switches can be controlled at the molecular level by interfering with its function and thereby, develop new ways of reducing inappropriately sustained inflammatory 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)?**

This work has the potential to result in new therapies against potentially debilitating inflammatory disease, such as asthma, arthritis and some heart diseases. Our previous studies have made considerable contributions to the understanding of the inflammatory response and our continued work will lead to a new understanding of these diseases, which will significantly contribute to new approaches to diagnosis, prevention and treatment.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice, approximately 15000 over 5 years in breeding protocols, with the majority used in procedures.

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

All experimental procedures are classed as mild or moderate. However, there is the potential for adverse effects to occur. Our work can be divided into two main areas and each area is associated with the potential welfare issues: 1. Breeding and maintenance of genetically modified animals, including germ free animals. We do not expect any harmful side effects purely from breeding genetically altered animals. Germ free conditions may lead to moderate problems with altered intestinal processes, leading to diarrhoea and dehydration. However the animals will be monitored daily and should any unexpected side effects occur (such as dehydration) the animals will be immediately euthanized by a pre-approved method. Where the immune status of the animals might compromise health, the animals will be maintained in a barrier environment, which will protect against infection. 2. Procedure designed to model human disease by administration of disease modifying substances. Any animal which undergoes any procedure which has the potential to elicit suffering will be monitored for signs of possible side effects and animals exhibiting signs of distress exceeding the humane end- will be immediately euthanized. Anaesthetics and pain-killers will be routinely provided to all animals when required. In models where analgesics cannot be given because their anti-inflammatory activities tend to inhibit the induction and progression of the disease (e.g. arthritis), opioids and additional veterinary support such as easier access to food and water (e.g. food pellets and water gel packs placed at floor level), and/or supplemental bedding may be provided during advanced stages of disease. Surgical procedures will adhere to strict protocols involving appropriate anaesthesia and administration of pain killers, so as to minimise any suffering. We will employ strict, pre-defined criteria in order to assess whether an animal is in distress and animals undergoing procedures will be monitored daily and will be euthanized using pre-approved methods should they show signs of ill-health.

## Application of the 3Rs

### Replacement

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

### Replacement

We seek to understand how master regulators of immune cells work in inflammatory diseases, but the steps leading to the development of the state of chronic inflammation are poorly understood and it is likely that multiple physiological processes are involved. This inevitably involves the use of whole organisms and, in particular, the use of animal models of inflammation. However, where possible, cells from human patients will be used to address questions relating to the mechanisms of action of compounds targeting the molecular regulators identified. For example, we

are currently using human ex plants from biopsies to monitor the effect of treating them with a kinase inhibitor on inflammatory cytokine production. To test a functional role for a given regulatory factor in cell activation, we will deplete or increase its levels in an appropriate cell line, using short interfering RNA or CRISPR-Cas9 (established in the laboratory) or treating with antagonists or inhibitors (such as specific antibodies or inhibitors of signalling pathways) and analyse target gene expression.

Lower animals, such as zebrafish or flies, can not fully mirror the heterogeneity of mammalian immune cells and the interactions between them.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will use the minimal number of animals to achieve 90% power to detect difference between mean clinical scores with a significance level of 5%. We will base our calculations on previous experiments with a help of a biostatistician. Whenever possible, untreated control groups will be shared between treatment groups. We will use randomisation and blinding in our experiments where possible.

We also pay a considerable attention to our mouse colony management. We keep a careful documentation of the number and type of breeders to help organize the colony and ensure no unnecessary breeding is carried out. Animal requirements are reviewed on a weekly basis at the lab meetings. We use homozygous genetically altered and wild type breeder pairs, which have been generated from original heterozygote breeder pair and hosted in the same environment. We re-set these pairs on a regular basis. Embryos of strains not currently in use are frozen.

## **Refinement**

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

### **Refinement**

Mice and rats are the lowest vertebrate groups on which well-established models of inflammatory diseases of interest have been developed. Mice are preferable to rats because of the greater availability of reagents (e.g. monoclonal antibodies) specific for this species. The specified models have already been refined to minimise the number of immunisations and the severity level of adjuvant. Animal welfare is of critical importance during these experiments and is essential to obtain high quality data; therefore we work closely with animal housing staff and veterinary surgeons to

ensure the highest standards of maintenance and welfare for mice under our care. Anaesthetics and pain-killers will be routinely provided to all animals when required. In models where anti-inflammatories cannot be given because their anti-inflammatory activities tend to inhibit the induction and progression of the disease (e.g. arthritis), opioid analgesics and additional veterinary support such as easier access to food and water, and/or supplemental bedding may be provided during advanced stages of disease. The most humane practice, however, is to limit the length of the disease to the shortest possible time required to answer a given experimental question. Occasionally, longer experiments will be carried out but in all cases, mice which have exceeded the humane end-points will be euthanized. Aseptic technique will always be used during surgical procedures and also when necessary on other procedures. Irradiation will be split in to two doses to minimize side effects.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 210. Hormonal and Growth Factor Control of Breast Cancer</b>
<b>Key Words</b>	Breast cancer, endocrine resistance
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Most breast cancers use female hormone oestrogen for growth. Patients diagnosed with oestrogen responsive primary breast cancers are treated with anti-oestrogens, which block the oestrogen signalling pathway and therefore tumour growth. Although effective, in around 40% of patients the cancer spreads to other parts of the body. Our aim is to identify the genes and proteins which change in the tumours that become resistant in order to identify new drug targets and new clinical 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)?**

These studies will allow us to identify mechanisms of resistance to endocrine treatments, to identify biological markers that can identify patients likely to relapse and allow us to develop new clinical strategies to treat these patients thus improving the patients quality of live and increasing their lifespan.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over 5 years we expect to use a maximum of 4400 mice. A typical study will involve 70 mice The number of mice required for a typical drug combination study is 70. This allows us to test 2 different drugs alone and in combination with each other plus a control group. A minimum of 6 animals per group are required in order to mathematically prove a treatment is effective. Given that a small number of animals may not develop tumours, 10 animals per group are deemed necessary. Mice with an impaired immune system are used as the species of choice for these studies. The limited immune system in these animals allows human tumours to grow without the natural immunity affecting them and therefore the scientific data produced.

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

Cancer cells are allowed to grow under the skin of the mouse on the flank or in the fatty area surrounding the mammary gland. These cells are either given by injection or by implanting a small fragment of tumour via a small incision. Agents, which support the tumour growth are given via injection under the skin. Treatment drugs are given by either an oral dose, or an Intraperitoneal injection. All procedures are carried out by skilled animal technologists using aseptic techniques. This reduces the risk of any adverse effects occurring as a result of the procedure. Sometimes drugs may have an adverse effect, pilot studies will be completed to ensure optimum treatment regimes are implemented, we continually monitor their weight and other physical signs of pain and distress. Any adverse effects are managed and dealt with promptly. At the end of studies animals are killed using humane methods carried out by skilled technicians. Samples of tumours are collected for laboratory analysis after the animals have been killed. The procedures we use may cause “moderate” distress and so animals sometimes show discomfort, abnormal behaviour and weight loss which we will use as indicators of poor wellbeing, systems are in place to alleviate stress if evident

## Application of the 3Rs

### Replacement

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

### Replacement

REDACTED Although these experiments provide insight into which molecules and drugs are useful they do not model the tumours “microenvironment” e.g. how the tumour makes a blood supply or how tumour cells are affected by surrounding normal cells. To do this we need to place human tumour cells into mice with an impaired immune system allowing us model the tumour in the patient. These studies cannot be carried out immediately in the clinic, as we must first be certain that these new drugs are effective at inhibiting tumour progression. Those drugs that are successful can then be tested clinically

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

The number of animals required for a typical drug combination study is 70. This allows us to test 2 different drugs alone and in combination with each other plus a control group. A minimum of 10 animals per group are necessary since in the Wilcoxon matched paired test, the most rugged statistical test for comparison within groups, requires a minimum of 6 animals for a change to be deemed statistically significant. Given that a small number of animals may not develop tumours, 10 animals per group are deemed necessary. This approach has provided us with good statistically acceptable data over a period of several years use of this model.

## Refinement

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

## Refinement

In some cases where we have limited information on a new drug, we will carry out a small study in a limited number of animals to ensure that the drug alone or in combination does not cause severe adverse effects.

To carry out these studies we use female mice which have a deficiency in their immune system. This allows them to accept and grow human malignant tissue without their bodies fighting the disease and them becoming sick.

To replicate the patient hormonal environment for the growth of tumours, the ovaries of the mice are removed under anaesthetic and a supplementary hormone therapy is provided to enable the tumours to establish and grow. In the past we have implanted a synthetic hormone pellet between the shoulder blades of the animal but we have documented that there are significant adverse effects from doing so due to a build-up of bladder calculi. Most of our tumour models are now modified to grow without oestrogen support thus eliminating the adverse effects completely.

Animals are monitored daily for signs of adverse effects from surgery and therapies. Surgery is carried out under aseptic techniques using a refined procedure which has been improved over many years. Animals are monitored closely during surgical recovery periods.

Any animals showing signs of adverse including loss of body condition and general demeanour are assessed and if necessary humanely killed.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 211. Production and maintenance of GM zebrafish lines</b>
<b>Key Words</b>	Zebrafish, Breeding, Production, Maintenance, Cryopreservation.
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The objective of this licence is to provide service and support to scientists through the generation, breeding and maintenance of genetically modified (GM) zebrafish. Technologies to create GM zebrafish, for example fish with fluorescent organs and tissues, make it simple to identify those tissues when studying them and underlying disease processes; such technologies are well known and well established and cause no known problems. Any problems detected will be dealt with before the animal is protected under the law. Once these zebrafish lines have been characterised they will be bred and maintained under standard husbandry conditions by dedicated husbandry staff. To facilitate the lines for future use the GM sperm may be frozen and used for IVF.

**What 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 zebrafish has proven itself as a reliable model for a significant number of human diseases and conditions, especially as the genetic comparison between zebrafish and humans genes shows approximately 70% similarity. Therefore, at the cell level or genetic level there are distinct parallels to be drawn, which make this is an important comparative research animal model for human disease. This licence will allow the development and production of the tools required to understand human disease. The application of non-protected embryonic stages, which are optically clear, allows easy examination of any manipulation to model human disease. Furthermore, the ability of zebrafish to regenerate all organs is an important and additional benefit of using this model system. More and more examples of comparative disease modelling are becoming available, allowing better understanding of disease intervention and management.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The vast majority of animals used on this licence will be used for breeding and maintenance of the GM zebrafish lines (approximately 8000 over 5 years), this will be animals with a non-harmful modification, and which behave and breed normally. Other protocols include generation of new GM zebrafish lines or the cryopreservation of lines for future use (4000 and 500, respectively over 5 years), again with a non-harmful modification.

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

All protocols listed on this application are Mild, although it is expected, as such modifications are non-harmful, that the severity limit could be considered and reported as sub-threshold. The majority of animals used will be for breeding of fish with genetic modifications, which is a normal breeding process and no different to what happens naturally. Protocols for cryopreservation of fish and generation of new zebrafish lines are considered to be non-invasive as this occurs externally (e.g. in a petri dish). Zebrafish are protected under law after 5-days post fertilisation prior to that they have not fully developed, consequently only fish with the correct modification will be raised to adulthood. Animals utilised for the freezing of GM sperm and subsequently IVF will again be a Mild protocol. In comparison to IVF in humans, which can be an invasive procedure, in fish this is non-invasive as fertilisation of the eggs occurs externally. Animals that show any abnormality or the incorrect genetic make-up will be euthanised by approved humane methods.

## Application of the 3Rs

### Replacement

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

### Replacement

Unfortunately, there are no alternative “non-animal” model systems that can be used for the purpose of this licence. However, amongst all the currently used model organisms in biomedical scientific research, we have chosen a lower vertebrate model species compared with mammals, zebrafish are considered less sentient than mammalian model species.

Most experiments are conducted on zebrafish larvae that are less than 5 days old and therefore not considered sufficiently developed to be considered protected animals.

The study of genes and disease in zebrafish aims to model human disease processes as accurately as possible. Due to the complex interactions of tissues and genetic systems, and because Humans cannot be manipulated genetically for research purposes, a whole animal model is required for this purpose and zebrafish are a good substitute.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Careful consideration will be made through discussion as to whether a new GM line is required to be made or whether this may be imported from any designated establishment, which may be worldwide.

The introduction of a new stock-management database will make it easier to assess population numbers, breeding success and aged populations; this allows careful management will prevent excessive breeding of lines.

Cryopreservation of GM zebrafish sperm will ensure the protection and future maintenance and safeguarding of such GM lines. This will negate the need to keep live fish breeding through successive generations

## **Refinement**

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

### **Refinement**

New genetic modification techniques and better techniques in support of this license will be continuously sought. Similarly, better stock management techniques will be employed to prevent excessive breeding of GM zebrafish.

Where practicable embryonic or less invasive sampling techniques will be used to determine the genetic background of a zebrafish population. Developments in such techniques are not only a refinement but the employment of such techniques will reduce the numbers of fish raised to adulthood.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 212. Omentum Derived Regenerative Cells (ODRCs) to prevent Anastomotic Leak following Gastrointestinal Surgery</b>
<b>Key Words</b>	Pig, Omentum, Regenerative cells, Gel, Anastomosis
<b>Expected duration of the project</b>	1 year(s) 6 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Surgery to the bowel often involves removal of diseased tissue with re-joining of the bowel ends, called an anastomosis. The most feared complication of this type of surgery is if the bowel join fails to heal properly and leaks bowel contents into the abdominal cavity (anastomotic leak). This can occur in up to 20% of cases when surgery is performed in humans and can cause the patient to become seriously ill with a 20% chance of dying. There is a need to improve the way that anastomoses are made so as to prevent anastomotic leak from occurring and making surgery safer for patients.

In this project, we will undertake a small, preliminary study to test whether a novel technology can help anastomotic healing and prevent leakage. The technology involves removing a small piece of fatty tissue from the animal's abdomen (the omentum) and extracting the cells from the sample in the laboratory. This process takes 1-2 hours. The cells are then incorporated into a fast setting gel that can be applied around a surgically created bowel anastomosis to encourage healing. Following a period of recovery (7 days), animals treated with the new technology will be sacrificed and the anastomoses removed for testing in the laboratory to see if better healing has occurred in comparison to untreated bowel anastomoses.

**What 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 new technology does improve anastomotic healing it could be applied in humans to prevent anastomotic leak from occurring following bowel surgery. This would be of benefit to some 50,000 patients in the NHS who undergo bowel surgery

each year. It would improve the safety of surgery for patients and minimise long term problems should an anastomotic leak occur. In addition to alleviating pain and suffering for patients, if the technology does prevent anastomotic leak, there will be immediate cost savings for the NHS through a reduction in remedial treatments needed to correct an anastomotic leak.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

In this small feasibility study, we will use 12 pigs over a period of 9 months

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

Animals will undergo an abdominal operation to allow a join (anastomosis) to be created in the bowel. Each pig will have two anastomoses created. One anastomosis will be treated with the new technology and the other will not have the active treatment. This will allow the effect of the new technology to be determined. All surgical operations carry risk. In the proposed studies, animals will be at risk of the general complications of anaesthesia and surgery. This includes heart and breathing problems, blood loss, and infections. Should any of these occur, they are expected to be temporary and treatable by simple interventions. Serious complications related to the study include anastomotic leak and incisional hernia (poor healing in the abdominal wound). Should an anastomotic leak occur it will be recognised by the animal becoming unwell and the animal will be sacrificed. Should an animal develop an incisional hernia, it is unlikely that this will cause suffering within the 7 days of recovery. All animals will be sacrificed on the 7th postoperative day to allow the anastomoses to be removed and examined in the laboratory to assess the extent of anastomotic healing.

## Application of the 3Rs

### Replacement

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

### Replacement

We have shown in previous laboratory models that the technology improves wound healing. Prior to using the technology in humans, we need to determine how it performs in a living animal. Specifically, we need to know, in a small number of animals, whether the technology can be used, if it is safe, and if there is evidence of improved healing.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Because this is a preliminary study, we only need to use small numbers of animals. To keep the number of animals to a minimum we have designed our intervention such that each animal undergoes two anastomoses - one anastomosis treated with the active technology and the other anastomosis not receiving the active technology. This will allow the number of animals used to be halved.

### **Refinement**

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

### **Refinement**

The pig has an abdominal anatomy closely resembling the human. This is particularly true for the left part of the bowel (the left colon and rectum). It is for this reason that pigs are frequently used to train surgeons. Like humans, pigs have fatty tissue (omentum) within the abdomen, which is frequently absent in other mammals. We need to sample this fatty tissue to obtain cells to put around the anastomosis and encourage healing. The pig is a suitable animal to perform the type of anastomosis (transanal stapled anastomosis) we wish to study. This anastomosis has the highest leak rate in humans and will give us the best indication if the new technology has any benefit.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 213. Mechanisms of neurodegeneration.</b>
<b>Key Words</b>	Motor Neurone Disease, Aging
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Motor Neurone Disease can be caused by genetic mutations – mistakes in the internal code that shapes all living things. The aim of this project is to test if one particular mutation found in humans can cause a similar disease in rats. If so, it may be possible to use these animals in experiments to better understand the basic process of Motor Neurone Disease and determine if there are mechanisms in common with other debilitating progressive motor disorders. For instance, it is not understood why the disease mainly affects just the ability to use your muscles; nor why it occurs more in older 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)?**

There are no effective treatments for this insidious disease, and it is invariably fatal. If we understood all the changes that took place in the body before the disease took hold and as it progressed, it might be possible to help develop new treatments or cures. This information is difficult or impossible to get from human patients. The short-term benefits of this work is new scientific information on the pathological mechanisms of neurodegeneration. These results will benefit researchers in fields such as neuroscience, cell biology, physiology and gerontology. In the mid-term, a detailed molecular characterisation of the pathological mechanisms of ALS may identify prognostic biomarkers that could form the basis of new diagnostic reagents and also highlight new potential drug targets. These may be exploited by the pharmaceutical industry, to produce new diagnostic kits and initiating new drug screens. These will have mid-term benefits to clinicians and patients by improving disease diagnosis and individual prognosis. In the longer term, the results from this work will stimulate rational drug design and provide a screening platform to quantify

therapeutic potential. There are no treatments for ALS/MND or other progressive motor disorders so this would be a major benefit to patients, clinicians and the NHS. If the, as hoped, there are mechanistic links between ALS and other neurodegenerative conditions then this longer term benefit could be even more significant and could influence public health policy developments.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 1500 rats and 500 mice across a range of ages will be used in the study, which will last 5 years.

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

The human diseases we wish to study are relatively slowly progressing and starts in latter life. Human patients do not appear to suffer any pain from the disease. As the experimental animals age, we anticipate mild to moderate effects of the mutation where they find simple tasks such as walking and gripping objects more difficult. Similarly, as they age, the general physical fitness of the animals will diminish. Animals would be humanely killed before their ability to eat or drink was significantly impaired. During the study animals will be tested on behavioural tests such as their ability to walk and balance. As the animals will be accustomed to being handled these tests should not result in significant adverse effects. Sampling of blood will require a hypodermic needle, which represents a mild stress for the animal. Sampling of CSF will require the animals to be sedated for approximately 5 mins, and a small incision to be made in the skin at the back of neck, just below the head. The incision will be closed and drugs will be provided post operatively to alleviate the discomfort from this procedure.

## Application of the 3Rs

### Replacement

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

### Replacement

Humans, like all animals are made from many different tissues such as muscle, fat skin and bone. These tissues themselves are made from many more different types of building blocks or cells. The cells affected in ALS control the voluntary muscles in the body. The way these control cells, or neurons, interact with muscles is quite different between animals with a central nervous system compared to other animals. We know very little about how this system collapses in ALS and other motor disorders, so at this point it is necessary to study a vertebrate rather than the related but distinctly different systems working in non-protected animal alternative.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Rats are strong and large enough that it is possible to monitor how a disease may start and progress in an individual animal that you can monitor closely over the course of its life. This makes it possible to design more reliable experiments that use less animals to learn reliable information as does using previously characterised mouse models with known timelines of disease progression.

## **Refinement**

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

### **Refinement**

To maintain a healthy body different tissues communicate with each other by sending small signals such as hormones through the blood and other biological fluids. These signals change in response to disease, and knowing the details of these changes can help to understand how the disease is causing its effect. Changes in the constituents of the fluid surrounding the brain have been found in motor neuron disease patients. Rats are the smallest experimental animals from which it is possible to sample this fluid without killing the animal. This allows multiple samples to be collected at times before and after the disease has taken hold. Making it much easier to determine which changes are important for the disease. Motor neuron disease is a progressive fatal condition. For the purpose of this study it is not necessary for the animals to suffer the full extent of the condition leading to respiratory failure. Once clear effects are evident animals may be humanely killed. In regards to the mouse models being used in this project they possess quantifiable features and yet the disease does not affect the general cage life of the mouse. Excellent care for the animals will be provided, specialised equipment used for all the behaviour and surgical procedures and advice from vets sought immediately for any welfare issue.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 214. Biomechanical in vivo models of osteoarthritis and bone healing</b>
<b>Key Words</b>	Biomechanics, osteoarthritis, wound healing, growth factors
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Osteoarthritis (OA) is a painful and debilitating disease leading to significant joint deformity and restricted mobility. Although the exact causes and mechanisms are still unknown, OA is associated with progressive degeneration of the cartilage lining the joints and destruction of the bone supporting the joint surfaces, and is one of the major causes of pain, disability and hospital treatment.

Existing models to look into the mechanisms that OA develops use either mice with a genetic defect predisposing them to OA development (so that they spontaneously develop OA) or surgically cutting the ligaments and other soft tissues which stabilise the joint about the knee joint to cause altered biomechanics and joint deterioration. However, genetic and chemical models have been criticised because they don't realistically represent true OA, and the effectiveness of the existing surgical models is under debate. It is our thesis that the proposed model will be more effective and realistic.

Having shown a new and more realistic model of osteoarthritis in living subjects can work, this project aims to further develop, validate and apply this model. In this model, the biomechanics of the knee joint are altered by cutting the bones just beneath/above the knee, re-setting it in the wrong alignment (under anaesthesia) and allowing the bone to heal (with secure fracture fixation) so that the joint is subjected to abnormal loads and motions. This has been shown to lead to osteoarthritis (OA) in rabbits, and is a more realistic disease process since OA in humans is linked with altered joint biomechanics as well as biochemical processes.

We also aim to validate a new fracture healing treatment using a biomaterial scaffold loaded with nucleic acids that can deliver and harvest growth factors from the patient's body and release them in the fracture healing zone, to accelerate and consolidate bone healing.

**What 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 the development of a more realistic and controllable in vivo model, it will be possible to evaluate novel therapeutic and rehabilitation interventions, and to develop preventative measures or monitoring strategies. The model can then be used to evaluate drugs and treatments for OA, which can be developed and evaluated in cell culture studies before resorting to animal trials. However, there is a the mechanics and the biology of joint disease are strongly linked which can only be fully studied in animal models, and the mechanism and stages of drug therapies need thorough evaluation in vivo before they can be tested for applicability in humans. Likewise, the development of the new protein based fracture healing therapy could enhance clinical choices for delayed- and or failed healing of fractures.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

It is expected that a maximum of 660 rats will participate in this study 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 levels of severity? What will happen to the animals at the end?**

Surgical wound breakdown or infection will be minimised with the use of standard sterile surgical techniques. Also, , preventative antibiotics and pain relief will be administered pre-operatively, and will be continued post operatively as advised by the vet. The use of X-rays and doses employed and scanning will be minimal so as to avoid harm to animals and the same animal will not be imaged more than three times. Animals will be closely monitored for signs of pain and distress by trained animal care staff and veterinary surgeon, with appropriate action taken to alleviate symptoms.

## Application of the 3Rs

### Replacement

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

### Replacement

The aim of this work is to develop a biomechanically induced Osteoarthritis of the knee joint, which involves the whole lower limb in functional and dysfunctional activities and cannot be achieved in any other manner.

Likewise, bone healing is a whole body response subject to many factors only present in an animal model

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

The use of blood sampling and tissue microscopy will preserve tissues for future analysis by biochemical and molecular analysis as developed.

The existence of gait analysis data for normal healthy rats ensures that only the animals required for the surgical model and for tissue samples need be employed.

X-ray Imaging will also be used to enable studying the same subjects over a long time interval in the same animals and therefore minimise the number of animals used.

We used a technique called Power Analysis to estimate the minimum number of animals needed to give valid scientific proof of the effects being studied, and hence no more than those calculated numbers will be used. This ensures a valid study with minimum numbers

### **Refinement**

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

#### **Refinement**

Rats have been chosen because they are the smallest animal with bone and joint properties and biochemistry sufficiently close to those of humans to allow realistic OA development and treatments. The surgery will be carried out under anaesthesia using sterile techniques, and pain relief cover will be provided during the fracture healing process, to minimise the pain and distress caused to the animals. On the completion of the study, the animals will be humanely killed and histology

of the joints will confirm the effects and mechanisms of OA achieved. However, by using computerised gait analysis to measure the walking biomechanics the altered joint mechanics and disease progress can be monitored non-invasively and longitudinally. Gait analysis uses computer graphical techniques which cause no pain and little discomfort to the animals.

All animals will be pre-screened to select natural runners and exclude natural non-runners; minimising the number of animals subjected to the surgery and healing phases.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 215. Skeletal homeostasis, remodelling and repair</b>
<b>Key Words</b>	Arthritis, Stem cells, Cartilage, Bone, Regeneration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Arthritis is a leading cause of disability. Joint injury due to trauma, and obesity, increasingly common in the western world, are important risk factors. Joint destruction is the result of an imbalance between tissue breakdown, often caused by inflammation or trauma, and repair. Throughout life, stem cells are specialised cells that maintain and repair tissues and organs of our body. Our knowledge of the location and functional regulation of the stem cells in the adult joint is limited. We propose to study the stem cells naturally present in the normal and diseased joint and to investigate their role in the maintenance, remodelling and repair of joint tissues in the adult life. For such studies, no system using a dish in the laboratory would be able to reproduce the complex environment of a living organism with continuous interactions among tissue and organ systems across the whole body.

In clinical conditions characterized by extended tissue damage/loss (e.g., cartilage defects or advanced osteoarthritis), the stem cells present in the joint may not be sufficient to ensure repair. In these circumstances, the transplantation of stem cells that have been taken out of their tissues and grown in the laboratory would be necessary for replacement of missing tissue components. Studies in humans support the utility of stem cells for bone and cartilage repair. A major problem, however, is the large variability in clinical outcome, partly due to inconsistency of the stem cell preparations. There is, therefore, an unmet pressing clinical need for development of quality controls for efficacy of stem cell preparations, a prerequisite for routine use in clinical practice.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The ultimate goal of this research is to develop cell-based treatments for patients with skeletal disorders such as arthritis, including osteoarthritis and rheumatoid arthritis. These conditions are characterised by extensive damage of cartilage and bone. Current treatments are often unsatisfactory. Medications can halt inflammation but are unable to achieve regeneration/repair of the damaged tissues. Our research could lead to novel cell-based therapies for replacement of damaged tissue via transplantation of stem cells or via the administration of drugs that target the stem cells that are naturally present in our body. Over the 5 years of this project, we expect to deepen our understanding of the stem cell populations present in the joint tissues and their roles in the maintenance of joint health and arthritis development, and to use this scientific knowledge to identify and evaluate drug targets and gain insights into the mechanism of drug interventions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice, which are most used for preclinical studies and allow assessment of function via genetic modification. We have estimated to use up to 3,000 mice for experiments 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 levels of severity? What will happen to the animals at the end?**

Joint injury, osteoarthritis and inflammatory arthritis in adult mice will be induced to study the role of stem cells present in the joint in these processes. The models of injury consist of damaging joint tissues such as the cartilage (a tissue that covers the bone end in the joint and is devoid of blood vessels and nerves) through minor knee surgery under anaesthesia. Mice recover rapidly (within hours) from the surgery and return to their normal activities. Depending on the mouse type or kind of damage, there is either repair (with return to a normal joint) or development of osteoarthritis long-term. Obesity is an important risk factor for osteoarthritis and to study the role of obesity, some mice will receive a diet rich in fat that will cause weight gain. Inflammatory arthritis is achieved via injection of substances that induce an immune reaction that affects the joints in a way that is similar to the human rheumatoid arthritis. In these models, mice may receive injections or undergo blood sampling to measure specific factors or detect cells under study. Mice may also undergo scanning such as x-ray under anaesthesia to monitor the disease. These conditions are typically well tolerated, and mice are monitored regularly. Like human patients with joint disorders, mice may develop joint pain and swelling. Side effects are rare and include skin lesions at the site of injections, opening of sutures (which can be successfully re-closed), and those related to the anaesthesia. Immune-deficient mice (unable of immune rejection) will be used for transplantation of human stem cells, obtained from adult individuals and grown in laboratory dishes, to assess their ability

to form joint tissues such as cartilage and bone in vivo. These protocols are well tolerated and the risk of adverse effects in our experience is very low. Mice will be kept in protective housing and all interventions will be carried out aseptically. Appropriate anaesthetic and pain relief regimes will be given as needed according to a regime recommended by the vet. Surgery will be carried out aseptically. Animals will be humanely killed at the end of the proposed experiments and tissues will be analysed.

## Application of the 3Rs

### Replacement

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

#### Replacement

Studies in living animals are necessary to study the stem cells that are naturally present in the joint and define their role in joint tissue maintenance, remodelling and repair. None of these experiments could be performed in a dish in the lab. Cell or tissue culture cannot mimic this, as the interactions between the different tissue and cell types within the joint and in the entire body are lost.

To study stem cells in arthritis there is a requirement to look at the cells in the full picture consisting of continuous interactions across multiple cell types, tissues and organs in a living body with circulation through bloodstream of a myriad of molecules. There are no suitable experimental models of arthritis in non-protected animal species.

In order to test the ability of human stem cells to make new joint tissues including cartilage and bone, we routinely employ several assays in laboratory dishes to determine the capacity of stem cells to form such tissues. However, evidence indicates that often they are an overestimation of the true in vivo capacity of stem cell populations. Hence, normally after extensive screening using dishes in the laboratory we proceed to confirm the findings obtained in the laboratory with appropriate experimentation in living animals, as this is a required step for any clinical translation.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The procedures involved in this study are well-established and can therefore be performed in a way that provides maximum information with the minimum number of animals determined using statistical analysis.

Experiments are planned very carefully. The availability of different optimised and specialised mouse models allows choosing for each circumstance the model, strain, sex and age that best addresses the scientific question and gives the most robust and reliable outcome, thereby allowing to obtain the information sought with the minimum number of mice.

The use of internal controls whenever possible (i.e. in a mouse arthritis is induced in one knee while the other knee of the same mouse is not treated and therefore used as internal control) eliminates the variability related to each individual mouse, and reduces the number of mice needed for the study.

In vivo imaging allows studies in mice that are longitudinal with multiple assessment in the same mouse at different time-points, thus reducing further the total numbers of animals used when time-point analysis is required.

## **Refinement**

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

## **Refinement**

The mouse is the most appropriate mammalian species and also now considerably more valued in preparation for clinical studies. The procedures in this study are internationally well-established and routinely used by academic and industrial institutions for preclinical studies and assessment of novel treatments. Several similar experiments have been carried out previously and have provided sufficient information to enable us to perform them in a way that provides maximum information but minimal distress to animals. Surgery is carried out by experienced individuals using sterile techniques to prevent infections. When needed, anaesthesia and pain-killers are provided. Mice are monitored regularly and, if needed, extra bedding is provided. When necessary, mice are provided with a diet with soft food for ease of eating. Veterinary staff is always accessible for advice and assistance in matters pertaining to the welfare of the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 216. Investigating gene regulation in colorectal, endometrial and associated cancers</b>
<b>Key Words</b>	cancer mutations, DNA variants,, colorectal cancer, endometrial cancer
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

1 in 14 men and 1 in 19 women in the UK will be diagnosed with bowel cancer during their lifetime. 1 in 41 women will be diagnosed with uterine cancer during their lifetime. Mutations that cause these cancers can be inherited or, more often, acquired during a patient's lifetime. New genetic technologies have helped to discover many new mutations that increase the risk of cancer, sometimes dramatically. There are also some common genetic variants (small differences found in the DNA of many people) that can increase the risk of bowel and uterine cancer by a small percentage. The specific mutations and variants found in a patient's cancer, not only play an important role in how the cancer develops, but can also affect the way in which the cancer responds to specific treatments.

The aim of this project is to generate mouse models which mimic the mutations in human bowel and uterine cancer patients as closely as possible. This will allow us 1) to find out how the mutation/variants cause cancer and 2) test which therapies work best for each mutation. In this way we will be able to develop personalised treatments for many cancer patients.

Our lab and many others have already started to investigate some of these questions by looking at bowel cancer and uterine cancer patient samples and cell lines derived from patients. We can understand some of the mechanisms of cancer development in this way and even test out some treatments. However tumour growth and the development of malignant cancers is dependent on many factors that cannot

be recreated in a laboratory dish. For instance the immune system, the blood supply and nearby cells are all factors in cancer growth and treatment response. This means that whole animal models are needed as well as cell lines.

We will create mouse lines carrying mutations that mimic, as closely as possible, bowel and uterine cancer mutations and cancer risk variants. We are particularly interesting in studying disruptions in genes involved in copying and repairing DNA and also variations in the DNA that can change how much or little a gene is expressed in certain cells. We will then assess how these mutations modify the development of tumours and the biological pathways that they alter. We will also test existing and new cancer treatments on mouse lines carrying specific mutations or combinations of DNA copying and repair mutations. We hope that new therapies that target the immune system may work well on these types of 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)?**

These experiments will help us in two ways. Firstly they will help us to better understand how mutations and common variations in our DNA can affect cancer development. The more we understand about how changes in DNA (both in the genes and in gene regulatory regions) drive cancer, the more accurately clinicians can give a prognosis and decide which treatment options to use. Secondly they will give us very specific models to test current and new treatments on. Promising new treatments or combinations of treatments may then be able to be trialled in human cancer patients identified as having matching mutation combinations.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 8,000 mice over the 5 years of the project. The majority of these animals will be used for breeding purposes. The numbers required are because the most realistic models of human cancers are those with accumulations of multiple faulty genes or variants. Accurate models of human cancers are required to replicate the tumour biology seen in human patients and to understand the response of tumours to drugs or therapy.

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

The majority of mice used for breeding will experience only mild severity. Some mice, will develop benign tumours or cancers and these could cause predictable symptoms such as anaemia, weight loss and general ill health. However, we can often predict when these tumours will develop and will humanely kill the mice before

they cause any pain or distress, but still gain enough information to answer the questions we are asking. For some mice in which the timing of cancer development is uncertain, we will regularly monitor for signs of tumours and intervene early at the first sign of distress and for a very few mice we will keep them until they show overt signs of cancer (hunched appearance, lethargy) in order to get information about cancer progression. A subset of mice will be administered substances to induce or make their cancer worse to reflect environmental factors that cancer patients experience. For example controlled doses of gut inflammatory agents (causing eg diarrhoea and weight loss) or known carcinogens (causing eg lethargy). Some mice will also be monitored by colonoscopy or magnetic resonance imaging (MRI), again these are procedures used to monitor human cancer patients. Some mice will be given cancer therapies and since many of the therapies used for human cancer patients induce side effects, mice are likely to experience these as well (e.g. lethargy, weight loss). Overall, we expect a large proportion of our mice to have sub threshold or mild experiences but a some will experience moderate pain or distress due to their cancer, or treatment with tumour promoting or therapeutic substances. At the end of the work, all mice will be humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

REDACTED However, there are good reasons why we cannot obtain all the information we need from humans. We can use cell lines derived from human cancers to investigate how mutations modify gene function and gene regulation, and the effect this has on cell pathways known to be important in cancer but cell lines lack certain very important features. We know that the interactions between cancer cells and normal cells are vital determinants of patient prognosis and how well the cancer responds to treatment. For example, the response of the immune system and changes in blood supply can dramatically change tumour growth. Only whole animal models can reliably imitate all these factors. Lower species can share some cancer causing pathways with humans but many do not live long enough to allow tumour growth. Their organs and immune systems can also differ significantly. Mice however do develop tumours in as a result of mutations in genes known to cause cancer in humans, and powerful new technologies now allow us to recreate precise mutations, and combinations of mutations in mice. Using these models we can follow cancer progression from the appearance of the first few tumour cells and investigate how, when and where these develop into cancers. We can also treat these mice with existing or new therapies to investigate which tumours respond well and poorly to each therapy.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will design our experiments to address well designed questions and carry out calculations to make sure we use the minimum number of animals necessary to get a reliable answer to our questions. Important steps in our design will be to control for or reduce any unnecessary variability, for example age or sex of animals. We will use the most up-to-date techniques, to make our mutants since these require much less breeding. We will also use up-to-date techniques to continuously monitor our animals which means we can get more information about tumour growth from each animal, therefore fewer animals are needed overall.

## **Refinement**

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

### **Refinement**

Mice DNA shows high levels of similarity with human DNA and we have very good tools to manipulate this DNA to carry the exact mutations found in human cancer patients. Many previous reports show that these mice usually show very similar phenotypes or symptoms to the human patients carrying equivalent mutations. They also show similar responses to cancer therapies.

We have many systems in place to minimise any suffering that these animals experience. They are housed a very controlled environment with optimal lighting, heating, food and with appropriate companions. We often use “conditional” mutations that are only activated at certain times and places in the animal meaning that many animals experience no adverse effects at all. We will always aim to induce these mutations in the least invasive manner, for instance in drinking water. For those animals that do develop tumours or associated phenotypes, we are usually able to predict accurately what symptoms they will experience and when they will begin. This means that we can humanely kill animals before they feel ill or at the very early signs and still gain the maximum amount of useful information from them. We use

regular monitoring to pick up early signs and also use up to date technology to monitor internal tumour growth making it easier to time the humane killing.

When we need to administer drugs or agents to mice we always pick the least invasive route, for instance in their food and drink or injections just under the skin.

We will also use the lowest effective dose when this is known. If we are using substances for the first time we will carry out pilot tests on typically 5-6 animals, always starting with the lowest dose and only increasing when necessary.

Anaesthesia and analgesia will be used for any procedures where the pain or discomfort could last longer than a few seconds and aseptic technique is always employed for invasive procedures. To avoid repeat injections to administer long term treatments, tools such as osmotic minipumps or slow release pellets can be implanted to release drugs or agents over a set period of time.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 217. Functional and Genomic Studies of Limbal Stem Cells</b>
<b>Key Words</b>	Stem Cells; Cornea; Gene Expression; Label-retaining cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

1/ We will establish the gene expression profile of corneal stem cells compared to non-stem cells (basal and differentiated cells). To achieve this, slow-cycling stem cells and actively dividing cells are isolated from transgenic mice and the RNA is extracted from each population for sequencing to map their gene expression profiles. Elucidating the gene expression profile of slow-cycling corneal stem cells could reveal new stem cell markers to isolate and study in human stem cells for transplantation.

2/ Secondly, we will reveal the distribution of slow-cycling stem cells and their cell cycle rates after they are activated to renew differentiated cells removed by corneal scrape. This will help us to understand how slow-cycling stem cells repair corneal injury, as well as determining whether there is a hierarchical mode of turnover in the corneal epithelium.

3/ Our final aim is to create a new mouse model of limbal stem cell deficiency (LSCD), which is a form of corneal blindness that is currently difficult to treat, however, it can potentially be treated by stem cell therapies. This LSCD model is developed by physically removing the stem cells from a mouse's eye. It is a more refined model than previous studies, where the entire limbus or cornea tissue are removed as opposed to specifically targeting stem 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)?**

This work will improve our understanding of stem cell gene expression and the mechanisms by which they renew epithelial tissues over a lifetime. We also hope to develop a mouse model of corneal blindness for use in stem cell transplantation

studies. This would allow us to move to cadaver human tissue to study stem cells and their efficacy as a stem cell therapy.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 650 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 levels of severity? What will happen to the animals at the end?**

Mice will be anaesthetised before the surface of their eye is scraped with a scalpel to remove corneal stem cells through non-invasive surgery, and the clinical effects will be imaged under a microscope. We anticipate that removing these cells will lead to corneal opacity and neo-vascularization that can be treated, in the future, by stem cell engraftment. The most severe outcome will be partial corneal blindness in one eye of the mouse, however these mice are already blind from weaning age as they have an inherited mutation causing retinal degeneration. Because they are nocturnal, vision is not vital for mice as they primarily rely on hearing, olfaction and their whiskers to sense their environment, so there are not expected to be any lifestyle changes. Any ocular pain will be alleviated at the injury site by the use of general anaesthesia (i.e., isoflurane) and systemic analgesics will be used to prevent post-operative pain. Administration of DNA-labels such as EdU and BrdU will be limited to 7 injections over 7 days so that their toxicity limits are not exceeded. At the end of the experiment, mice will be killed by a schedule 1 method to study the limbus after stem cell removal.

## Application of the 3Rs

### Replacement

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

### Replacement

There is currently a complete absence of an antibody that is able to mark epithelial stem cells in mammalian tissue. H2B-GFP/K5tTA transgenic mice are the only available model to label epithelial stem cells based on the functional marker of stem cell quiescence. In this study, we aim to develop new markers that can be used to isolate stem cells from cadaver human tissue.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The gene expression and surgical experiments have been optimised at the University of California, Irvine, USA, which allowed us to determine the number of mice used in this project. To obtain enough RNA (approx. 1µg) to perform gene expression analysis of epithelial stem cells, approximately 30 mice are required for each time-point of 28 days chase. An *a priori* power analysis based on corneal opacities up to 20% corneal opacity indicates that a minimum of 12 mice are required for surgical experimentation to statistically confirm the induction of LSCD.

### **Refinement**

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

### **Refinement**

These transgenic mice are bred from the FVB strain, which are homozygous for the retinal degeneration 1 allele of *Pde6brd1*, which causes blindness by weaning age (21 days) but does not affect the corneal stem cells in this study. In general, mice are not considered visual animals as they are nocturnal and primarily rely on hearing, olfaction and their whiskers to sense their environment, therefore, corneal injury is not expected to significantly impact the lifestyle of the experimental mice.

Previous LSCD mammalian models have relied on complete excision of the peripheral corneal region or chemical burn of the entire cornea, which does not specifically target stem cells and damages the entire ocular surface. We aim to more specifically target stem cells through *in-vivo* fluorescence microscopy, so we can attempt to recapitulate LSCD and avoid any injury to important underlying or adjacent structures, such as the corneal stroma and endothelium, trabecular meshwork, or the conjunctiva, which are not implicated in LSCD.

All surgery will be performed on mice under general anaesthesia (e.g. isoflurane). Systemic analgesic and topical antibiotics recommended by the named veterinary surgeon will be used to prevent any discomfort or infection to the mouse ocular surface

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 218. Angiogenesis and vascular remodelling in disease</b>
<b>Key Words</b>	Angiogenesis, Eye disease, Cancer, Vascular biology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No

(d) protection of the natural environment in the interests of the health or welfare of man or animals;

No

(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No

(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No

(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

During foetal development, blood vessels grow into the developing tissue in a controlled manner to supply the surrounding cells with oxygen and essential nutrients. Once established these new vessels rapidly stabilise and become functional permanent structures. In most diseases, however, the structure and function of existing blood vessels is altered resulting in harmful consequences such as fluid leakage and oedema. Moreover, in many pathological situations new blood vessels grow and these, unlike during development, are highly abnormal contributing to disease progression and hindering the delivery of blood-borne therapies. It is still unclear why blood vessels grow normally during development and remain functional but in disease become abnormal or are prompted to grown in a chaotic and dysfunctional manner. Abnormal vessels contribute the severity of diseases such as age-related macular degeneration and diabetic retinopathy as well as many solid cancers. There is a great need, therefore, to find new approaches to prevent vessels from becoming abnormal and contributing to disease severity. The aim of this project is to understand what mechanisms are responsible for causing abnormal vessel formation. Such information will enable us to identify new therapeutic targets and to develop and test new drugs that will inhibit unwanted and damaging vessel growth, induce normal vessel growth where it is needed such as in ischaemic disease and ultimately reduce disease burden.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The aim of this project is to obtain a greater understanding of the disease process, to identify new therapeutic targets, to develop new therapies and undertake pre-clinical evaluation of such therapies. New blood vessel growth in diseases of the eye (known as ocular neovascularisation) and vascular dysfunction brought about through remodelling of existing blood vessels is a major contributor to the pathogenesis of several eye diseases. These include wet age related macular degeneration (wAMD), diabetic retinopathy (DR), corneal graft rejection and retinopathy of prematurity (ROP), of which wAMD and DR are the leading causes of blindness in developed countries. The overall prevalence of late AMD over the age of 50 is 2.4%, equivalent to 513,000 cases in the UK with 71,000 new cases estimated per year. Diabetes is an increasingly important condition globally that will affect an estimated 552 million people by 2030 with 22-37% exhibiting diabetic retinopathy. Therapies developed to inhibit abnormal vessel growth – the so called anti-angiogenics - have been successfully used to treat wAMD, retinal vein occlusion and diabetic maculopathy but many patients do not respond or develop resistance over time. There is also concern over the longer term safety of some of these drugs. New therapies, therefore, have the potential to reduce significantly the burden of vision loss and associated socioeconomic costs to society. In recent decades significant improvements have also been made in the 10-year survival rate of solid cancers including testicular, breast, prostate and bowel. Despite this progress, prognosis remains poor for many other cancers such as stomach, brain, oesophageal, lung and pancreatic. Moreover, the incidence of cancer in the UK has risen alarmingly by more than a third over the last 40 years. In 2011 over 331,487 people were diagnosed with cancer and approximately 85,400 died, accounting for 1 in every 4 deaths in the UK. This is more than coronary heart disease, respiratory disease and stroke, making it a major cause of morbidity and mortality. The societal, psychological and emotional cost to patients, family and friends and the economic burden is substantial, with the total annual fiscal cost to the UK being estimated in the order of £16 billion. The growing incidence of cancer and the continued refractive nature of many cancers to therapy, demonstrates the urgent need for new therapies. The work proposed under this licence is aimed at understanding why vessels go wrong, use this information to identify therapeutic targets, and to develop and test new potential therapies. Most of our work will focus on a molecule (called LRG1) that we have identified as a blood vessel disrupting factor and on a therapeutic we have developed to block its vessel damaging action. Any successful therapy also brings with it a potential economic benefit as the combined cancer/ocular market where targeting the vasculature is feasible amounts to many £billions per annum.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Choice of species, animal models and number of animals will be carefully considered based on various sources of information. Aside from our own experience and knowledge we will be further informed in our decision making by applying the

CAMARADES-NC3R's Systemic Review Facility (SyRF; <https://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf>). This is a free-to-use online platform that enables us to undertake a systematic review of relevant published information regarding the use of animals in our area of interest. It permits us to identify animal alternatives, the most appropriate animal models to use to address our questions and the number of animals needed. In conjunction with this tool we will also apply the PREPARE guidelines for planning animal research and testing therapies that will help in experimental design. Based on this information we plan to use well-established rodent models of disease, both mice and rats, in this study. We anticipate that we will use approximately 9,724 mice and 250 rats during the 5 years of this licence (total 9974).

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

Every effort has gone into reducing the need to use animals and to reduce suffering. Where feasible, we will employ an escalating go/no-go approach whereby we will only proceed if there is evidence that we need to move to a more complex system to provide further critical data. Accordingly, in the first instance we will gain as much information as possible from growing cells in culture and using tissue derived from culled animals. The complex nature of blood vessels and what controls their function is very difficult to replicate in a dish as it requires interactions of many different cell types all arranged in the correct configuration as well as potential influences from distant organs. It is imperative, therefore, that to address our hypotheses fully we utilize animal models of blood vessel dysfunction. To reduce suffering we will, in most instances, undertake studies in the eye models (moderate severity) and grafted cancer models (moderate severity) as these are generally short-lived experiments. Only when we have evidence that the preceding data warrants it, will we proceed to genetically engineered models of cancer that, whilst also moderate in severity, are more protracted. It is anticipated that using this approach will minimise the number of animals being used in severe procedures. This overall approach is designed to reduce animal usage and suffering whilst maximising the patho-biological information we obtain. During all animal procedures we will use robust monitoring to ensure that the animals fall within the permitted severity limit and do not exceed the allowed duration. Any animal that experiences adverse effects during the experiment will be treated or killed by a schedule 1 procedure. Upon completion of the permitted experiments all animals will be terminated through schedule 1 killing.

## Application of the 3Rs

### Replacement

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

## Replacement

The complexity of the vascular system we are investigating is part of a complex structure that is influenced by many cell types as well as its environment. This is impossible to completely recapitulate in more simple model systems. We always conduct as much of our work as possible in cell and tissue culture experiments and we continually review the research literature, through aids such as the on-line SyRF facility, to ensure that we encompass, where feasible, new methodology that will replace the need for *animal* work. Appropriate cell and tissue culture approaches provide us with the ability to control carefully the environment and can answer a number of fundamental questions without the need for animal research. Once such proof of principle data has been acquired, however, it is then necessary to establish whether this translates into the more complex setting in the living animal.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Pilot experiments using *cell and tissue* culture systems will be used whenever possible to provide data and reduce animal numbers. The use of living animals will be restricted as far as possible through ensuring the the correct choice of animal species and model system is made. Poorly designed experiments can lead to unnecessary animal use and so we will apply various aids to ensure that our experimental design will minimise the number of animals needed to test robustly a hypothesis. Accordingly, we will make use of the CAMARADES-NC3R's Systemic Review Facility (SyRF) that will assist us in identifying the most appropriate animal models to use and, in conjunction with a statistician, conduct power calculations that will enable us to determine likely minimum numbers required to achieve significance. We will also apply the PREPARE guidelines for planning our animal research and testing therapies as this will also help to determine the most effective and efficient experimental design and avoid bias. Animals will be bred on a need basis only.

## Refinement

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

## Refinement

We have chosen rodents (rats and mice) as they replicate most of the specialised features of the vasculature of the eye and of tumours in general. Some insight may be gained from studying non-mammals such as the zebrafish but these do not fully translate to humans. Rodents are the lowest species in which suitable models are available which can be applied to replicate human disease. We are not aware of any alternative to animal models that provide the necessary complexity to enable us to achieve our objectives.

Undertaking careful monitoring of all animals that have undergone procedures in order to evaluate welfare within the permitted limits will be a principle objective. We will apply standard established assessment methodology to minimise harm to the animals. As part of an ongoing process we also aim to identify ways in which improvements can be made by using aids such as SyRF and the PREPARE guidelines. Alongside close monitoring of outcomes, especially during pilot studies, this will ensure that refinements to the experimental design and/or methodology can be made.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 219. Cardiac disease, recovery, regeneration and ageing</b>
<b>Key Words</b>	Heart failure, Regeneration, Ageing
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Heart disease is the main cause of premature death in the UK and the biggest risk factor is age. As the population continues to live longer heart disease will become an even bigger problem. Older people are more likely to suffer from heart disease and do not recover as well following a heart attack. It is now known that the heart has the ability to make new muscle or 'regenerate'. However, we have shown that unfortunately this ability may be impaired by ageing and heart disease. We want to understand how the heart regenerates and to better understand how this regenerative process is affected by heart disease and 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)?**

As the ultimate aim of these studies is to identify new ways to treat people who have heart failure, and therefore improve the standard of life for the many people who suffer from this disease. We hope that the work proposed in this project will also help identify new therapeutics both directly and indirectly, by providing data for the scientific community who are working to similar aims. We aim to begin to present data obtained from the project at national and international meetings within the next 2 years and to be published in high quality peer review journals in the next 3 years. We believe that the data obtained from this project could influence clinical trials within the next 5-10 years. In terms of each objective. Understanding the mechanisms of cardiac regeneration and recovery. A better understanding of how

the heart repairs itself after injury will allow us to start to investigate ways to enhance this potential. This will lead to the ability to improve recovery of patients suffering from heart disease. Given the ultimate potential for these novel therapeutic approaches, this project has a clear route to have beneficial societal and economic impact. The role of senescence in heart disease and ageing. As we age our cardiac function declines, we have a higher chance of suffering from heart disease and if we do our chances of recovery decrease. As the population continues to age this will present a significant societal and economic burden. If we can understand why the heart ages this will help in the discovery of therapeutics to prevent these processes. Enhancing cardiomyocyte regeneration. The aim of the two objectives above is to identify the mechanisms of cardiac recovery and age related disease. This will allow the discovery of potential therapeutics. The effectiveness of these potential treatments will require testing. The final objective is therefore to use animal models to analyse the effect and effectiveness of new and novel therapies for the treatment of heart disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The mouse will be used as our model and it is estimated that no more than 3000 adult mice will be used throughout the 5 years of the project licence These are genetically modified mice which allow us to target genes of interest in particular cells of the heart. The majority of these mice are used solely for breeding or to produce offspring for experiments.

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

Most of the mice used in this project will need to be identified by examining the mouse's DNA. This will be extracted from an ear clip from each mouse. This procedure is quick and should only cause mild, short-lived pain. Delivery of substances to rescue the clinical problems will be given via the least stressful method possible e.g. via the food or drinking water. Alternatively, where injection is required, multiple doses will be given using a minipump. Ligating a coronary artery will be used to model a heart attack. The surgery is complex and on some occasions this can lead to respiratory distress. If this occurs the animals are humanely killed. Some animals will be imaged using MRI or fluorescent methods, and these imaging methods are not normally associated with adverse effects. Cell transplantation studies have the potential to lead to the formation of tumours, animals will be closely monitored and if rapid tumour growth is detected they will be humanely killed. We keep within moderate severity limits and all animals are humanely killed at the end of the work.

## Application of the 3Rs

## Replacement

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

### Replacement

We have considered using non-living alternatives to our mutant mouse models but at present this is not possible. The advantage of the mouse models is that they replicate important human diseases. We can then study these mouse models in a more complete manner than is possible in humans, gaining valuable insights into human diseases with the ultimate aim of relieving suffering and death in patients.

The main non-animal alternative to these studies is to use cells in culture. Unfortunately, these culture systems currently cannot accurately represent a complex organ such as the heart which is made up of a number of different types of cells and in which the 3-dimensional structure is required for its function.

Further, a number of groups use Zebrafish as a model organism. However, as Zebrafish have the ability to fully regenerate their hearts following injury these are unsuitable for the study of the effects of ageing and disease on cardiac recovery.

Where appropriate (for example when investigating processes that occur within individual cells) then cell culture experiments are used.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We have selected Protocols which maximise the benefit from each animal such that the minimum number of animals will be used.

We use the minimum number of animals required for our experiments and regularly consult a statistician for advice. We use online tools (e.g. <https://eda.nc3rs.org.uk/> as discussed in Nature. 2016; 531(7592):p128) to predict group sizes needed to detect differences with statistical significance.

## Refinement

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

## Refinement

Mice are being used in this project because this is the simplest organism that has a similar heart and blood vessels to human. This means they can be used to investigate the roles of different genes in cardiovascular function and can mirror the events that occur following a heart attack.

Furthermore, the mouse is the best characterised and most widely used experimental model, and there are a number of genetically modified mice available which are required for our research. There is also an extensive literature of baseline studies using these models and we can correlate them directly to *in vitro* studies.

At every stage we take care to ensure minimum harm to the animals and regularly review our refinement measures to keep the highest standards that we can. Refinements in this licence include (i) taking advantage of special genetic techniques, the majority of mice are indistinguishable from wildtype mice, and detrimental mutations are introduced only for the limited time period of the experiment, and in the limited numbers of animals necessary; (ii) using appropriate anaesthesia and pain relieving drugs the protocols cause the minimum possible discomfort; (iii) any animal found to be showing signs of distress despite the provision of pain relieving drugs will be promptly and humanely killed.

Finally, our methods have been selected to deliver the maximal benefit whilst keeping animal suffering to a minimum. Techniques likely to cause suffering or major distress (cardiac injury model) are done under general anaesthetic.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 220. Immune responses in helminth infections</b>
<b>Key Words</b>	Infection, Immunity, Immune regulation, Tropical Disease
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

More than 2 billion people are infected with a parasitic worm. This is particularly the case in poorer countries, and many people are currently treated with deworming drugs. However, these drugs do not stop most people from becoming quickly re-infected. An alternative approach is to develop vaccines against these parasites, but no human anti-worm vaccines exist. One reason for this is that these worms are able to turn-off our immune response, and so prevent our body from killing them. We want to try and stop these worms from inhibiting our immune system, so we can then kill the parasite. We will try this in normal mice and in mice that contain human immune cells. This way, we can examine whether our treatments stimulate human cells to kill these parasites.

**What 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 help us understand better how parasitic worms manipulate our immune response to their benefit. In the longer term, it is possible that these findings may contribute to the development of new drugs to treat human parasite infections. It is also likely that these findings will apply to parasites of livestock, so potentially leading to better livestock welfare and increased food production.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We estimate we will use approximately 300 mice per year. The majority of these (~80%) will be infected with a parasitic worm to help us understand how we can stop these infections. The remaining animals will be used to study how the parasite alters our immune response.

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

In humans, infection with a parasitic worm can cause ill-health that develops over a long period of time. This is also true in laboratory mice, where infection results in a long-term disease (rather than death). We will ensure that the mice do not become very ill by keeping infection levels low. We will be giving mice treatments that we hope will reduce the level of infection. Some experiments will involve mice being given bone-marrow transplants. This procedure can make mice more likely to get a bacterial infection, but we will prevent this by giving the mice antibiotics and housing them in a extremely clean environment. If mice appear to be suffering, humane endpoints are used and all animals will be euthanized using an approved method at the end of an experiment.

## Application of the 3Rs

### Replacement

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

### Replacement

In order to study parasitic worms, we need to keep their lifecycle going in the laboratory, and this cannot be done without an animal host. How parasitic worms are killed involves lots of different immune cell types and tissues, and it is necessary for us to study this in a whole organism.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

REDACTED We will use statistics to estimate how many animals to use in an experiment, and then check these numbers in the light of new data. More broadly, new scientific techniques allow us to generate ever greater amounts of data from smaller numbers of animals.

### **Refinement**

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

### **Refinement**

The mouse is the best small animal host for these parasites because they naturally infect mice, develop as they would in a human, and cause disease that is very similar to that which occurs in human infection. The mouse immune system is well characterised and has great similarity with the human immune system. Differences between mouse and human immune cells can be examined using mice containing human immune systems. We constantly monitor the welfare of these mice and we use humane endpoints if they become sick.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 221. DNA repair in development and tissue homeostasis</b>
<b>Key Words</b>	stem cells, ageing, neurodegeneration, DNA repair
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project has 3 aims:

1) to understand the DNA repair mechanism underlying Fanconi Anaemia (FA), a human genetic illness (currently incurable); 2) to uncover sources of natural factors that we produce, that damage our DNA; 3) to understand how these molecules alter our genomes in fundamental ways.

FA patients stop producing blood and also have a very high predisposition to cancers and a cure is yet to be discovered. Over the last ten years, our research with cells and animals (mouse) has led to the discovery of a natural source of DNA damage that could explain why FA patients stop producing blood and develop cancer. These natural substances are known as aldehydes which are produced in our body and they can damage our DNA. First, cells are equipped with factors that repair this damaged DNA, in order to keep the genetic information intact in our cells. Fanconi Anemia patients are unable to repair some forms of DNA damage. It is the accumulation of damaged DNA that ultimately leads to the loss of blood and the onset of cancer. Second, other proteins are tasked with the “mopping up” of aldehydes. It is the joint action of DNA repair and aldehyde detoxification that allows our bodies to remain cancer-free and produce blood for several decades.

We have shown that the blood stem cells (that reside in the bone marrow cells and that produce blood throughout life) are easily damaged by these aldehydes. Thus, blood stem cells need both efficient DNA repair and aldehyde clearance to remain healthy and continue to make fresh blood throughout life. We want to know if other organs also protect their DNA in the same way: the skin and the brain are two major tissues for which we have preliminary data supporting this hypothesis.

We think that, as we get older, aldehydes (or other chemicals) produced in our bodies accumulate and might be a contributing factor to ageing. It is also important

to mention that aldehydes present in our environment (alcohol, food, air pollution etc) could play a role. We will develop mouse models to study this phenomenon.

Finally, we will investigate how aldehydes can impact the immune system (the cells that help combat infection by killing germs and producing antibodies). Indeed, a mutation in the detoxifying gene *ALDH2* is very common among Japanese and South East Asian individuals (over 400 million people). Paradoxically, preliminary work suggest that having a mutation in *ALDH2* helps to fight an infection. We will test this hypothesis in mice by mimicking 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)?**

It is clear that being able to fix damaged DNA is crucial: patients of FA develop anaemia and have a 1000-fold risk of cancer. By understanding how DNA is repaired by the FA proteins in a small mammal like the mouse, we will be in a better position to understand cancer and loss of blood production. This will benefit research focussing on other genetic diseases where DNA repair is also faulty. Lastly, the identification of new sources of DNA damaging agents (environmental, dietary or produced in our bodies) could have public health implications.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We use genetically altered mice in our research. We anticipate that we will need ~100,000 animals over the next five years. This estimate is based on our usage in the past decade.

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

All the mice generated throughout this programme of work are ultimately killed. Because we breed mice to obtain mutants, a large number of mice do not carry the mutation and are of no use (so-called wrong genotype). These animals are killed as early as possible. The mice that are useful to us are used in experiments and are killed at the end of the experiments. We anticipate that some of our mice may suffer developmental defects, loss of blood production (anaemia) and cancer. This will happen while breeding animals to produce mutants, and by letting these mice age. In the last ten years we have developed accurate methods to measure signs of disease in mice and from these signs to predict outcome. This allows us to kill mice before their welfare is compromised, allowing us to gain maximum information from every mutant mouse we generate. A first example of experiments we perform is bone marrow transplant, similar to what is done in humans. Here, “donor” mice are killed and their bone marrow is harvested. “Recipient” mice are exposed to radiation to destroy their bone marrow. Cells from the donors are injected in the recipients to allow them to continue making the blood and survive the irradiation. The blood of

recipients is analysed every 4 weeks for 4 months to assess the capacity of donor bone marrow to produce blood. After 4 months, the recipients are killed. Adverse effects: irradiation often leads to transient weight loss. If the donor bone marrow is not rejected, the recipient makes a full recovery from the irradiation. Rarely, the transplant is not successful and 10-14 days after the irradiation the mice show signs of radiation sickness and are killed promptly to avoid suffering. A second example of experiments involves giving alcohol to the mice. This is because inside the body, the alcohol is converted into an aldehyde that will cause DNA damage. By treating mice with alcohol, we can study how DNA is protected and repaired. Usually, the alcohol is given by replacing water with a mixture of alcohol and fruit juice to make it palatable for the mice. Another way to administer the alcohol is to inject a dose into the abdomen, using a syringe and a very fine needle. In some instances, we will inject alcohol in pregnant female mice. This is to study the effect of alcohol and aldehyde during pregnancy. In this type of experiment, the mice are injected at a time equivalent to the first trimester of human pregnancy. The pregnancies are terminated a week later or in some cases, they might be carried to term. Pups that are not healthy will be humanely killed. Adverse effects: the injection procedure results in mild and transient discomfort. When treated with alcohol, the mice are unsteady on their feet, hunched and their fur bristles. These symptoms cause mild and transient discomfort and within 30 minutes to 1 hour, the animals have made a full recovery. Finally, we want to understand how the immune system might be a source of aldehydes. To this end, we will perform experiments where mice will be given infectious agents to trigger an immune response. Since we do not know which pathogenic agent(s) might lead to the production of aldehydes, we will need to test models of viral and bacterial infections. Besides infection, inflammation is also a possible source of aldehydes. We will therefore perform experiments where mice will be given drugs that can induce an inflammatory reaction. Adverse effects: the effects of infection and inflammation are variable and depend on the nature of the infectious/inflammatory agent. Generally, weight loss and subdued behaviour are to be expected. Mice will be closely monitored and humanely killed if the severity limit is to be reached, as defined by the humane endpoints for each model of infection or inflammation.

## Application of the 3Rs

### Replacement

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

### Replacement

The main reason for the use of this animal model is that we intend to investigate how such DNA repair pathways enable normal development, help mammals to deal with

common toxins present in our environment and diet, preserve stem cells and finally protect against DNA changes that lead to cancer.

It is really only possible to study the development of embryos/fetuses (pregnancy) in the context of a whole animal. Furthermore, to study stem cell biology and cancer in a way that can be compared to the human situation, it is necessary to use animal models that are mammals like us humans. For these reasons, the mouse is the best model at our disposal.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

When we plan an experiment, we make sure we use the least number of mice that allows us to make a valid observation (confirmed by statistical calculations).

We carry out small pilot studies to refine our experiments.

All of our Fanconi mice are sterile and born at low ratios compared to what is expected. This necessitates large breeding programmes with many mice with the incorrect mutation being generated (wrong genotype). Over the past 3 years we have devised strategies that allow us to greatly reduce the number of mice that we have to breed to get animals with the useful genetic modifications.

Finally, we will cryopreserve our strains when they are not needed so that we can thaw out embryos and produce new mice when needed. This technique means that we do not need to maintain many mice alive.

## **Refinement**

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

### **Refinement**

We use the mouse as a model because it is a mammal and its genome (DNA sequence present in each mouse cell) has been decoded and is very similar to the human genome.

The development of a mouse and the function of their stem cells is also comparable to that of humans.

Our mouse models are the best available models to study the effects of DNA damage caused by aldehydes. Furthermore, these are the only mouse models that recapitulate the key features of Fanconi Anaemia as seen in humans, which makes

them relevant models. For the first time these models enable us to study the physiological role of the Fanconi DNA repair pathway.

As mentioned above we have invested in genetic modifications that allow us to control where/when the mutation has an effect. This allows us to further refine our models.

In these mice, the DNA repair pathway can be “switched off” in response to an inducing agent (e.g. tamoxifen). This allows us to generate mutant mice when we need them, further reducing the possibility that the mice will develop disease when not in an experiment.

As outlined above, our mutant mice that may develop signs of disease will be identified very early (14 – 21 days old). These mice will be monitored carefully by daily inspection for signs of disease and also through weekly weighing. Mice that develop signs of disease will be killed and analysed promptly to avoid unnecessary suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 222. Investigation of serotherapy as an alternative treatment for streptococcal necrotizing soft tissue infections</b>
<b>Key Words</b>	Therapeutics, Necrotizing, Mice, Streptococcus pyogenes
<b>Expected duration of the project</b>	2 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Necrotizing soft tissue infections (NSTIs, commonly known as “flesh eating bacteria”) are caused by bacteria which release tissue destroying toxins. Of the people who contract these infections, 34% die, while all survivors suffer permanent physical disabilities and disfigurements that require prolonged care and NHS investment.

Once NSTIs take hold, they spread rapidly (>1inch/hour) and are 100% fatal without rapid and extreme surgical intervention (amputation, tissue removal) in a matter of days or hours.

Current treatments such as antibiotics, target the bacteria and not the toxins causing damage, and thus extreme surgery is the only assured method of containing disease spread.

The aim of this project is to determine if either proteins called antibodies, commonly used in antivenoms to stop toxin effects in people bitten by venomous snakes, or protein inhibitors, which disrupt the function of certain toxins, can be used to limit NSTI bacteria toxin damage and spread. It is hoped such a therapy will be easily applied to suspected cases of NSTI, to stop tissue damage before 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)?**

The key benefit from these experiments will be the potential for an early intervention therapy, to be applied at the first suspicion of NSTI, to reduce the need or limit the extent of surgery required. Such a therapy would substantially improve survival and subsequent quality of life for patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

2050 mice over the course of two years. These numbers will allow us to: 1) Assess multiple interventions. 2) Ensure we have statistically accurate and robust data. 3) Develop our model to reduce the numbers of mice required in any subsequent testing and to reduce the level of pain and discomfort experienced.

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

The animals will be infected with *Streptococcus pyogenes* bacteria, which is the most common cause of NSTIs in humans. Due to the tissue destructive nature of NSTIs, the expected level of severity for these experiments is severe. Infected mice will develop a necrotic wound at the site of injection within 2 days. Depending on the inoculating dose and route of infection, some animals will develop sepsis-like (severe bacterial infection of the blood) disease and rapidly reach the point where they will be euthanised (around 1 day post infection). In all cases, euthanasia will occur at a point when animals showing moderate clinical signs, before they become moribund and are likely to die. All animals will be euthanized at the end of predetermined infection protocols. This will allow samples to be retrieved from mouse blood and tissues so we can assess the effectiveness of experimental interventions.

## Application of the 3Rs

### Replacement

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

### Replacement

NSTIs are a complex disease involving invasion, disruption and damage of many different tissues and organ systems (e.g. muscle, circulatory, respiratory and immune systems). It is currently not possible to replicate multiple different tissues and organ systems outside an animal model. Whilst some non-protected animal alternatives do possess some of the above features, they cannot accurately represent the full spectrum of NSTI infection and allow a thorough analysis of proposed interventions.

Despite this, we will extensively test our candidate interventions in simple non-animal laboratory experiments, to ensure that the intervention, in a controlled environment, does stop toxin function. This will allow removal of any intervention that will not work in an animal model.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Our experimental design is such that if an intervention fails at a certain step, it will not be taken forward to a more subsequently more severe test – reducing the number of animals used overall.

We will extensively assess all our candidate interventions in laboratory tests to make sure we only test therapies with the best chance of reducing infection and symptoms in mice. This reduces the number of mice used as it ensures no mice will be non-productively used on poor candidate interventions.

For all experiments, we will use the minimum number of animals based on prior experience and statistical calculations.

Throughout our experiments, observations will allow more confidence in our statistical predictions, further reducing the numbers required for robust, accurate data. For example, in experiments where we need to determine the impact of an intervention, we will run small pilot studies to assess the variation, which we will then use in statistical calculations. Small pilot studies will also quickly identify any intervention in which it is clear provides no protection, therefore reducing the number of animals subjected to it.

## **Refinement**

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

### **Refinement**

Mouse models for NSTIs are well established and have extensive data on how infection affects mice and what to expect during infection.

This knowledge allows us to apply refinements to the experiments which otherwise would not be possible with a new animal model. For example, refinements include; implementation of shorter experiment lengths (to a few days, or shorter), apply pain relief throughout and, in the case of lethal infection, apply the earliest termination of

the experiment prior to animals becoming moribund, without compromising the outcome of the experiment.

General measures to minimise harms to animals include:

Clipping of hair and Initiation of infection whilst under anaesthesia to minimise distress.

Application of strong, long lasting pain relief prior and throughout infection.

To minimise length of suffering, application of euthanasia at points well before animals become very sick

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 223. Assessing pro- and anti-inflammatory mechanisms of chemicals in zebrafish</b>
<b>Key Words</b>	Toxicology, drug safety, inflammation, zebrafish
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Inflammation is a major factor underlying many different diseases. Anti-inflammatory drugs, such as aspirin or ibuprofen, are used daily by millions of people worldwide, both alone and in combination with other drugs. Recent research has suggested that also other types of drugs, outside the traditional group of anti-inflammatory medicines, can exert anti-inflammatory effects in the body. Understanding the exact secondary mechanisms by which these drugs work may significantly improve their clinical use. The overall aim of this project is to characterise the pro- and anti-inflammatory mechanisms of existing human drugs using zebrafish as the experimental model. The two objectives of this project are:

Objective 1. To determine the effects of common anti-inflammatory drugs on healthy zebrafish.

Objective 2. To determine the effects of chronic low-grade inflammation on zebrafish, and to test the ability of different classes of drugs to prevent inflammation-mediated 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)?**

The primary potential benefit of this project will be the generation of new knowledge about the pro- and anti-inflammatory properties of existing drugs. Ultimately, this project will help to improve doctor's decision-making regarding the right choice of drugs to prescribe as well as safe combinations of anti-inflammatories and other drugs.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use both typical and genetically altered strains of zebrafish. We estimate that we will use approximately 10,300 zebrafish during the course of this 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 levels of severity? What will happen to the animals at the end?**

We propose five different protocols. 1) The maintenance and breeding of the genetically altered fish we intend to use in this project pose no harm to the animals. At the end of the optimal breeding period, most fish will be humanely killed. However, some of the genetically altered animals may be supplied to other establishments for use, helping to minimise the use of animals. 2) Generation of genetically altered zebrafish lines involve the alteration of the genetic characteristics of the animal. Specifically, we will insert fluorescent tags in specific cells making them more easily visible under the microscope. This procedure should not cause adverse effects or abnormal alterations of the physical characteristics of the fish. However, newly generated embryos and larvae will be closely monitored to ensure that any fish displaying abnormal physical features is humanely killed as soon as possible. 3) Drug exposure for testing therapeutic or toxic effects is usually carried out in healthy fish by immersion in water containing the desired amount of drug. At the end of the exposure period, fish are assessed for any specific physiological or molecular changes potentially associated with drug exposure. The test used allow us to quantify various changes, including effects on the immune, cardiovascular, and gastrointestinal system, as well as on behaviour. Following these tests all animals are humanely killed. Drug effects are predicted to be of mild or moderate severity. 4) Drug effects in healthy animals can be different than those caused in animals with a given disease condition. Hence, we also test drugs in fish in which we have induced chronic inflammation. This level of inflammation is not anticipated to cause any severe effect. As far the drug exposure is concerned, the expected adverse effects are the same as described above. The overall effects of the treatment is expected to be of mild or moderate severity. Following final effect assessment all animals are humanely killed. 5) Before initiating a study with fish, we generate predictions using computer models so that we minimise, or even eliminate, the risk of causing severe effects during our experiments. However, in some cases we do not have enough information to generate computational predictions. In those cases, before starting large experiments with animals, we will perform small-scale experiments to make sure that the drug concentrations we select do not cause any severe adverse effects on fish. Following final assessment, all animals are humanely killed.

## Application of the 3Rs

### Replacement

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

### **Replacement**

Many of the drug-mediated effects that we will investigate in this project emerge from the interplay between biological responses at many levels in the body. In vitro systems are not able to capture these complex responses, neither they are able to predict the effects of long-term drug administration. Where possible, we will maximise the use of the vast amount of existing in silico/in vitro/in vivo data generated during drug discovery and development, and we will apply computer modelling so that we will perform animal experiments only when strictly necessary to advance our current understanding of drug mode of action.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Every aspect of the project and of the experimental design has been thought through with the goal of reducing animal use. For the breeding of zebrafish, we will ensure best practice husbandry methods to reduce natural losses. For the generation of genetically altered fish, we will make sure that we use the most efficient and state of the art methods that optimize transmission of the fluorescent tag proteins and reduce the number of excess, non-genetically altered animals. For the exposure experiments, we will design the most statistically robust designs in collaboration with our in-house mathematician/statistician.

### **Refinement**

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

### **Refinement**

This project uses the zebrafish as substitute for mammalian/human models. Most zebrafish organs perform the same functions as their human counterparts, and their immune systems are similar. A major strength of the zebrafish model is that immune and inflammatory responses can be observed in the living organism using fish that express fluorescent markers. This approach allows us to establish the relationship between inflammatory processes and changes in organ physiology and functions. We will minimise any potential animal suffering by using the best husbandry practices possible, for example ensuring optimal environmental conditions (e.g. water temperature, water quality, day length and light levels) and daily visual inspection of fish to promptly identify any possible welfare problems. The

experiments in which we induce inflammation will mainly involve low intensity long-term inflammation; this level of disruption is not predicted to affect the normal day to day life of the fish. Before testing any drug, we will carefully consider all existing information generated during drug discovery and development, so that we will be able to expose fish to doses of drugs that are already considered to be safe in humans. We will humanely kill animals showing symptoms such as, infection, abnormal swimming behaviour, poor body condition in order that they do not suffer.

## JUNE 2018 - NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 224. Production of Pregnant Time Mated Rabbits using administration of Luteinising Hormone</b>
<b>Key Words</b>	Time mating, rabbits, reproductive toxicology, luteinising hormone
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

To supply groups of rabbits in early pregnancy to users who continue work under their own project licence authority e.g. these rabbits go onto regulatory studies evaluating the effect of test compounds on pregnancy.

To ensure high success rate of pregnancy we administer luteinising hormone (LH) to the doe immediately after successful mating.

This hormone injection is to facilitate successful conception. It would be a waste of animal life to undergo scientific procedures only to find that the animal is not pregnant and the data needed cannot be collected.

**What 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 rabbit has many advantages as a non-rodent and second model for assessing the effects of toxic agents on fertility, developmental toxicity and teratology. In some respects the rabbit extraembryonic membranes more closely resemble the human than do rodents. The animals supplied under this licence will, therefore, aid the work carried out on safety testing of compounds to prevent and/or treat diseases. Luteinising hormone aids the onset of ovulation to increase the chances of pregnancy. It helps to ensure that ovulation successfully occurs (the stimulus associated with mating may not be enough) and thus maximises the potential pregnancy rate thus ensuring a successful regulatory acceptable study and avoiding animal wastage. Regulatory requirements stipulate that for certain studies pregnant animals are used for testing compounds. Animals are only injected with the LH to order, thus ensuring the minimum number of animals are supplied to clients. The LH aids the onset of ovulation to increase the chances of pregnancy.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rabbits, approximately 7,000 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 levels of severity? What will happen to the animals at the end?**

Luteinising hormone will be administered by an injection into a superficial vein by a licensed technician. No adverse effects are expected from the administration of the LH to the rabbits and is classified as a mild procedure. All licensed technicians giving the injection are trained and competent, this ensures a successful procedure and reduces stress to the animal. The animals are then placed back into their original environmentally enriched housing and monitored until despatch to customer. Animals will be supplied into the project licence authority of other establishments in the UK and bone fide establishments abroad to contribute to the assessment of any adverse effects on reproduction of potential new medicines and other chemicals.

## Application of the 3Rs

### Replacement

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

### Replacement

The animals supplied under this project licence are generally required for toxicology studies on pregnant animals, including the embryonic and foetal development of the offspring. Currently this research cannot be replaced by non-animal methods.

The studies are a chemical, pharmaceutical and crop protection regulatory requirement and therefore part of the development process.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Rabbits will only be time mated and treated with the luteinising hormone to order e.g. for specific regulatory studies.

### Refinement

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

## Refinement

The choice of rabbit for use on this licence is governed by the industry regulatory requirements and is the preferred non rodent mammalian choice. The rabbit has many advantages as a non-rodent and second model for assessing the effects of toxic agents on fertility, developmental toxicity and teratology. Rabbit extraembryonic membranes closely resemble humans and differences in maternal embryonic exchange contribute to understanding mechanisms of action for developmental toxicants.

The collection of in-house production data assists in refining our breeding processes in order to understand the bucks' performance within the breeding colony, this assist the selection of best performing studs for time- mating, to help ensure customers receive pregnant animals.

Information on success rates provided by the customer will allow unsuccessful males to be removed from the breeding colony. We also maintain records on the environmental conditions in the facility, and also of the animal technician carrying out the technique so that should unsuccessful pregnancies occur we can investigate the cause.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 225. Studies of anti-malarial drug and vaccine targets</b>
<b>Key Words</b>	Malaria, Plasmodium, Gene-function, virulence, therapeutic
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Malaria remains one of the most serious challenges to global human health and new approaches to the development of anti-malaria therapies are urgently needed. Our research aims to address this challenge by identifying and investigating the function of malaria parasite proteins that play important roles mediating parasite development and virulence and so are potential therapeutic targets. To achieve our objectives, we will take advantage of relevant and amenable mouse malaria model systems which share many infection and disease characteristics with human malaria 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)?**

The major benefit from these studies lies in increasing our understanding of parasite biology and infectivity, and, importantly, in validating and prioritising new targets for anti-malaria therapeutic development.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use approximately 5650 mice over a 5 year period to accomplish the project objectives

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

The expected adverse effects will result from malaria infection as we need to use in vivo models of malaria to achieve the objectives of this project. In a minority of cases, mice may experience severe clinical symptoms, including hypothermia, anaemia, reduced activity & responsiveness. However, severe disease is usually transient, and almost all mice make a rapid and full recovery. Importantly, all infected mice are closely monitored during disease (at least twice daily) and mice not expected to recover are immediately euthanised. In this way, no individual will experience > 48-hours severe disease. Nevertheless, the majority of infected mice (approx 90%) are not expected to experience any severe symptoms. We work closely with veterinary staff (consulted whenever unexpected adverse effects are observed), and the endpoint for all mice is a humane method of killing.

## Application of the 3Rs

### Replacement

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

#### Replacement

The interaction between malaria parasites and their mammalian hosts, including humans, is highly complex and is dependent on an appropriate immune response and the structural integrity of the vascular system and tissues in which the parasite accumulates. Therefore, to fully understand the role of parasite genes mediating interactions that contribute to parasite virulence and immunity to malaria, they must be studied in the context of the complete malaria parasite life cycle; in the liver following transmission and in the bloodstream during proliferation. These studies cannot be pursued in detail in humans for ethical reasons and there is currently no *in vitro* system that can recreate these complex interactions between mosquito, parasite and host. Therefore, the objectives of this project can only be achieved through animal research.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We calculate the minimum numbers of mice that can be used to achieve our objectives by drawing upon our extensive experience in the design and analysis of molecular malaria experiments and by analyzing all available relevant data sets

(including our own previous and pilot studies) which can provide indications of expected effect sizes and observed levels of variation between experiments. We then use advanced statistical and modelling methods to calculate the size of experimental groups and to maximize the quality of the data obtained from each mouse used. We have also developed new fluorescence based and multiplexed technologies to generate and functionally examine transgenic parasites. These new methods significantly reduce the number of animals used (~50-60%) while still delivering statistically significant data. We also work closely, and share resources, with other malaria researchers to reduce overall mouse numbers used and to ensure that each mouse is used to answer as many research questions as possible.

### **Refinement**

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

### **Refinement**

Malaria infections in mice reproduce many of the important features of malaria in humans and are therefore the most appropriate choice of model system for studying aspects of parasite biology and parasite gene function that may have relevance in the design of anti-malaria therapies in humans. I have worked to improve and refine mouse models of malaria throughout my career and, in particular, have pioneered new molecular techniques that have allowed us to move away from using mouse models of malaria that give rise to severe disease, to using a malaria model that more often causes mild or moderate disease symptoms and more closely reflects the complexity of disease and symptoms of human malaria. These refinements have allowed us to obtain more relevant data using fewer animals at a lower degree of severity. Furthermore, we continually revise experimental design and monitoring to further reduce the number of mice used and to minimise suffering, whilst still achieving our project aims.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 226. Investigating the status, migration, connectivity and ecology of Elasmobranch populations.</b>
<b>Key Words</b>	Elasmobranch, shark, skate, tagging, migration, management
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Several species of elasmobranchs (sharks, skates and rays) in Scottish waters, and the wider North East Atlantic, have suffered severe declines in numbers and range contraction with at least one species that will be the focus of this research, the common skate *Dipturus batis*, being declared locally extinct. Scotland has a higher proportion of threatened (designated as either Vulnerable, Endangered or Critically Endangered by the IUCN) of elasmobranch species (23%) compared to the global average (17%).

Due to the relative insignificance of elasmobranchs in our commercial fisheries, there has been little effort on data collection for many species and 18% found in Scottish waters are listed as 'Data-deficient' by the IUCN. This lack of information has also prevented several species being put on SNH's Priority Marine Feature List, promoting them as a conservation concern.

The main aims of this project focus around generating much needed data on the elasmobranch species in Scottish waters (and the wider UK) with special focus on movement and habitat use. Special focus will be given to how elasmobranchs use the habitat within an MPA and how effective this form of management is for such large animals that have the potential to undertake big migrations. Choice of species

is determined by conservation priorities as directed by the Scottish Government, notably through Scottish Natural Heritage (SNH) Priority Marine Feature list and by the International Union for the Conservation of Nature (IUCN) Red List. No species will be used that appear on Annex A of Council Regulation EU 338/97 or CITES Appendixes I or II.

One of the best ways to understand how elasmobranchs move and interact with their environment is to use tags to track an individual's movements. Tagging may or may not include anaesthesia depending on the specific welfare benefits of doing so. For example, for surgical implantation of tags, anaesthesia (either full or local) is required, but for external attachment of tags, notably on large sharks and skates, anaesthesia is not practical or necessary allowing tagging to be a quick process, returning the animal to its natural environment quickly. The tagging methods have been chosen to be as mild, with as little short-term suffering as possible. Highly refined technical solutions for catching, handling and holding the fish that minimise stress, discomfort, and damage will be used.

Types of tag:

The aim of this part of the project is to quantify the behaviour, movement and environmental experience of free-ranging elasmobranchs in the wild with a view on spatial management measures. This will be achieved by the use of conventional and electronic tagging technologies. The tags are of five types;

- data storage tags (DSTs) which record temperature and pressure (depth),
- acoustic 'pinger' tags that relay a coded signal to hydrophones positioned in the area, thereby allowing passive monitoring of tagged fish movements,
- 'pop-off' satellite tags that record data and at a pre-programmed time detach from the fish and send that data via satellite.
- PIT tags that carry a unique electronic identifier.
- Conventional (non-electronic) 'Floy' identification tags or similar.

Tag type will be carefully selected to ensure that they have as small an impact as possible on the tagged animal and that as few tags as possible are deployed while being appropriate for answering the research question. The number of tags to be deployed is determined on the basis of past experience with tag return rates and the likelihood of obtaining sufficient data for robust results.

To maximise the cost/benefit of captured animals, a small tissue or blood sample will in some cases be taken. This is to allow for genetic analysis that can provide more information on elasmobranchs' movements and the connectivity between populations and areas. Genetic material may also provide information about the impacts of spatial management on a population and act as a precursor in identifying how a population will respond to rising sea temperatures.

**What 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 data for advising on the most effective management measures for the conservation of the study species as well as refining handling and procedure techniques to maximise post-release survival. Effective management can result in the long-term population recovery and recolonization of former ranges, helping to maintain a healthy and balanced marine ecosystem. The data also has the potential to allow for the re-classification of several species from data-deficient (by the IUCN) to a more informed conservation status, allowing conservation priorities to be identified and focus management and research on species that are in urgent need of protecting. The data will help inform models that allow us to predict a species range and distribution beyond the study site, helping to guide future research efforts. For example, in species that are highly mobile, initial identification of areas where species repeatedly visit or remain resident for long periods of time suggest that spatial management may be suitable tool to contribute to the species conservation. Future research will be focused on these areas to prove the effectiveness of such a spatial management strategy. The aim of the group involved in this proposal is to continue to research into elasmobranch spatial ecology, continually building on this research gained in this project. We will work with government and other stakeholders with the aim of providing as much relevant data as possible to help towards appropriate and effective management of elasmobranchs in UK waters.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use a range of elasmobranch species, both sharks and skates. Over the 5 year life time of the project we do not anticipate using more than 1500 individuals.

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

We do not anticipate any adverse effects other than short term, mild suffering caused by tag implantation and/or attachment. Tagged elasmobranchs recaptured after short durations at liberty have not shown infection around tag implant sites and tagged individuals recaptured after more than a month at liberty have shown full healing, providing data for periods in excess of 1 year. There have been some rare cases where external tags have initiated a protective reaction whereby the elasmobranch has formed scar tissue around the tagging site in order to protect the skin. This has not shown any signs of infection. Tagged individuals are released to the wild and are either never recaptured, recaptured dead by commercial fisheries or recaptured alive by anglers in which case tags are removed and the fish returned to the wild once more.

## Application of the 3Rs

### Replacement

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

### **Replacement**

Without tagging studies of live elasmobranchs we can infer little about their spatial dynamics in relation to human activities and management plans. Spatial data collected from tagging data will be used to develop models of fish movement and population response to climate change and will help reduce the use of wild fish in marine science

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We have data from past projects that we use to assess the minimum number of animals to achieve statistically robust results. By ensuring we initially tag a suitable range of different sizes and sex of elasmobranch, we can reduce the number of individuals that need to be tagged in future studies.

### **Refinement**

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

### **Refinement**

Technological improvements of electronic tags (greater memory, more sensors, flexible recording schedules) means that for every animal used we now get a far greater quantity and quality of data being returned. The size of tags continues to decrease thereby meaning that smaller incisions and fewer sutures are needed for implantation. We use the smallest, least invasive tags available that are capable of storing sufficient data to yield robust results. Furthermore, the appropriate tag is chosen carefully to answer the research question to prevent unnecessary tagging.

The tags are either attached or implanted by the least invasive means and/or in a way that is least likely to interfere with natural behaviour. The protocols have been carefully developed after the extensive field experience of several experts with animal welfare and minimisation of suffering at the forefront. The development of lifting cradle for large skate and general handling practice of wild-caught elasmobranchs are all examples of this.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 227. Genetics of Cardiovascular Development and Function</b>
<b>Key Words</b>	Heart development, heart disease, genetics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Problems associated with the heart are one of the most common birth defects, and in adults heart disease is the main cause of death in the UK. Many of the causes of heart defects in newborn babies still remain undiscovered. It is also thought that adult heart disease may be associated with the abnormal development of the heart, but this has not been extensively researched. Using mouse models, the aims of the project are to study and understand the instructions, ie. the genes that are important for the development of the heart and to investigate what happens to the heart, when they go wrong. Furthermore, this will be extended to explore the role of these genes in the adult heart and investigate the link between their role in development and in a diseased heart. We will also study the processes going on within the cells of the heart and what happens when something inside these cells goes wrong. Therefore, this research aims to identify and understand genes, their signalling pathways and cellular processes, which are important for heart development, but are also involved in the progression from a healthy normal heart to a diseased heart in the adult.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Abnormalities in heart development are often life threatening, and can result in the need for the immediate surgery when a baby is born and can have a large impact on the quality of life of the individual. Adult heart disease also can lead to surgery and debilitating health problems and therefore both conditions are a huge healthcare burden. Therefore, by building on our understanding of the genetic pathways that

control heart development and function it may be possible to devise screening programs for unborn babies and adults to identify any potential genetic risk factors.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will require complex breeding between different mouse strains. Over the duration of the project, up to 10,000 adult mice will be utilised. These will be used to set up the complex breeding program, to maintain the mouse colonies and to be used for mating to generate the required litters for the collection of embryos (less than two thirds through development, not regulated) and fetuses (in the remaining third stage of development, up to 4,000) and newborns (up to 1,000). Adult mice will also be used to monitor cardiac function of the mice generated from breeding genetically altered mice.

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

Most of the mice used in this project will need to be identified by examining the mouse's DNA. This will be extracted from an ear clip from each mouse. This procedure is quick and should only cause mild, short lived pain. A main focus of the research will involve the use of embryos or fetuses for the analysis of the heart. This will involve mating genetically altered mice and then killing the pregnant females to collect the unborn embryos/fetuses, which are subsequently humanely killed prior to any analysis. In some cases the pregnant female will be administered substances by mouth, by injection or by a slow release implant, and we do not expect this to have any adverse effects, but they will be closely monitored. A number of genetically altered mice will be allowed to live into adulthood. Their heart function will be assessed and will be compared to wild type mice and again the incidence of adverse effects will be very low. The severity level is set at moderate as some mice may develop heart disease, but these mice will be closely monitored and humanely killed if there are any signs of suffering. At the end of the experiments, the mice will be killed and the hearts collected for analysis.

## Application of the 3Rs

### Replacement

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

### Replacement

For our research, we are dependent on examining the development of a mammalian heart in an attempt to understand how heart defects in newborn babies and adult

heart disease develop in humans. The mouse is an ideal model as it has a similar heart and blood vessels to humans and it can be used to investigate the role of different genes in the different cell populations, which form the heart. There are limited suitable cell culture systems that could be used as an alternative as it is important the heart remains intact as a 3D model to identify any abnormalities. Occasionally results generated from the mouse studies will be applicable to further experiments in cell lines. In these cases we will use either commercially available cell lines, or generate our own cell lines from mouse embryos which can then be used to look at how individual cells behave. For example, testing hypotheses using drugs using *in vitro* approaches will act as a replacement to using further mice. We will also use human tissue as an alternative for looking at gene expression when tissue is available.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

All of the experiments will be carefully planned, using statistical analysis, to use the minimal number of mice. The number of litters collected for the analysis of the embryonic hearts will be closely monitored. Approximately 8 embryos are present within each litter, and this allows us to minimise the number of adult mice used to generate offspring for analysis and each embryo will be used for a number of experiments, maximising the information which is obtained, therefore minimising the number of pregnant females used.

## **Refinement**

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

### **Refinement**

There are a number of genetically altered mice already used extensively in research. There are a number of different cell types needed to form the heart and these mouse

lines allow us to remove the function of a gene of interest in each of these cell types. The mouse heart closely mirrors the human heart and is therefore the most complete representation of a functional heart in a mammalian model, for understanding the role of genes and cellular processes required to make a normal functional heart. The pregnant female mice will be killed humanely and any mice that have been injected, or subjected to any form of cardiac stress will be closely monitored. A further refinement, could also involve using zebrafish as an alternative model to further study a particular gene in for example the heart muscle cells, as these are similar in mouse and zebrafish.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 228. Improving the housed environment for farmed dairy cattle</b>
<b>Key Words</b>	Dairy cow, Housing, Environment, Health and welfare, Productivity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this project is to generate evidence based recommendations to enhance the housed environment of the UKs farmed dairy cows to optimise animal health, wellbeing and productivity. Specifically the project will address two key area:

1. How does the type and area of loafing space (the amount of space each animal has in a barn which isn't used for lying down) affect an animals health, well-being and productivity
2. How does the stocking density (the number of resting stalls in a barn relative to the number of animals present) affect an animals health, well-being and productivity

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

More than 95% of the UK dairy cows are housed during winter (when no grass is available for them to graze) and increasing numbers are housed year round. This research will lead to advances in how to design and manage the housed environment of dairy cows to maximise their health, welfare, productivity and the sustainability of dairy farming. Key beneficiaries of the research will be the UKs 1.8 million dairy cows and dairy farmers; there will be direct benefits to animal welfare when our findings are implemented on farm. Consumers will benefit from the assurance that dairy products in the UK are produced under acceptable conditions. Finally we expect our finding to influence policy, legislation and industry guidelines at a national and international level.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use up to 420 adult dairy cows over a 5 year period of experiments

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

We aim to test the impact of different environments on dairy cows in a flexible dairy cow housing facility which has two identical mirror imaged pens which can be set up with different layout. Animals will be housed in either a test or control environment, designed to replicate pen layout and conditions present on commercial farms. Typically these layouts will exceed normal on-farm conditions, i.e. typically, our objective will be to test the potential benefits of increases above minimum industry standards, which will be used as control conditions. The health, behaviour, physiology and productivity of cows in each group will be monitored and compared. In order to assess the impacts of the different environments we need to monitor and assess the animals and collect a range of samples from them to understand how their health, behaviour, physiology and productivity is affected. Overall, we expect the work we conduct to have a mild impact on the animals; as a result of a number of the procedures we will conduct, animals may experience short-term mild pain, suffering or distress (although it is of note that much of what we will do is likely to lead to no significant impairment of well-being in most animals). The potential for suffering in the worst case scenario would be the cumulative effects of a number of mild procedures over the maximum duration of an experiment (up to 13 months). At the end of the study, the animals will be examined by a vet; if deemed healthy and unaffected by the study they will be returned to our main dairy herd. Because of the nature of the research we are conducting, we would expect this to be the case in all but exceptional circumstances. To reduce the total number of animals subjected to experimental procedures, the same animals may be re-used on this project or on other projects. We consider that animals are suitable for re-use as they will have been subjected to only mild procedures and will have fully recovered before they are considered as suitable candidates for further experiments.

## Application of the 3Rs

### Replacement

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

### Replacement

This work aims to investigate the health, well-being and physiological responses of dairy cows kept under differing environmental condition in order to optimise housing

for farmed dairy cattle. Dairy cows are the subject of, and direct beneficiaries of the research; as such it is not possible to conduct this work without using animals and no other alternatives are suitable.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

REDACTED. We will design and conduct all of our experiments carefully and in accordance with best research practice to ensure we minimise the number of animals required, whilst at the same time ensuring our results are robust so we do not use animals unnecessarily.

## **Refinement**

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

### **Refinement**

Dairy cows are the subject of, and direct beneficiaries of the research. The effect that changes to the housed environment has on lactating dairy cows cannot be assessed in another species. Holstein cows have been chosen as this breed makes up the majority of milk producing cows in the UK so the results of this study will be applicable to most animals in the UK.

The welfare costs to study animals will be minimised by conducting only those procedures necessary to gain the information we require. As experiments progress protocols will be critiqued and further refined to reduce the number and frequency of procedures to those absolutely necessary for the validity of the study. This will reduce an individual animals cumulative exposure to the minimum required to generate robust results.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 229. The Neural Basis of Memory Formation in the Rodent</b>
<b>Key Words</b>	Learning, Memory, Brain, Plasticity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of the research in this project is to understand how the brain acquires and stores memory information and to identify the necessary interactions between specific brain regions for learning and memory to occur.

Understanding the neural basis of memory is one of the foremost challenges of science. We know that different types of information are acquired and stored within different neural networks within the brain. However, we do not know how information is stored, i.e. the cellular processes that occur within subsets of neurons, nor how information is communicated between different areas of the brain.

To achieve this aim we will undertake sequences of studies in which specific neural processes are disrupted in a selective manner and then assess how this disruption alters memory formation. To assess the effects of the interventions on memory function, the rodents (rats or mice) will be tested using memory tasks that examine the animals' ability to recognise an object or remember different places. To further advance our understanding of the processes within brain cells that produce memories, brain tissue will be examined using state of the art microscopic and electrophysiological 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 results of this project will advance understanding of how learning and memory occurs in the healthy adult brain. The knowledge gained will advance understand of how learning and memory fail during ageing and in neurological and psychiatric disorders such as dementia, depression and schizophrenia. The development of therapeutic strategies to treat such conditions depends on a clear understanding of how memory information is stored in different brain networks.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

For this project rats and mice will be used. It is estimated that approximately 680 rats and 320 mice will be used per year of this project licence. To ensure that the minimum number of animals are used we will, wherever possible, use a within-subject experimental design whereby each animal acts as its own control.

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

The severity limit for this licence is moderate. Mice and rats will be bred and maintained under a mild protocol and therefore are expected to develop no adverse symptoms. During aseptic surgery the animal will receive a general and local anaesthetic and following surgery (apart from during the immediate post-surgical period) the animals are expected to look and behave normally and to live a normal life. After a two-week recovery period, memory performance in the animals will be tested. Thus some animals may be kept under conditions of either mild food or water restriction in order to motivate them to engage with behavioural tasks, however, all animals are expected to grow and live perfectly normally. Some animals may receive compounds (drugs) which will be delivered directly into specific brain regions to interfere with the activity of the brain cells. The general pattern of motor and sensory behaviour is expected to be normal. At the end of the testing period the animals will be killed.

## Application of the 3Rs

### Replacement

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

### Replacement

We can only understand the chemical and cellular processes that are essential for memory by studying the intact brain in living animals. Hence, the objectives in the project cannot be achieved using cultured neurons or by using computer simulations. In addition, the memory processes in which we are interested cannot be investigated in lower animal species, such as the fruit fly, as they do not possess a sufficiently developed brain.

To understand the cellular processes of memory formation, *ex vivo* experiments will be conducted using brain tissue. The results of these studies will inform the direction of the behavioural experiments in whole animals, thus all experiments on living

animals will be dedicated and specific to cell types or pathways thus reducing the overall number of in vivo experiments.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

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 we will, wherever possible, use a within-subject experimental design whereby each animal acts as its own control, thus half the number of animals will be used to meet the stated experimental objectives. In addition we will test the same group of animals across a number of spontaneous recognition memory tasks. As these tasks exploit the animals natural tendency to explore objects, and are not aversively motivated and the duration of testing is relatively short (minutes), the animals show no decrement in performance with repeated testing and the stress of behavioural testing is minimised.

To maximize the information from a single animal and to minimize suffering, we will take slices of brain tissue post mortem and ex vivo experimental will be conducted to investigate the cellular processes involved in memory formation.

### **Refinement**

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

#### **Refinement**

As stated rodents (rats and mice) will be used throughout the project. Rodents will be used as they are the species with the lowest degree of neurophysiological sensitivity that are able to perform the memory tasks upon which the work depends.

All surgeries will be conducted under strict aseptic conditions, with the animals under a surgical plane of general anaesthetic. Post operatively the animals will receive analgesia to provide pain control. Animals are expected to make a rapid and

unremarkable recovery following surgery and to continue to live a normal life. Apart from the immediate post-operative period the animal will be kept in social groups.

The animals, which will be group-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 while they are on task.

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.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 230. Studies of experimental small ruminant TSE</b>
<b>Key Words</b>	TSE, STRAINS, TRANSMISSIBILITY, PATHOGENESIS, FARM ANIMALS
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Naturally occurring transmissible spongiform encephalopathies (TSEs) , an infectious degenerative brain disease is caused by a number of distinct, identifiable 'strains' resulting in classical and atypical bovine spongiform encephalopathy in cattle and classical and atypical scrapie in sheep. If these strains were to cross from one host species to another, either naturally or through animal feed, we need to know what these would look like, and whether or not our current surveillance sampling and testing methods are robust enough to detect and classify them.

**What 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 important to know which species are susceptible or resistant to specific infections because it helps the policy makers and risk managers to decide which disease controls and food production measures are necessary to protect animal and human health. Knowing as much as possible about what a disease looks like increases the chance of us being able to detect it effectively if it should occur, further strengthening animal health and human food chain protection.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The project will use 3 pigs, 16 cattle and 14 sheep for up to 5 years.

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

The majority of animals have already been challenged with various TSE strains under a previous project licence, and due to the long incubation period in most TSEs

are now being housed under normal conditions until they show signs of disease. They will be monitored daily by the animal care staff, and have regular more detailed observations and clinical examinations by scientific and veterinary staff. When any abnormality, such as a change in behaviour, movement or eating habits, is noted the clinical monitoring frequency will increase, so that clinical progression can be monitored and appropriate action taken. When the animal is showing unequivocal signs of disease it will be killed humanely, and a full range of samples taken for a range of tests to characterise the disease type. Some additional animals will be inoculated intracerebrally under general anaesthesia and appropriate analgesia and will be treated the same way as the animals above

## Application of the 3Rs

### Replacement

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

#### Replacement

Farm animals are used to investigate whether they are resistant or to know what the clinical disease will look like in the particular species that may be naturally exposed to these TSE strains.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

These are pilot studies that aim to establish resistance or susceptibility to a particular agent, or studies to confirm the type of disease produced in previous studies where the sample size can be minimised because the experience of previous experiments means that disease will occur in all the animals used. Statisticians have been consulted to determine optimal numbers.

### Refinement

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

#### Refinement

Farm animals have to be used to describe the disease that can be produced because they may potentially be exposed to these strains naturally.

All animals will be closely monitored for any clinical or behavioural abnormality and such changes carefully recorded. CCTV will be used to look for infrequent or subtle signs which may not be displayed when observers are physically present. We have established clearly defined, disease specific endpoints based on previous experience with various TSE strains in these species and continue to review these as new data emerge. The decision point for culling an animal is when any abnormality is deemed to adversely affect the animal's normal activity.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 231. Preclinical development and assessment of Bacillus species medical interventions</b>
<b>Key Words</b>	Vaccine, Bacillus, Therapeutic
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

New vaccines and other interventions are required to be developed against *Bacillus species* infections. The objective of this licence is to develop new vaccine therapies and treatments. Human trials are ethically and practically very difficult to conduct due to the infrequency as well as severity of some *Bacillus species* infections. This project aims to assist in the assessment of the new interventions using animal models of these infections. Candidate vaccines and therapies that show promise in these models will be taken forward by sponsors in order to provide doctors with new treatments against 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)?**

This project will enable testing of vaccines and therapies against the most serious forms of *Bacillus species* infections. *Bacillus species* are bacteria which primarily can cause disease in herbivorous animals, although all mammals are susceptible to infection. In humans, the disease can affect the skin and, rarely, the respiratory or gastro-intestinal tracts. The bacterium is spread by spores. Spores can be found in animal products such as wool, hair, hides, skins, bones and bone meal, and in the carcasses of infected animals. The spores can also contaminate soil and may survive for many years. In the UK, human disease from infection is rare, and was historically almost entirely an occupational disease affecting those handling imported infected animal products or working with infected animals. Sporadic outbreaks of severe disease have occurred amongst drug users following injection of heroin contaminated with spores and isolated cases of inhalational infection have been reported in individuals making drums with imported animal skins. Human infections occur in countries where the disease is common in animals including those in the

Southern and Central Americas, Southern and Eastern Europe, the Caucasus, Asia and Africa. Inhalation of some *Bacillus* species carries a close to 100% mortality rate without medical intervention. Human trials are not possible due to the severity of the form of disease being investigated. Regulatory bodies will, however, accept preclinical data generated in animals in such circumstances so this project may assist in the licensure of new or improved interventions. Many experimental vaccines or therapeutics do not successfully pass through the rigorous tests required for modern medicines and this project may help prevent potentially hazardous or ineffective treatments reaching the clinic. Conversely, this project may also highlight the experimental products which may potentially become globally accepted antimicrobials or vaccines which will save and protect many human lives.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Although it is very difficult to predict how many treatments we will test during the life of this project, our past performance indicates that we will use at least 400 animals each year. The level of usage may increase in any year due to the possibility that a candidate vaccine or therapy may suddenly need a lot more testing as it enters clinical or other critical studies. As a result, we predict to use the following; Mice 6460 and Rabbits 850.

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

Animals will be sympathetically handled, with or without anaesthesia, in order to inject or give them vaccines or therapies. This process may cause short term distress to animals but this will be minimised by using only highly trained staff. In animals that are not protected by the vaccine or therapy, respiratory infection is likely to develop very quickly and may overwhelm the animals due to a rapid blood poisoning from the bacterial toxins. The likely adverse effects include a period of fever, a combination of clinical signs of infection including ruffled fur and closed eyes. If clinical signs include signs of advanced disease, the animals will be euthanised in accordance with Schedule 1. Regular health monitoring will detect these signs, but in some cases animals may die before such signs are evident. Our previous experience indicates that although this can happen, the majority of our procedures will be Moderate in severity due to the application of rigorous clinical monitoring and humane end-points. The levels of severity experienced by animals protected by a vaccine or therapy will range from Mild to Moderate, depending on efficacy. Due to the regulatory restraint associated with the infectious diseases we are studying, all animals will be humanely euthanised at the end of each study and will provide an extensive range of tissues for further analysis.

## **Application of the 3Rs**

## **Replacement**

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

### **Replacement**

We will try to use human sera wherever possible to assess human vaccines for biological activity. However, to establish evidence of efficacy of new treatments, it is essential to have the full range of host-pathogen-treatment interactions which mammalian model systems offer. As clinical efficacy trials would be too dangerous it is necessary to conduct this work in a suitable animal model

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The number of animals required for a study will be determined by how variable the infection model outcome is. We reduce our model variability by optimising our infection procedures using good microbiology and world class instrumentation. In addition, we use a sequential approach to ensure that candidate interventions that are found to be performing poorly are eliminated at an early stage.

## **Refinement**

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

### **Refinement**

In carrying out these studies we will seek methods that minimise the pain, suffering, distress or lasting harm that may be experienced by research animals, and which improve their welfare.

Animals tend to reach death very quickly from an apparent healthy state when infected with the more virulent *Bacillus* species. Every effort is made to prevent suffering by frequent monitoring of clinical signs and using these to determine and refine humane endpoints.

The majority of our work will be conducted in mice. Mouse models are very useful as they are widely used by the scientific community because there is such a large body of information already published about mouse biology. Sometimes, however, mouse models are not adequate or not closely matched to human disease in which case we need to use other species such as rabbit.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 232. The Breeding, Maintenance, Genotyping and Genetic Monitoring of both Genetically Altered and Wild Type Rodents</b>
<b>Key Words</b>	Genetically, Altered, Rodent, Breeding, Maintenance
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This service licence will facilitate the breeding and maintenance of genetically altered animal lines in a managed and controlled environment, utilising the highest standards of welfare, colony management and husbandry practices allowing animals to be kept at a high health status and for their genetic status to be correctly monitored. Subsequently the animals are supplied for research with the knowledge that the animals have been bred to a high standard making them suitable for scientific research.

The use of genetically altered animals in biomedical research allows for the specific traits of certain genes to be studied in a complex physiological environment that cannot be achieved by laboratory methods, further allowing for a greater understanding of the function of genes in disease and ill health.

As a result of naturally occurring genetic mutations, certain animal strains will display similar diseased states to that of humans e.g:

- rats whose mutation results in hypertension, allowing for advancements in the treatment of high blood pressure.
- mice whose mutation results in reduced functionality of their immune system, thus allowing for rapid uptake of cancer cells and the resulting growth of tumours, allowing for the development of new cancer treatments.

Skilled animal technologists who are fully trained in caring for laboratory animals of this type will be responsible for managing colonies in accordance with the guidance outlined by various groups with expertise in this field.

**What 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 prevent unnecessary breeding of animals by carefully monitoring researcher's usage needs and forecasts, alongside good breeding colony management. This is in line with the 3R's (refine, replace and reduce). The management of this centralised service will provide benefit to those institutions that do not have the necessary expertise or infrastructure to produce their own animals. It also prevents duplication of colonies at multiple establishments and allows the research facilities to focus on the refinement of their experimental programs. Overall this results in a lower number of animals used in both breeding and experimental areas. The use of high quality animals in research is critical in reducing variability in the data or results obtained. It dramatically reduces the need for repeat experimental programs where results are inconsistent due to the quality of animals utilised e.g. variability in the genetic status of animals may result in significant variation in the results obtained within a single group of animals

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice = 742,000 Rats = 35,000

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

This project will predominantly focus on the breeding and care of mice and rats up to 12 months of age, with a small number of animals maintained from 12-18 months of age when characterisation of disease pathology at various life stages is required. All programs will be managed and monitored within conditions according to their health status, as well as the functionality of their immune system e.g. the use of barriered (high health status) environments that reduce the risk of infection by bacterial, viral or parasitic agents within animals whose immune state is compromised by their genetic alteration. In order to establish the genetic status of animals produced, tissue samples will be taken from animals utilising the most refined method (ear punch system) that focuses on the welfare of the animals but also ensures sufficient DNA can be obtained to analyse their genetic state. Physical tests to confirm the presence of a genetic alteration that results in hypertension will involve the restraint and warming of animals within specially designed equipment for no more than 30 minutes. This allows for dilation of the blood vessels and for the subsequent accurate reading of blood pressure to ensure the procedure takes as short a time as possible for the animal. Some animals produced will display similar cognitive deficits to that of an ageing human, this may result in reduced spatial learning and memory deficits in line with symptoms of neurodegenerative disease e.g. Alzheimer's. One particular genetic alteration has been noted to result in spontaneous death within <5% of all animals carrying the genetic alteration born between 4-7 months of age, the cause of death is non-convulsive seizures brought on

by physiological changes in the brain and is as a result of their genetic alteration (neurodegenerative disease model). These seizures induce a state whereby animals are not aware and die without suffering. Procedures are in place to reduce the risk of triggering seizures e.g. reduction of noise and sudden changes in lighting. Animals produced will be supplied into the project licence authority of other establishments in the UK and bona fide establishments abroad.

## Application of the 3Rs

### Replacement

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

#### Replacement

Non-animal methods are not always able to model or replicate the complete array of behavioural, cellular, molecular and physiological interactions required to fully understand how genetic alterations result in normal or abnormal processes. Mice and rats bred or maintained under this project will be subject to scientific justification in the researcher's protocols demonstrating that the goals cannot be met with the use of non-animal methods.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The use of effective and stringent colony management systems will result in a reduction in the number of animals required in the breeding aspect of this project. Colonies will be planned according to the demand of end user groups and will be subject to continual review to ensure production levels are in line with the forecasted demand. Should usage reduce and remain sporadic, colonies will be closed and embryos frozen to preserve the model of interest.

The supply of high quality animals according to client specifications will result in more effective experimental programs where variability would ultimately impact upon the results obtained. The ability to offer this as a service will also result in a reduced need for duplicate colonies at various establishments, also lowering the number of animals of a similar type needed for breeding programs.

### Refinement

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

## Refinement

End users will be required by the project licence holder to satisfactorily justify the need for the animals that will be bred and maintained under the authority of this service licence. The choice of species/strain is driven by end user requirements.

Prior to the receipt of any animal model, stringent data collection will be performed to ensure all known traits and observations due to the genetic alteration are known, this will allow for specific refinements to be made in terms of breeding practices, husbandry, nutritional requirements, enrichment and handling. For example, animals that carry a genetic alteration which results in reduced functionality of their immune system will need to be housed within specific barriered environments to maintain their high health status and reduce the risk of infection e.g. with opportunistic bacterial agents.

Stringent colony management systems are in place and controlled by animal technologists fully trained in the breeding, care and husbandry of specialised animal colonies, utilising their experience and expertise as well as guidance from experts in the field of genetically altered animals.

Prior to the start of any breeding program all responsible members of the team will ensure specific details related to the animal model are known and used to set up the specific breeding and maintenance plan. The breeding plan will be subject to changes throughout the lifetime of any colony and will be in line with the forecasted usage. Breeding systems that minimise overproduction of unwanted genetic status animals will be used.

Animals will be housed in optimal social groups, allowing for a reduction in potential aggression or overt dominance behaviours, thus reducing any associated stress.

When determining the genetic status of both genetically altered and wild type colonies, the least invasive and most refined method, ear punch system, will be used for the retrieval of tissue, whilst the most advanced methods and technology will be used for the analysis of DNA to maximise the likelihood of success in this procedure, therefore reducing the need for re-sampling.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 233. Interventions against Tuberculosis.</b>
<b>Key Words</b>	vaccine, therapeutic, efficacy, Tuberculosis
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Chronic infections are leading global causes of death and disease. This project aims to

- a) Identify correlates (e.g. immunological and inflammatory) as predictors of successful protection against bacteria that cause chronic infections
- b) Identify host markers that could be correlates of risk of developing chronic infections.
- c) Demonstrate the immunogenicity and / or efficacy of therapeutic interventions against primary infection, co-infection with other diseases, reinfection and reactivation of latent infection.
- d) Generate data regarding immunogenicity or efficacy for formal submission to relevant regulatory authorities.

This project will play a critical role in the development of new vaccines and drugs against bacterial diseases responsible for chronic infections and will accelerate their progression into human 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 models and the information resulting from the studies will be used to progress development and evaluation of new vaccines and drugs to combat diseases caused by chronic bacterial infections. Evidence of the effectiveness of new vaccines or treatments will support their progress into clinical trial. These outputs will accelerate new interventions through to clinical application and effective interventions against respiratory diseases such as tuberculosis, would be of enormous benefit to mankind

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will use approximately 30 -100 animals each year 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 levels of severity? What will happen to the animals at the end?**

The subjects in these studies will receive new vaccines and drugs. Small volumes of blood, clinical measurements (weight and temperature) and images using medical scanners will be collected for health monitoring purposes. The efficacy of these vaccines or therapeutic strategies will be evaluated by challenging subjects with the relevant pathogens. In unprotected animals this may result in infection and potential progression of disease. However, we have sufficient knowledge of our models to limit the level of disease that is required to determine whether a vaccine or therapy has been effective. The use of realistic low challenge doses, close monitoring of animals for clinical signs, all serve to allow early intervention before progression to severe disease. At the end of each experiment animals will be humanely killed so that the levels of disease in treated versus untreated animals can be accurately determined.

## Application of the 3Rs

### Replacement

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

### Replacement

Whilst every effort will be made to use in vitro systems such as cell culture wherever possible, only in an animal model can we currently demonstrate efficacy in terms of alleviation of clinical signs and systemic pathology.

In order to study the complex inter-reactions between cells of the immune system during the course of vaccination and infection, it is necessary to reproduce as closely as possible this complex system in an experimental situation. Currently the only way to reflect this complexity and to define the dynamics of what is occurring in real time, is to use animal models that are reproducible and have been demonstrated to show the changes seen in man.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Rigorous statistical systems will be applied to minimise the number of animals used whilst maximising the data gained from each experiment. A large archive of stored samples such as cells of the immune system, serum, plasma and tissues obtained from previous studies are available to be tested by new and improved assays as they emerge, reducing the need for further animals to be used.

### **Refinement**

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

### **Refinement**

Only the most promising new drugs and vaccines will be tested in the models in this project and all will have been shown to be safe and effective in other systems.

All procedures will be carried out by highly trained and experienced personnel and animals will be sedated for these to minimise stress. Vaccines and treatments will be given in the same way that they are to humans and blood samples to show that the vaccines have induced an immune response or that drugs are present at suitable levels, will be collected under sedation. The research establishment has extensive experience and understanding of the diseases being studied and animals will be monitored carefully for signs of infection and a number of clearly defined clinical observations are established that will predict those that will progress to disease.

The slow nature of disease development coupled with the excellent health monitoring processes that include the use of advanced imaging scanners similar to those used in human medicine enable intervention prior to the development of disease.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 234. Pre-clinical evaluation of cellular immunotherapies for human disease</b>
<b>Key Words</b>	Cancer, Immunotherapy, CAR T-cell, gamma delta T-cell
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Although recent developments in cancer therapies have improved the survival rate of many patients, cancer remains set to overtake heart disease as the biggest killer of adults in the Western world. Simply put, currently available treatments are inadequate for most patients with advanced cancer. Thus, there is an overwhelming need for new and more effective means of treatment.

The overall aim of this project is to develop cancer immunotherapies that harness white blood cells from the patient's immune system to control the tumour. Within the patient, these white blood cells are suppressed by the environment created by the cancer. However, if they are removed from the body and manipulated in the laboratory, they can acquire potent anti-cancer activity. The approach we wish to test may involve the use of cells that naturally recognise and kill cancer cells. Alternatively, we will boost the ability of white blood cells to undertake this task by delivering new genes to these cells ("genetic engineering"). In either case, white blood cells are removed from patient blood and are expanded and sometimes modified in the laboratory so that they can recognise and kill cancer or leukaemic cells.

In this project, we will develop systems to simulate human cancer and leukaemia in mice, either by injecting cancer cells in the mice, or by introducing genes that cause the mice to develop cancer. These so called 'mouse models' are essential to allow us to test safety and effectiveness of newly developed white blood cell-based treatments. We will then proceed to test a range of white blood cell-based treatments for their effectiveness and safety in mice. This will involve scanning and taking

samples from the mice in order to determine the state of the cancer and of the white blood cells that have been used to treat 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 most effective treatment for chemotherapy unresponsive acute lymphocytic leukaemia involves the administration of white blood cells that have been removed from patients, genetically engineered in the laboratory and then re-infused back into the patients bloodstream. Terminally ill patients who are treated in this way achieve remission in 80-90% of cases – a level of effectiveness that has never been seen before when a new cancer medicine is tested for the first time in man. We hope to develop similar treatments for other cancers that are based upon this principle. Currently however, solid cancers and other forms of leukaemia are much more difficult to treat using this approach. In the short term, we will be able to optimise new white blood cell-based therapies, demonstrate their effectiveness in mice with cancer and obtain safety data required for initiating clinical trials in cancer patients. Promising treatment approaches will be advanced to clinical studies in patients with advanced and otherwise untreatable cancer, while ineffective approaches will be discontinued. We have already advanced once such white blood cell-based treatment to a clinical trial in patients with advanced head and neck cancer, so we have the necessary experience to translate our experimental work to the patient setting. In the longer term, we will continue to develop promising treatment approaches through larger clinical studies in which these experimental treatment approaches are compared to (and perhaps combined with) to the best available treatments for cancer patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will only work with mice. We expect that we will use 35,000 mice over the 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 levels of severity? What will happen to the animals at the end?**

Expected adverse effects are infection, transient flu-like symptoms due to immune treatment, transient weight loss, transient roughening of fur and transient reduced mobility and/or diarrhoea. The most severe adverse effects that we expect to see are classified as moderate in nature. Experiments are planned such that, whenever possible, mice will be humanely killed before symptoms become apparent. Failing this, mice will be humanely killed as soon as significant symptoms develop. At the end of every experiment, mice will be humanely killed.

## Application of the 3Rs

### Replacement

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

#### Replacement

Whenever possible experiments are performed using tumour cells grown in the laboratory and immune cells taken from healthy volunteers or cancer patients. However, the immune system is very complex and it is impossible to reproduce this in laboratory experiments, so we need to use living animals. Moreover, testing experimental therapies in animals is an essential pre-requisite prior to initiating early stage trials in patients. Although a considerable amount of information can be obtained from experiments performed in the laboratory, the interaction between a new treatment and the whole organism can only be defined in animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

To minimise the number of mice, pilot experiments will be carried out to determine the minimal numbers of mice required to give statistically valid results, thereby minimising the number of repeat experiments required.

Whenever possible tumour growth will be monitored using technology applied to imaging patients' tumours, greatly reducing the need to kill mice at defined time-points.

### Refinement

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

#### Refinement

Mice are the only animal species used in this programme of work. Mice are less sentient than higher mammals but are still relevant to human biology. Moreover, increasingly complex mouse models are available that closely mimic human cancer or which have specific defects in the immune system that allows us to ask questions about the role of that immune component in treatment response or failure. Use of pilot experiments (with smaller numbers of mice) and imaging (allowing monitoring of

disease in the same animal, without the need to kill the mouse) should ensure that, whenever possible, mice will be humanely killed before clinical symptoms are evident. Mice will be regularly monitored and humanely killed immediately if they develop signs of distress.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 235. Reproductive and Developmental Toxicology</b>
<b>Key Words</b>	Determine, reproductive/developmental, toxicity, hazards
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>Yes</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project is being performed to detect test substance related impaired mating performance/fertility, damaged reproductive organs or, impaired survival, development or growth of fetuses/offspring, as required by the regulatory authorities.

Authorities can then make informed risk based decision concerning the known reproductive/developmental toxicity hazards associated with a drug and whether the drug should be approved for use on clinical or veterinary trials, or approved for marketing, and to prescribe conditions for its safe use.

For chemical and agrochemicals, the project will enable regulatory authorities to make an informed risk based decision concerning the known reproductive/developmental toxicity hazards associated with the substance and whether the substance should be approved for marketing, and to prescribe conditions of safe use and handling of the substance].

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Safer human or veterinary drugs will be approved for clinical trials and marketing. The regulatory authorities will thus be able to better prescribe conditions for the safe use of drugs. Critically, on the basis of risk assessments completed, based on information made available through animal testing, those drugs that are deemed unsafe will be precluded from further development/marketing. Safer chemicals and agrochemicals will be approved for marketing, and regulatory authorities will thus be able to better prescribe conditions for the safe use and handling of these substances.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

124000 Rats 6000 Mice 8500 Rabbits Over the 5 year life of the project licence. A small proportion of these animals may be genetically altered.

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

Rats, mice or rabbits are dosed (e.g. by oral gavage, injection or inhalation) with test substances. The majority of animals are expected to show little or no reaction to treatment. A few animals may show transient body weight loss and reduced appetite following the start of treatment with the test substance, but are expected to recover quickly. Rats and rabbits may have minor surgery to implant a cannula into a vein or a device under the skin that can release a medicine slowly. They are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital. Animals may be subject to restraint or restriction of free movement during exposure to test substances (e.g. held in inhalation chambers, in jackets or tethered to a drug delivery system or may be housed in a metabolism cage for short periods of time to enable collection of urine and /or faeces for analysis); none of these procedures are expected to affect the clinical condition of the animals or elicit more than transient body weight loss and reduced appetite. Animals will be killed at the end of all regulatory studies for reproductive/pathological evaluations to achieve defined regulatory endpoints.

## Application of the 3Rs

### Replacement

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

### Replacement

We need to use animals because non-protected animal alternatives cannot replace the extremely complex series of events involved in reproduction and/or subsequent development of young and reproductive organs: these processes cannot be

effectively modelled in the laboratory in test tubes/dishes or by the use of sub-mammalian animals.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The regulatory guidelines usually indicate the number of animals in a study; otherwise, the number used is the minimum to achieve the aims of the study.

When testing several closely related test items in several different preliminary studies, use of a common control group/less control animals can decrease numbers of animals used per study. So can the experimental design which is optimised, being mindful of regulatory requirement. If a study is cancelled, in some circumstances animals may be reused on a different study rather than using an entirely new batch of animals. Animals which have only had restraint training and have received no treatment may be used on another study. Re use is subject to satisfactory veterinary assessment and is such that scientific outcomes can be reached with the most efficient use of animals and without data or welfare compromise. Methods used to measure the level of a drug in blood which are compatible with small blood volumes (less than 0.1 ml) will be used where available, and this can lead to a reduction in animal use.

## **Refinement**

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

### **Refinement**

Rats (Rodents) and rabbits (non rodents) have been chosen for use of these studies because rats and rabbits are the species preferred by the regulatory authorities for testing. Mice are rarely used when either the rat or rabbit are demonstrated to be unsuitable e.g. due to higher levels of drug being present in the blood of mice or, the drug target responds strongly to the test item in mice but only weakly in rats or rabbits.

Studies are performed in a stepwise manner, starting with preliminary studies using small numbers of animals where there is limited information, and building on the information obtained from these initial studies doses to be used, group sizes and procedures are optimised in the next phase/definitive regulatory studies. This gives the highest prospect of refining and optimising the programme to achieve the desired

scientific outcomes (data) whilst minimising any pain, suffering, distress or lasting harm experienced by the animals on study.

The clinical condition, body weight and appetite of all animals are regularly monitored for signs of any adverse effects on their health or wellbeing to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test item, or humane killing of affected animals).

Other examples of refinement are exposing the animals to test item in water or diet rather than dosing. Dosing of a drug via an infusion system rather than repeated injections can despite initial surgery involved to place catheter/delivery device be more refined as it avoids frequent repeat injection over the study period which may be up to 7 weeks.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 236. Identification of pneumococcal genes involved in nasopharyngeal colonisation and disease</b>
<b>Key Words</b>	Streptococcus, Infection, Colonization, Vaccine, Pneumonia
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Streptococcus pneumoniae (the pneumococcus) is a bacterium that colonises the nose of a large proportion of children and adults. In many cases this does not cause a problem and may actually be beneficial. However, in some cases the bacteria can cause invasive diseases such as pneumonia and meningitis. Cases fatality rates of adults admitted to hospital with pneumonia can be as high as 44%. The aim of the work described in this project is to identify bacterial molecules which are important in the ability of streptococci to colonize the respiratory tract and cause pneumonia and use this knowledge to develop new vaccines and 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 identification of targets for use in new vaccines to prevent streptococcal colonisation and disease in man and animals. Optimisation of vaccine strategies and therapies to prevent and treat pneumococcal disease. The data generated will be of benefit to science in promoting the understanding of the biology of Streptococcus pneumoniae and how it causes disease in both humans and animals (S.pneumoniae also causes disease in horses). Understanding of the disease process will also help to educate in best practice for treatment of infection. Other benefits will include optimisation of antibiotic therapy in combination with adjunctive therapy to improve outcome. This information will be useful to transfer into humans and other animals

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice. 4500 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 levels of severity? What will happen to the animals at the end?**

Two models will be used. One involves colonisation of the upper respiratory tract of mice with the pneumococcus. There are no adverse effects associated with colonisation of mice. Mice used in colonisation experiments are killed at the end of the experiment. The second model used is a pneumonia model. When mice are infected into the lower respiratory tract they develop pneumonia and this can progress to sepsis (bloodstream infection). A system for scoring disease severity is used and symptoms are monitored at regular intervals. There is an endpoint defined by a severity score when animals are killed. • Repeated anaesthetics may lead to dehydration of the animal. To control the risk of animals becoming dehydrated during anaesthesia, fluid replacements will be given as advised by the NVS. • Adverse effects of dosing e.g. peritonitis, damage to the trachea. The risk will be controlled by using experienced licensee to carry out these techniques. If signs of adverse effects do appear (hunching, reluctance to move) the mice will be killed. • With knowledge of the expected time of death in control animals (3-4 days post infection), the mice will be infected at a time of day and week when the animals will be able to be inspected at their most critical times. The signs characteristic of the disease in mice will be assessed, e.g. inactivity, lethargy and starey coat with hunching. A system for scoring disease severity is used and symptoms are monitored at regular intervals. The interval between observations is shortened closer to the end point. At the defined endpoint defined by the clinical score the animals are killed. • Use of other parameters such as presence of bacteria in the blood are also used to define endpoint for some bacterial strains. • The colonisation model has a minor impact on the animal and animals return to normal following recovery from the anaesthetic used to restrain the animal. • In the later stages of the infection model the animals become lethargic and less active in movement. Animals are humanely killed when they reach this stage (as determined by the scoring protocol). • Using the scoring system means that the majority of animals are culled at a set stage of the infection and do not die from the infection itself. In very rare cases an animal may die early in the infection process. • The main harm to the animal occurs at the end of the infection process which is determined by clinical symptoms. These are monitored more frequently as the animal progresses through the disease and killed at the selected endpoint which is when symptoms are relatively mild. Animals are killed when there is an obvious difference between the control and treatment group at early time points. • The animals also undergo blood sampling and may be injected with antibodies or other agents. These cause minor discomfort to the animal from which they recover.

## Application of the 3Rs

### Replacement

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

## Replacement

Bacterial strains are tested in tissue culture models. We also use other alternative methods where possible and to do initial studies in non-protected animals (eg *Galleria mellonella* larvae) where possible. Animals will only be used in the final stages of studies where intact animal and immune system are required.

Continued review of the scientific literature is 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 studies in animals.

We will use SyRF the free online platform for researchers to perform a systematic review and meta-analysis of animal studies. This will allow us to keep up to date with any improvements in protocols and techniques which would reduce or replace the use of mice.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Bacterial strains are first tested in tissue culture models. This allows selection of appropriate strains for further investigation in vivo. Use of statistical data from previous mouse studies is used to minimize group sizes. Use of statistical data from our previous studies to minimize group sizes. The optimal group size using data from our and other previous studies for most of the experiments is 5. When assessing new bacterial strains of unknown virulence, groups of 2 animals are used to give an indication of virulence before larger groups at multiple time points are used. We will also use the NC3R's experimental design tool to aid our experimental design and we will also publish according the ARRIVE guidelines for reporting these studies.

## Refinement

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

## Refinement

The mouse is the main model for experimental studies of the infection concerned. This makes our data comparable with other research groups around the world. The infection model used is well established and end points have been refined using a clinical scoring system to minimise welfare costs to the animals.

In our proposed programme of work, we will systematically 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 subsequent experiments

Use of a non-invasive colonisation model for some initial screens of bacterial mutants and vaccines. For invasive disease experiments can be refined experiments by choice of bacterial strain. Some bacterial strains cause less severe disease in the mouse and these can be used for initial testing and optimising some vaccination/intervention strategies before final testing in the sepsis model.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 237. Genetically engineered mouse models of cancer</b>
<b>Key Words</b>	Oncology, Tumour, Efficacy, mechanism, cancer
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Whilst current cancer treatments provide some survival benefits (50% survive cancer for 10 years or more in 2010-2011), they are often associated with significant side effects. Thus there is a clear need for improved and better tolerated medicines that can be used either alone or in combination with existing or other new therapies. By combining 2 or more drugs it may be possible to see an enhanced benefit in treating cancer. Traditional cancer models do not accurately reflect all aspects of the tumour microenvironment observed in patients. Many new drugs are aiming to specifically target aspects of the tumour microenvironment that cannot be assessed in these traditional models. Genetically engineered mouse models have been shown to have a tumour microenvironment that more accurately reflects that seen in patients. The aim of this project is to develop new models and to profile potential new drugs and/or combination in genetically engineered mouse models to investigate efficacy, development of resistance and support the design of future 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)?**

In this project we will generate new mouse models. Using these models we will be able to better understand the efficacy of potential new drugs and drug combinations within a tumour that is clinically relevant. As such we can better define how to give compounds alone and in combination to have the optimal impact on tumour growth. This will significantly improve the cancer patient's quality of life and overall survival.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Only mice will be used on this project. Up to 1000 mice will be used over 5 years in the pilot studies.

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

Pilot studies will be performed to define the most optimal welfare endpoints for each different model type proposed to be used under this licence. The plan is to generate approximately 10 new models over the course of the licence. In these studies we will monitor growth by relevant methods e.g caliper measure, palpation, imaging. Animal welfare, including weight loss and/or changes in clinical signs (e.g social isolation or ruffled fur) will be closely monitored in these studies. Animals will be closely monitored and humanely culled if adverse effects are observed. Animals are monitored by trained staff, with referral to the Named Animal Care and Welfare Officer, veterinary staff and Project Licence Holder as necessary. The mice will be genetically engineered in a way that means that they will form tumours either spontaneously over time or in response to an inducing agent. Depending on the model this may be via a number of routes but is most likely to be via drinking water or orally. Inducing agents may be administered by a route under short term anaesthesia e.g directly into the lungs. In some rare instances there may be a need for mice to undergo surgery for the tumour induction. The least invasive route possible will be used and surgery will only be used when all other alternatives have been investigated. After tumour induction mice will be monitored for the growth of tumours. Animal welfare during tumour formation will be carefully monitored in pilot studies. The endpoints for each model will be defined and agreed with named animal care and welfare officer (NACWO) and veterinary staff prior to performing the larger scale studies. To get a better understanding of the models we will test known anti-cancer drugs to determine how well the tumours respond to therapies. These drugs will typically be given orally, but may be via other routes including intravenous injection (IV), injection under the skin (subcutaneously) or in to the peritoneal cavity (intraperitoneal). These studies will help us to define how many mice would be needed to perform the optimal experiment to profile novel anti-cancer drugs in each new model. Once these experiments have been performed the licence will be updated to clearly define the welfare endpoints for each model. These tumour models will only be used where there is a scientific rationale for using these more complex model systems. Animals will be culled if the tumour results in significant pain or distress. In these studies clinical signs related to the compound may be seen and mild to moderate signs of toxicity are possible. Animals will be humanely killed if this persists. All animals will be regularly monitored for weight loss and general condition. Weight loss as a result of repeat anaesthesia may occur and this will be minimised by correct dosing and good maintenance of body temperature. The protocols are classified as moderate severity. Animals will be humanely culled at the end of the study.

## **Application of the 3Rs**

### **Replacement**

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

## Replacement

Non-animal alternatives are used in the identification and selection of compounds and generally include measurements of the likely effect of the agent on the target cells.. There are more complex in vitro systems, including 3D culture systems or co-culture systems that can be used where traditional 2D culture systems can not be used to increase understanding of a compound. These systems are used to reduce the numbers of compounds that are required to be used in vivo as only compounds that show the appropriate activity in cells are progressed further. To date there is no cell culture system that is able to predict the likely *in vivo* activity given the complexity of issues such as bioavailability, metabolism and elaborate physiological interactions associated with tumourgenesis and therefore the whole animal is needed for the studies proposed in this licence. We will continue to work with groups that develop assay systems that have the potential for replacement. Additionally we will continue to keep up with the latest developments by attending conferences and monitoring websites such as pubmed for the latest advances.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

To maximise the scientific integrity of data generated and use the minimum number of animals, an in house statistician will review experimental design and analyses. The initial pilot studies will be used to drive these discussions with the statistician and perform power calculations. Based on historic data from these types of models we would expect to have group sizes of n=6-10.

There will be multiple projects within the portfolio that would gain additional insight to support progression by profiling compounds in the genetically engineered mouse models. We will maintain a list of required experiments for each project and ensure all preliminary work (e.g tolerability studies) is in place for these. This will reduce the risk of not having a use for experimental animals if projects are unexpectedly terminated.

As part of initial characterisation of the models we will generate cell lines and fragments determine whether we can generate transplantable models. This will reduce the requirement to specifically breed mice to support these experiments. Given potential numbers of mice that are wasted during the breeding of these complex models this has the potential to significantly reduce animal numbers.

### **Refinement**

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

### **Refinement**

Only mice including transgenic are used on this licence. Using non-mammalian species is not possible since they lack the appropriate tissue physiology. Therefore they cannot be used to predict exposure and efficacy in humans.

Animal condition will be monitored daily. Any mouse that exhibits 15% weight loss compared to peak weight for more than 2 consecutive days or 20% on any one day will be humanely killed.

Within this project genetically altered animals will be used in order to achieve the scientific objective. The most appropriate mouse models will be chosen based on previous in-house or external data for each model / project. In this project we are establishing models which aim to reflect the tumours seen in breast, ovary, lung, pancreas and prostate cancer. The models will be developed by two different methods.

One method is classically used in the literature where mice are bred that form specific tumours in the organs of interest. In some cases the tumours will be induced when mice reach adulthood. In >95% of cases the induction of the tumour will be via a non-invasive methods. On very rare occasions it may be necessary to induce the gene by administering directly to the target organ. This is when tumours may form more systemically if the induction is not done in a specific location. This will only be done when there is no alternative.

The alternate system is a novel method where mice are bred with an agent that enables us to administer a gRNA that can induce tumours with specific mutations and target organs. This enables the development of multiple different tumour models from one breeding colony. For some tumour types it will be possible to add the gRNA via a non-invasive method that result in minor transient impact on the animal e.g lung and breast models. For other target organs e.g ovary, pancreas and

prostate it may be necessary to administer the gRNA directly into the organ using surgery. We are working closely with a group that is looking at methods to reduce the requirement of surgery and use alter. Where this is possible it will be avoided.

It is believed that 10% mice on this licence will undergo surgery and each mouse will only undergo one surgical procedure. Surgery will be performed using aseptic techniques to minimise risk of infection. We will work closely with the vet and the named animal care and welfare officer to ensure the pain relief is optimal and the mice will be monitored more often until they are fully recovered from surgery.

Pilot studies will be performed to ensure that most refined endpoints are defined for each model. The most refined model that can be used to address the specific scientific questions will be used.

A tolerability assessment in vivo will have been performed for all compounds profiled under this project licence. Where possible data will have been generated to confirm the doses used under this project licence are sufficient for target engagement. This will ensure that the minimal adverse events are observed after compound administration and also that the level of compound is sufficient to achieve the scientific objective.

The use of microsampling where possible has refined the process of collecting blood. Where appropriate, to reduce the number of animals used, multiple tissue samples for PD analysis will be taken from each animal.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 238. Understanding MeCP2 function and its role in Rett Syndrome</b>
<b>Key Words</b>	Rett syndrome, Mouse models, Epigenetics
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We know that alterations in a gene called Mecp2 cause a devastating neurological disorder in humans called Rett Syndrome (RTT). We have shown that different mutations in the Mecp2 gene can cause RTT in diverse ways and with different severity. We aim to find out why this happens by trying to better understand the function of MeCP2 in the brain. We can best address these aims by modelling the Mecp2 changes in mice and studying the 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)?**

A greater understanding of how Mecp2 works will constitute a significant advance in basic scientific knowledge of how genes can be regulated. We have previously shown that in principle RTT could be cured. The work here also hopes to identify and test potential therapeutic options that could transfer to the clinic where the benefits may be life changing

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will be using 5000 mice per annum of which 95% are 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 levels of severity? What will happen to the animals at the end?**

It is known that mice with genetically altered copies of the *Mecp2* gene develop clinical signs like Rett syndrome. The resultant severity in mice is moderate. Most of the animals generated will be on breeding protocols, to maintain the line, with nothing happening to them. Animals will not be allowed to develop clinical signs unless this state is essential for a specific experiment. Some animals will develop signs such as impaired mobility. A minority of animals will be administered prospective therapeutic substances via the least invasive appropriate route possible. All animals will be euthanized using authorised procedures.

## Application of the 3Rs

### Replacement

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

#### Replacement

RTT is a neurological disorder and we wish to study the consequences of the proposed genetic alterations in the brain. The complex biology of such multi-cellular organs cannot be modelled *in vitro*.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

In general we conduct preliminary molecular studies in cultured cells and only progress the more promising approaches into live animal work. Consideration will be given to all breeding strategies to ensure we can produce sufficient animals with the desired genetic status most efficiently, thereby reducing the numbers of animals produced overall. When novel approaches are being tested, we will conduct pilot studies with smaller groups of animals prior to further experiments. Careful experimental design and statistical analyses will enable us to determine the smallest number of animals required to give us meaningful results.

### Refinement

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

#### Refinement

Mice remain the best system in which to study the consequences of changes to *Mecp2* as they provide us with the most extensively characterised model of the human disorder, Rett Syndrome. The precise nature of the genetic modifications that we make, copying those found in humans, constitute significant refinements.

Mice will not be allowed to develop clinical signs unless specifically required for an experiment and in all experiments use of early humane end-points is implemented using clearly defined scoring systems to minimise suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 239. Closed-loop neural interfaces</b>
<b>Key Words</b>	Spinal cord injury, Stroke, Epilepsy, Medical implants
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Closed-loop neural interfaces are electronic implants that sense electrical activity in the brain and deliver feedback to the nervous system. These devices have numerous therapeutic applications in debilitating neurological conditions for which we currently have only limited treatment options, including spinal cord injury, stroke and focal epilepsy. For example, a closed-loop interface that senses in the brain the intention to move and relays this information artificially to the spinal cord can act as a replacement for motor pathways disconnected by spinal cord injury, restoring the ability to make voluntary movements of the limbs. Alternatively, a brain implant that senses the beginning of a seizure and delivers appropriate stimulation to abort that seizure could be used to treat epilepsy, which is important because many people with epilepsy find that no currently available drugs are able to control their seizures. Finally, closed-loop neural interfaces can drive lasting changes to connections within the nervous system through mechanisms of associative plasticity (whereby the connections between brain cells active at the same time become strengthened), with possible applications in rehabilitation following stroke and incomplete spinal cord injury. In other words, the implants could in principle retrain the nervous system to be able to make movements unaided. This project will investigate the basic science underpinning these applications, develop new therapeutic approaches based on closed-loop neural interfaces and perform safety and efficacy testing to enable them to be used as clinical 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)?**

As well as advancing our basic science understanding about brain function, the control of movement, and mechanisms of plasticity during waking behaviour and sleep, this project aims to provide demonstration of the efficacy and safety relating to new therapies that use closed-loop neural interfaces to treat spinal cord injury (by restoring voluntary control of upper-limb movements), epilepsy (by detecting and

suppressing seizures), and stroke (by inducing lasting changes in brain connectivity to support the restoration of lost brain functions). If successful, this information will be used to support subsequent trials in human patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately fourteen macaque monkeys over five years. The maximum number of animals we would use is eighteen.

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

Monkeys are trained to enter a primate chair, become acclimatised to the laboratory, accept further restraint (loose neck collar and chest/arm harness) and perform specific behaviours with the hand/arm. All training uses positive reinforcement with food rewards for voluntarily engaging with restraint and tasks. As training progresses we will likely restrict food availability in the home cage to maintain motivation in the lab. Animals will always receive enough food to keep them healthy and the longest an animal will go without any food is 25 hours. Laboratory sessions typically last 2-3 hours (maximum 5 hours). Up to 5 awake sessions and/or 1-3 sessions under sedation per week will proceed over a period of around 36 months. We find that monkeys adjust well to the routine, do not show outward signs of distress in the lab and calmly perform behavioural tasks. Animals will have an MRI scan followed by surgery to implant devices. Surgeries will be combined where possible, but up to three major procedures, and some minor repairs may be carried out. Possible surgeries include a head implant attached to the top of the skull, retained for life, craniotomies to allow access to the brain, electrodes to record or stimulate brain tissue, tunnelled wires for recording/stimulating arm muscles and nerves, and electrodes within the spine combined with fusion of 3-5 neck vertebrae. All surgeries are conducted under general anaesthesia with post-operative monitoring, antibiotics and analgesics as in human surgery. In some sessions, neurological impairments are temporarily simulated by reversibly inactivating parts of the nervous system. This allows us to simulate some of the effects of a stroke or spinal cord injury (e.g. paralysis of the hand) for a few hours to test our therapy. After the drug wears off, the animals' movements are back to normal. In other experiments we may inject into the brain a gene therapy designed to allow control of brain activity with light, which we believe may be used to prevent seizures. In around 30% of animals, the use of implants is associated with wound problems and/or infection of skin margins. These may require antibiotic treatment or in rare cases further repair surgery. There is possibility of more serious infection in the brain or spinal cord, and around one in ten animals may be humanely killed early if these cannot be effectively treated. Surgery and implants in the nervous system can potentially cause paralysis, weakness or seizures. These complications are uncommon, and will result in the animal being humanely killed if welfare is severely affected or veterinary treatment does not

rapidly resolve the issue. Around one in five animals undergoing surgery may experience short-term neurological problems (most commonly mild weakness in one limb). Where neck vertebrae are fused, animals may experience reduced range of neck movement but typically adjust quickly (within a week) with no lasting impairments to natural behaviours. Animals will be used in a final experiment under non-recovery anaesthesia, which may involve epilepsy induction or creation of nerve damage. This may last up to three days during which the animal is continually monitored and totally unconscious, and after is killed without regaining consciousness. The protocols are classified as up to “Severe” severity, with one “non-recovery” protocol.

## Application of the 3Rs

### Replacement

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

#### Replacement

Wherever possible we use alternatives to animal experiments such as computer modelling, accelerated lifetime testing of devices on the bench, and experimental investigation in human subjects using techniques such as transcutaneous spinal stimulation, transcranial magnetic stimulation and electroencephalography. In addition, we perform experiments using tissue collected from human patients undergoing resection surgery for epilepsy. However due to the invasive nature of the clinical devices we are developing, limited testing in animals to optimise the technology and demonstrate it to be safe and effective is required prior to human trials.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The experiments are carefully designed to maximise the amount of data obtained from each animal. Multiple experimental sessions over different days are used to collect a substantial dataset in each animal, allowing us to demonstrate statistically significant results with a small number of subjects. At the end of the experimental sequence, experiments under non-recovery anaesthesia allow us to collect further data with no additional welfare cost. In addition we share our datasets widely with other researchers in order to maximise the benefits accruing by these valuable experiments.

### Refinement

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

### **Refinement**

Experiments with monkeys are only performed when it is not possible to use other species. In this case, the similarities in the use of the limbs, the neuroanatomy of the brain and spinal cord, and their genetic profile makes data from non-human primates essential to prove these approaches are safe to use in humans. The animals are preselected for behavioural performance, housed in large enclosures in groups of at least two, and receive expert veterinary care. Training procedures are carefully optimised to minimise distress and extent of food control. Our implanted devices (developed for human applications) allow us to collect data using pain-free techniques while the animals are awake, in some cases during natural behaviour in the home-cage. Our surgical and experimental procedures have been optimised over many years to minimise the risk of adverse effects. Pharmacological techniques allow us to temporarily simulate the neurological conditions we are trying to treat without causing permanent impairments.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 240. Discovery of new treatments for pain and inflammation</b>
<b>Key Words</b>	Inflammation, Neuropathic pain, Drug discovery, Therapeutic agents, Analgesia
<b>Expected duration of the project</b>	3 year(s) 9 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to identify and characterise potential new candidate drugs for clinical testing in human inflammatory diseases (such as rheumatoid arthritis) and chronic pain (such as pain from damaged nerves, known as neuropathy). Existing medicines for the treatment of these human diseases have drawbacks, often having serious unwanted or unpleasant side-effects (such as sedation, cardiovascular complications or damage to the digestive system) and new therapeutic targets are required.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The ultimate benefit of this project is that compounds could go forward for pre-clinical development and ultimately clinical testing in humans. These will form the basis for new treatments for chronic inflammation and neuropathic pain.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will only use rodents for these studies, predominantly rats. On occasions, we will need to use mice when this species is more appropriate because of the activity of the compounds being more comparable to the expected activity in humans. We plan to use a maximum of 11000 rats and 2400 mice during the 5 years 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 levels of severity? What will happen to the animals at the end?**

The experiments are designed and conducted by highly trained personnel to ensure that animals suffer the minimum amount of distress whilst meeting the scientific objectives of the study. In most cases the endpoints of the experiment will be measurements acquired from behavioural tests which are considered minimally traumatic to the animals and are of short duration. Throughout the tests the animals will have full escape routes from the experiment and will be well monitored for signs of discomfort/distress.

## Application of the 3Rs

### Replacement

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

#### Replacement

Pain is a multi-cellular process involving the peripheral sensory nervous system, the spinal cord and higher brain areas. Pain generation and transmission involves numerous cell types, including: neurons, glial cells, inflammatory cells and other supporting cell types. Analgesic medicines may act at any point in this network; hence it is currently not practicable to model this process *in vitro*. Realistic measures of success such as pain scores, as measured by such systems as von Frey hairs, and inflammation scores (plethysmography) are not possible in, or transposable to, *in vitro* systems and there are no reliable biomarkers for analgesia. The analgesic or anti-inflammatory effects of compounds must be measured in a mammalian model system. Currently, there are no alternatives to this process, although we constantly review the possible alternatives to the use of animals. A variety of *in vitro* tests (such as target activity, selectivity, measurements of permeability etc) are applied prior to any *in vivo* procedure in order to filter the most appropriate compounds.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Animal numbers have been optimised through statistical power calculations to ensure that the results are meaningful and allow to make decisions moving the compounds forward with the minimum possible number of animals. Through the use of the screening cascade applied to the project priority will be given to animal

procedures that eliminate the maximum number of compounds at the earliest stage of the screening process. Subsequent efficacy experiments will be conducted on single compounds at multiple doses, usually 3, compared vs a vehicle control and potentially tested alongside a positive control. A decision on whether a compound is efficacious will be made on the basis of achieving statistical significance vs the vehicle group. All studies will be done under the strictest experimental design: randomised, blind to the treatment, including positive and negative controls and with the appropriate statistical power.

## **Refinement**

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

## **Refinement**

Rodent models, especially rats, are the lowest species that is likely to produce reliable results that may be predictive for human disease. Rats have a well-documented pharmacology, and the utility and the predictive nature of this model organism has been validated repeatedly through the successful development of a large number of human drugs. Rats respond in a reliable and predictable manner to behavioural tests, allowing group sizes to be lower when compared with other animals, minimising animal suffering. The procedures described in this project (such as inflamed paw using Complete Freund Adjuvant or neuropathic model such as Chronic Constriction Injury) are restricted to one paw and aimed at minimising any potential animal suffering. The testing methods to be used to assess hypersensitivity do not elicit acute pain and are short in duration, minimising any suffering. Throughout the tests the animals will have full escape routes from the experiment and will be well monitored for signs of discomfort or distress. During the tests the novel compounds will provide analgesia and during the surgery marketed analgesics will be administered.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 241. Cell proliferation, death and cell senescence in cardiovascular disease</b>
<b>Key Words</b>	Atherosclerosis, aneurysm, cell death, Cell proliferation, Ageing
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Atherosclerosis is a disease that causes thickening of the arteries, and is responsible for heart attack and strokes, the commonest cause of death in the UK. Arterial aneurysms are a localised expansion of the artery that may cause rupture and sudden death in humans, and we have no current treatment for them. The primary goal of the project is to understand how cell processes such as cell death, cell proliferation and cell ageing (senescence) contribute to diseases such as atherosclerosis, formation of an aneurysm, vessel injury and vessel ageing, and to identify treatments for 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)?**

We will explore ways to reduce or increase cell death and cell ageing (senescence), or increase cell proliferation to inhibit disease progression. If we can do this we may be able to develop both new strategies and new treatments that could reduce the burden of heart attacks and strokes, and reduce aneurysm formation or progression.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mouse. Total numbers are approximately 16850 over 5 years. However, the vast majority of animals will be simply bred to get the required genotype.

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

Most of the animals are just bred to generate the required genotype. Some of these animals are then fed a diet that leads to atherosclerosis, and / or may undergo

additional procedures such as drug administration, artery surgery or bone marrow transplantation. Most of these procedures result in few adverse effects, and most protocols are no more than moderate severity, although development of a vascular aneurysm may result in a severe phenotype. At the end most animals will be killed humanely, although some mice on experimental protocols will undergo perfusion fixation under terminal anaesthesia.

## Application of the 3Rs

### Replacement

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

#### Replacement

The research plan involves in vitro work using tissue culture techniques to understand cell death, proliferation and ageing, to determine the genes and molecules responsible, and validate against human tissues and cells. However, the complex processes involved in atherosclerosis, aneurysm formation, and vessel ageing cannot be reproduced in vitro. We therefore need animal models to mimic human disease and to test the impact of novel therapeutic strategies on disease development and complications in vivo.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The number of animals is minimised by careful experimental design according to extensive previous experience in the models, and is determined according to pre-defined and appropriate statistical analyses. We have used imaging to sequentially follow the same animal, such that each mouse is its own control for changes over time. This also markedly reduces number needed.

### Refinement

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

#### Refinement

The mouse models have genetic alterations to make them susceptible to disease, or have over- or under-expressed genes. Most protocols are no more than moderate severity, but the aneurysm protocol could be severe, which reflects the severity of the consequences seen in humans (aortic rupture and death). In these protocols, surgery is done under general anaesthesia, so the animal does not suffer, and animals monitored at least every day and up to 28 days after surgery to detect any sign of cardiac failure (reduced movement, hunching, breathless at rest), aneurysm rupture or signs of limb necrosis. If this occurs, the animals will be killed by a schedule 1 protocol.

To reduce suffering we have also reduced the study ages of animals on ageing protocols who had an accelerated ageing phenotype and would become ill at later time points. In addition, The surgical procedures have been refined over the years and the operators are very skilled at the procedure. New personnel undergo stringent training beforehand on cadavers.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 242. Testing new Clostridium difficile directed therapies and vaccines.</b>
<b>Key Words</b>	Clostridium difficile, Therapy, vaccines
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

*Clostridium difficile* associated disease (CDAD) is a significant cause of morbidity and mortality in humans worldwide. Vaccines to prevent infection and the choice of antibiotic therapies is limited and may be further compromised by the emergence of resistance. The aim of this project is to investigate novel and emerging therapies for the treatment and prevention of *Clostridium difficile* infection.

**What 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 animal models will allow testing of the efficacy of new therapies generated by ourselves or collaborators. This could lead to more effective treatments for human disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Hamsters – 2280 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 levels of severity? What will happen to the animals at the end?**

The animals will show symptoms of *Clostridium difficile* infection (including wet tail, diarrhoea, and hunched posture). With an expected level of moderate severity. Once the animals are showing non-recoverable symptoms of *C. difficile* infection they will be humanly killed using a Schedule 1 method.

**Application of the 3Rs**

## Replacement

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

### Replacement

The combination of the requirements for an anaerobic environment and the gut specificity of *C. difficile* has meant that suitable non-animal alternatives have not been identified.

The information that can be obtained from established tissue culture assays, is limited as the infection process involves complex dynamic interactions between host and bacterium which are impossible to model effectively in non-animal alternatives at present.

Testing of vaccines involves the generation of immune responses and delivery of drugs to the appropriate tissues which also requires living animals to determine clinical response.

We have considered the use of *Galleria mellonella* (wax moth larvae) as an invertebrate model to replace the use of animals. So far we have found that this organism is not susceptible to *C. difficile* toxins when injected however oral inoculation with *C. difficile* results in a degree of infection. However since the intestinal tract of the wax worm differs significantly from that of higher vertebrate organisms, the full spectrum of disease symptoms is not observed in the *Galleria* model. We are continuing to develop this model however to determine its relevance as a colonisation model.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We aim to reduce the number of animals used by carrying out power calculations and implementing a block design for each experiment, initially using groups of 3-4 animals per treatment group and common control groups, with all experiments being blinded. Once the initial experiment has been completed and the data analysed, additional groups of animals of an appropriate size (up to the number obtained from the power calculations and to a maximum of 15 animals per group) will be used and the data combined for statistical analysis.

To date we have typically used combined group sizes of between 8-15 animals. Where possible the data generated from the control animals will be used across a number of studies.

In order to ensure that high quality, reliable and valid data is extracted from the minimum number of experiments, the ARRIVE guidelines (Kilkenny, 2010) will be followed (<http://www.nc3rs.org.uk/page.asp?id=1357>). The following websites will also be utilised to provide additional information on experimental design and statistics; The NC3Rs experimental design assistant <http://eda.nc3rs.org.uk/> and the 3Rs-Reduction.co.uk site <http://www.3rs-reduction.co.uk>

## Refinement

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

## Refinement

Hamsters are the lowest vertebrates in which the pathology of infection by *C. difficile* resembles that manifest in humans. This includes the germination of spores and colonisation of resultant vegetative cells in the intestine, inflammation due to toxin release and transmission of spores in faeces. The hamster is particularly susceptible to CDAD and develops wet tail; diarrhoea being a classic symptom of CDAD in humans, and rapidly progresses to the defined end point. The hamster is therefore invaluable in quickly assessing the efficacy of interventions in preventing disease onset or delaying the time from infection to the defined end point.

The scientific objective is to administer therapies or vaccines to the hamsters and determine if they are efficacious in treating or preventing CDAD. The condition of the infected animal, degree of colonisation of *C. difficile* (as determined by the degree of shedding of this bacterium) and extent of pathology in the gut (as determined by tissue histological studies) of treated animals versus control animals will provide an indication of the success of the treatment. Ultimately the scientific end point is to achieve complete prevention of CDAD.

The procedures proposed to be conducted in hamsters were previously optimised by the applicant. The practice of these methods has been closely observed by the NVS, NACWO and Home Office Inspector with minor modifications implemented upon recommendations. An existing monitoring scheme established and widely used for hamsters will be strictly adhered to ensuring that appropriate non-lethal end points are achieved. Based on this scheme, a specific scoring sheet will be used to assess a number of parameters. A score will be given between 0-3, and any animal scoring 15 or more will be euthanized using a Schedule 1 method.

Animals will be handled by trained competent staff at all times and will be returned to their home cages as soon as possible following any procedure in order to minimise stress. LASA guidelines will be followed for dosing and blood sampling.

Animals will be administered a maximum dose of 20ml/kg of sodium bicarbonate by the oral route to neutralize the stomach acid an hour before administration of therapy or vaccine. Topical local anaesthetic cream will be applied 30 minutes prior to any blood sampling to prevent any pain from the venepuncture. The volume of blood withdrawn will not exceed 10% of the blood volume of the animal as recommended by the LASA guidelines. Immune response markers will also be sampled from faecal samples and if a direct correlation can be established between the levels seen in blood serum and faecal samples, blood sampling will no longer be conducted.

Hamsters will be group-housed until oral administration of Clindamycin after which time they will be individually housed. As hamsters are typically solitary animals, single housing should not adversely affect their normal behaviour and is required for the experiments as the monitoring of infection requires analysis of faecal samples collected from individual animals. In addition, single housing is required to ensure that any infection observed is as a result of the planned infection and not as a result of animal interactions.

All animals will be provided with paper nesting material, cardboard tubes and wooden chew blocks in the cages. Hamsters will also be provided with wheels as a way of providing stimulation and environmental enrichment.

Animals will be given free access to food and drinking water at all times, unless withdrawal of food is required to minimise stomach acidity for the experiment. If the removal of food is required, it will be returned after the shortest time period possible typically 2 to 4 hours.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 243. Novel Conduit Repair of Rat Sciatic Nerve Defects</b>
<b>Key Words</b>	Nerve Guidance Conduit (NGC), Peripheral Nerve
<b>Expected duration of the project</b>	2 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

In this project, the key aim is to study peripheral nerve regeneration. This occurs after nerve tissue is injured and the nerve itself will regrow. However, this is not always efficient and complications arise when nerve tissue does not regenerate fast enough resulting in loss of muscle action or touch sensation.

We wish to test the use of a new synthetic nerve graft material which can be implanted into a nerve injury site to help regenerate the tissue more efficiently. The nerve graft material will be 3D printed in nylon and contain a blend of natural gel materials used in peripheral nerve regeneration research. We propose to test the nerve graft in a Rat Sciatic Nerve gap injury model, where will transect the nerve to leave a gap and then graft in the proposed materials. The suitability of the new materials will be measured against the gold standard method of repair, which is used in clinics currently. The gold standard involves grafting the injured nerve with a patient's own healthy nerve tissue which results in two operation sites and is only effective in 50% of cases. Currently, the scientists in this field look to increase effectiveness of nerve grafts, but this must be tested for safety in animals first. This is our aim, to test in rats and then translate the technology to human trials.

This synthetic nerve graft is called a Nerve Guidance Conduit (NGC) and has never been tested in an animal study. The effectiveness of the NGC will be measured before surgery and during a 3 month recovery period at regular intervals so a trend can be drawn and the results published in a scientific journal.

The broad aim can be further split into six objectives of this study, as we wish to get as much information from each animal as possible:

1) We will carry out a surgical procedure to implant the new nylon NGC into the Rat Sciatic nerve.

- 2) We will investigate the capability for the animal to use muscles to manipulate its toe spreading
- 3) We will determine if the sciatic nerve has been repaired by measuring electrical impulses derived from the new nerve tissue under light general anaesthetic.
- 4) We will carry out a touch response test to determine if the animal feels physical stimuli after nerve repair
- 5) We will carry out thermal test to determine perception of heat following the nerve repair

Following behavioural tests (objectives 2-5), we will sacrifice the animals by a Schedule 1 method and image the new nerve tissue to show evidence of repair.

**What 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 proposed nylon and collagen/ chitosan NGC is successful in aiding sciatic nerve regeneration then the novel NGC could be developed to be included in clinical trials in humans. At present, the gold standard procedure is the standard method for repairing long gap injuries, but this involves the use of healthy nerve from the patient and so causes a second injury site to the nerve being repaired. This can result in pain experience, loss of sensation or loss of muscle action and will cause long-term damage to the nerve from which the healthy nerve is taken. We propose the use of a safe and functional synthetic designed NGC for use in humans after rigorous analysis in the animal study proposed. In terms of benefits directly for the patient, we would like to publish these findings in a scientific journal in which surgeons will be able to read our findings and we can encourage some surgeons to use our technology in a clinical trial on patients from REDACTED. The technology should reduce the need for the current gold standard method of repair which is suboptimal.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We would like to use the minimum number of Wistar Rats required to provide statistically sound results so that we can publish our findings in a respectable scientific journal. Therefore, after consulting experts and other scientific journals, 14 animals will be used in each experimental group. We have three experimental groups in total: autograft repair control group; nylon NGC group; and nylon NGC containing chitosan/collagen gel group. Therefore, the approximate number of animals is 42. We would be using these animals before the nerve repair procedure for handling and training to minimise stress, carry out the surgery and then monitor behaviour over a 3 month period at regular intervals, so the experimental time period will be up to 4 months for each animal. We would like to use a minimal number of 14

animals per experimental group, therefore we would expect to use approximately 60 adult rats. This takes into account some pilot studies before the full study is carried out, to ensure high levels of safety and animal care in the procedure.

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

This study relies on transection and repair of the sciatic nerve which supplies muscles that are involved in walking. Therefore, the adverse effects of the surgery will affect sciatic nerve function and potentially affect the rat's walking motion. Additional adverse effects of the surgery may include post-operative swelling, and possibly pain which will be monitored and treated accordingly. Animals will be checked daily for signs of pain, suffering, distress or lasting harm and any of these signs will be referred to the vet for advice. When necessary the appropriate analgesia will be administered, on the vet's instruction. This surgical technique has been carried out and published previously and has been shown to be a moderate procedure in terms of severity. At the end of the experiment, the animals will be humanely euthanised by Schedule 1 methods ending the protocol.

## Application of the 3Rs

### Replacement

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

### Replacement

Previously, before applying for this licence, we have carried out extensive in vivo tests to determine the suitability of the three materials being used in this study for their biocompatibility. Specifically, we have tested the materials effects on two cell types and grown cells up to 2 weeks. No adverse effects of the materials have been reported thus far. To understand the effects of the proposed design, i.e. inclusion of the chitosan/collagen gel, we need to study how real nerve tissue in the animal responds to the novel NGC structure. We have attempted to see the effect of the material on harvested nerve tissue from rat embryos in Petri dishes, however, the nerves in these experiments only grow to 0.5mm at most which are insufficient to predict the suitability of the materials to aid repair of a gap injury that is 10mm long. If we cannot study the materials at an appropriate scale, which would be supplied by the animal model, we cannot translate the technology confidently to human trials as we would not know the cellular-material effects in vivo over a long period of time (months) and longer gap injury length.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

As stated previously, we have consulted with statistical experts, training staff at the REDACTED and the Named Veterinary Surgeon as well as scientific journals to ensure the minimum number of animals can be used. However, the number of animals must be appropriate to allow for statistical analysis of the data we will retrieve so that we can publish the findings in a respectable scientific journal. Therefore, we have concluded that 14 animals per experimental group is a statistically sound number. Furthermore, we would like to carry out a pilot study to ensure that procedure is fully comprehensive and the behavioural tests can be carried out. This can ensure that no unnecessary waste of life occurs.

## **Refinement**

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

### **Refinement**

The Rat Sciatic nerve model has been calculated to be the most widely used model in the field of peripheral nerve regeneration. Furthermore, the rat has been chosen as opposed to the mouse due to its larger proportions, ease of handling and to lessen the risk of error in the surgical procedure as the nerve is larger and easier to see/operate on. The rabbit was not chosen as a model as the animal is considered a higher species, however, the results we require can be easily obtained in the rat. We believe this is for good reason as it is the largest diameter nerve available in the Rat, and also is easily accessible via the mid-thigh region. With respect to the objectives, the toe spread analysis is only useful following sciatic nerve transection. The other behavioural tests can be easily carried out on the sciatic nerve, however, on smaller nerves this would be difficult as the muscles and skin supplied by these nerves would be smaller in volume and less accessible externally.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 244. Defining correlates of infection to reduce respiratory infections</b>
<b>Key Words</b>	Infection, Lung, Vaccine, Antibiotic
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of the project is to understand how the immune system protects us from lung infections in order to develop new treatments and preventative approaches (such as vaccines and antibiotics).

The response to infection is complex and multi-factorial. These components all contribute to prevent infection or reduce its impact. We will use a number of different approaches to dissect the role of individual components.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Respiratory infections are a major cause of disease and death globally. Of particular concern is the rising prevalence of antibiotic resistant infections. The knowledge developed from this project will help us to develop new vaccines, new anti-viral drugs and new antibiotics.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mouse ~ 8400 animals

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

The mice will be infected with a range of infectious agents. This is classified as moderate severity. The most marked adverse effect is transient weight loss from which the animals will recover. The animals will be humanely killed at the end of the procedures.

## Application of the 3Rs

### Replacement

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

#### Replacement

The interactions in the human immune system following infection and vaccination are complex and not fully understood. This incomplete understanding therefore means that experiments using white blood cells alone or computer models cannot fully reflect what is taking place in vivo. In order to gain a better understanding, manipulations involving the depletion / replacement of immune system components need to be performed which cannot be done in human volunteers. Specifically for studies of infancy, human studies are impossible because it is ethically unacceptable to experiment on babies. Therefore there is no viable alternative to using animal models. The work itself is justified because of the impact of infant disease and the potential benefits to be gained from understanding it.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will only perform studies on animals when there is no other alternative. We will reduce the numbers of animals used by extensively testing our hypothesis in experiments without animals before confirmation studies in animals.

When it comes to the use of animals, we will use statistical advice and our longstanding experience to minimise the number of animals needed to answer each research question.

### Refinement

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

## Refinement

Mice have been chosen for this study for several reasons, there is a large number of tools available to interrogate the immune response, they are widely used so responses are comparable between different studies, they are the lowest mammal in which these studies can be performed and they recapitulate most features of the human immune response.

Where procedures may cause animals more than minimal or momentary distress or pain, animals will undergo brief anaesthesia prior to such procedures being carried out. Housing: we will ensure use of appropriate bedding and nesting material so comfort of animals is maximised. Handling: Good, sympathetic animal handling techniques will be used at all times, injection and blood sampling techniques will be carried out only when required to answer the research questions and will be done using techniques to minimize discomfort. Based on our experience and knowledge of previously performed studies, we will ensure the suffering of animals is minimal in the context of the work being performed.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 245. Developing Genetic Technologies in Mice</b>
<b>Key Words</b>	Genetic Technologies
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Proteins carry out many of the functions that help cells within an organism function, and aberrant protein function can cause disease. Proteins are composed of different combinations of 20 amino acids. Our ability to study proteins is limited by our ability to control them and to see what they are doing. We have developed technologies in cells that allow us to put new amino acids, that are made by chemists in the laboratory, into proteins. These technologies have proven useful in cells for understanding what proteins are doing in isolated cells- they have allowed us to see proteins, to see what they interact with and to control them. Isolated cells have very different properties from cells within a whole animal, because cells within an animal interact to give rise to the complex and important behaviours we associate with animals that are not present in isolated cells. It is therefore important to understand what proteins do in cells within the context of an animal and we are therefore planning to extend the approaches we have developed for introducing new amino acids into protein from cells to the mouse. The majority of the work will be in establishing a robust technological foundation for the approach. We anticipate that this technology may have broad impacts in how protein function is studied, which may in turn have broad impact for studies of health and 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 project is aimed at technology development. It aims to develop technologies that will help us to study processes at the molecular level in a physiological context. The technology we aim to develop will allow us to introduce new types of amino acids into proteins inside the mouse. These amino acids can give new properties to the proteins. For example they can allow us to see the protein, or turn its function on or off. Using these methods, researchers can gain increased insight into what proteins are doing in the cells of animals. In one example of a potential application of a technology we are developing we would be able to label and identify all the proteins produced in specific cells in an animal. This is particularly important in the

brains of animals where there are many different cell types producing different proteins. These proteins are believed to underpin the different functions carried out by different parts of the brain. Defining the proteins produced in different cells in the brain may therefore enable us to learn about how the different parts of the brain function differently. It is also believed that different proteins are produced in specific regions of the brain when mammals learn. Our approaches may allow us to label and identify those proteins that change when a mouse learns and thereby provide insight into learning and memory.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 year course of this licence up to 9,800 genetically altered mice will be born. The majority of these will be examined with mild, non-invasive techniques. Up to 800 may be given substances that alter the way genetic alteration behaves. A few hundred will have blood samples taken and all will ultimately be humanely killed. Up to 2,000 female mice will be humanely killed to donate eggs and embryos. Another 50 will be used to make sterile males, which are required for the breeding. Therefore the total maximum number of animals likely to be used over 5 years is 13,350.

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

We do not expect the animals harbouring our technology to suffer as result of the gene manipulation. Indeed, the aim is to generate a system that shows no deviation from the normal, as this would interfere and possibly complicate the subsequent experimental use. However, since the technology is novel, we cannot fully predict the outcome of particular gene combinations. Similarly, the administration of substances for this technology is not expected to generally cause harm. Substances found to be harmful will not be useful and their use will be stopped. Occasionally some of the substances may make the food/drink taste unpleasant, in this case, food flavouring will be applied to make the food and water taste better. When the administration of substances through food and/or drink is not suitable, we might use intraperitoneal, intravenous or subcutaneous injections, or topical applications on the skin in the form of creams or ointments. Some mice may have minor surgery to implant a device under the skin that can release a substance slowly or will undergo an injection of small volume of solution in their brains. For the latter, during half an hour surgery we will typically make a very small window in the skull, to enable the needle to reach the brain. Mice undergoing minor surgery are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital. All of our mice will be kept in an establishment with controlled temperature and air humidity. Their cages will be equipped with fun tunnels, nesting materials and other environment enrichment tools, which promote natural behaviours and animal well being. Usually animals won't be kept beyond one year of age. At the end of the experimental procedures all animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

#### Replacement

Prior to mice, we have used single cell organisms, tissue cultures and invertebrate animal models to tackle a number of fundamental biological problems. Their power comes, at least in part, from their simplicity, which will allow us to use them to optimise the assay conditions before applying the assays to mice. However, this is also one of their main limitations. In particular, invertebrate models lack an immune system, while other aspects are very different such as development and neurobiology.

Research in diseases using invertebrate models is limited to those areas that are conserved. Thus, the mouse provides an appropriate model as its basic biological and pathological processes are similar or identical to those in other mammals, including man.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Our experiments will comply with the ARRIVE guidelines. To reduce the number of animals required initial experiments will be conducted in cultured cells, which may be taken from genetically altered mice. Pilot studies, involving a very small number of mice will be designed to explore the feasibility of each experiment.

To maximise the information from a single animal, we will collect samples from all major body organs once the animal is humanely killed. Further, by using viruses we can generate directly a new transgenic animal without the lengthy breeding of transgenic mouse lines. Cryopreservation is being used routinely to preserve important mouse lines and to remove the need to breed mice just to maintain a given line.

### Refinement

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

## **Refinement**

Our transgenic mice are unlikely to display any abnormal features. However, to avoid unexpected pain and suffering, we will control the transgene expression wherever possible and the mice should not display any side effect until gene expression is induced.

When new mouse lines are generated and bred for the first time, animal technicians will be specifically informed and the first litters carefully monitored. Any unexpected effects will be discussed with the NACWO, veterinarian and if appropriate, the Home Office inspector.

Genotyping will be undertaken from ear biopsies and in rare cases, where more DNA is required, from tail biopsies.

The surgery will be carried out in sterile conditions and we will aim to follow "Guiding principles for preparing for and undertaking aseptic surgery" (2010) as closely as possible. Mice will be given painkillers prior to recovery from anaesthesia and whenever necessary to alleviate pain as advised by the veterinarian.

All animal experimentation conducted under this project licence will comply with the document entitled "Animal Usage Guidelines". This document has been adopted by the local Ethical Review Process in order to inform researchers of the bounds within which their animal work should be conducted, and to provide practical recommendations on various aspects of animal experimentation.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 246. Mechanisms of Central Nervous System Development</b>
<b>Key Words</b>	Brain, Development, Gene, Embryo, Mouse
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

About 1-2% of people develop neurological or psychiatric diseases such as epilepsy, schizophrenia, cognitive disorders and autism whose origins lie in defects of early brain development. Although we can link these diseases of the brain to mutations of developmentally important genes, we do not understand how the mutations cause the defects. Given this lack of understanding, we have little chance of developing effective therapies. Our work aims to improve the opportunities for therapy. Our previous work identified some likely mechanisms by which key genes regulate normal development and, therefore, how their mutation causes disordered development. We aim to test these possible mechanisms.

The objective of this project is to increase our fundamental understanding of how genes control brain development. We focus on two major classes of genes: those that control the function of DNA and those that control essential signalling between developing cells. Defects in both of these processes can cause neurodevelopmental disorders. We want to know how genes of these two types normally work and what happens when they are defective and why.

**What 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 increase our fundamental knowledge of the mechanisms that generate the mammalian brain. Current knowledge in this area is relatively very poor due largely to the extreme complexity of the brain. In addition, many of the human neurological diseases originate during development, making impossible any study in human subjects. Advances in molecular genetics and the progresses of the understanding of the development of the mouse brain over the last 15 years have provided new ways to investigate this topic and improvement in understanding the mechanisms of brain development are likely to increase our ability to tackle currently incurable diseases of the human brain in the future.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice About 15,000 Over 5 years

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

Animals that have been genetically modified will be generated and bred so that we can learn about the consequences of the genetic defects, and hence uncover the biology of the affected genes. We mainly used so-called conditional mutants in which the defects are limited exclusively to the region of interest (the brain) with little overt sign of general harmful effects for the animal. To assess the consequences of the genetic modifications, animals may be administered with substances to label specific cells, but the majority of animals will have no invasive procedures carried out while they are alive. The adverse effects will be brain defects resulting from the gene mutations that we are studying; i.e. these defects are the subject of our study and relate to human disease. In a minority of cases, animals may receive compounds that aren't expected to cause any side effects in themselves, but that alter how genes are expressed. These substances may be injected or dosed orally, but in some instances we need to be able to place them directly into the brain of the embryos. Here, the adverse effects will be those associated with the surgical techniques required to access fetuses through the womb and to their brain. Pain killers will be given on veterinary advice to ensure suffering is minimised. The maximum severity is classified as moderate. At the end, the animals will be killed and they or their offspring will be analysed or their tissues will be cultured.

## Application of the 3Rs

### Replacement

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

## Replacement

Overall, this research project aims to increase our knowledge of how the brain develops in animals including humans and, as such, can not be done without studying animals. Neuronal development *in vitro* does not recapitulate the patterned development seen *in vivo* and *in vitro* techniques are often inappropriate for our studies. Similarly neuronal cell lines are inappropriate because their immortality means that the very processes that we are striving to understand have been disrupted. The use of *in vitro* methods will, therefore, be a useful adjunct to our overall approach but can not replace *in vivo* techniques.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

The numbers of animals required will be the least required to give statistically significant results. Any less would be a waste since conclusions could not be drawn. The numbers required to give statistical significance depends on the effect size – the smaller the effect, the larger the numbers of animals required. Thus, for any specific experiment, pilot experiments are needed to assess the likely effect size and then numbers can be determined.

## Refinement

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

## Refinement

The mouse offers the advantage of being an excellent genetic model and, most importantly, its brain recapitulates the major features and developmental pathways of the human brain. This is important if we are to learn about mechanisms that relate to human diseases. Much of our work (probably 75%) involves the analysis of post-mortem material and animals are always killed rapidly and humanely. Where surgical methods are required, we carry these out as quickly as possible with attention to the use of appropriate measures to minimize the risk of pain and suffering. Where we see evidence of unreasonable harm, animals are killed humanely.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 247. Reciprocal tumour-host interaction in cancer metastasis and therapy response</b>
<b>Key Words</b>	Cancer, Stroma, Metastasis, Therapy Resistance
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Cancer consists not just of malignant tumour cells but also many number of normal host cells that are not malignant. These include cells that provide nutrition and growth factors to the tumour cells and that form an essential support to the tumour as it progresses to be able to escape from the local environment to a distant site. This process is known as metastasis and is the primary cause of death among cancer patients. Unfortunately overall survival for these patients with metastasis has not changed for twenty years because this stage of disease cannot be operated surgically and is often resistant to anti-cancer therapies. This indicates that the current medicines are ineffective for treatment of metastatic disease and this demands development of new medicines in order to effectively cure the disease.

The natural history of cancer is that they progress from benign (not life threatening) to malignant (life threatening) stages. Our and others studies have indicated that these transitions to the most dangerous stages of the diseases is speeded up by the normal cells in the tumour environment. Our previous research has indicated that killing these non-malignant cells in the tumour can inhibit tumour cell metastasis. Our experiments have mainly focussed upon the sub-populations of a type of blood cells called macrophages that reside within the tumour. In particular we have indicated that these tumour-associated macrophages are important for the spreading of cancer cells. At each step of this process macrophages provide support to the cancer

cells. Consequently our general experimental plan is to use mouse models of cancer to define the molecules involved in the tumour cell-macrophage interaction and the relationship of these cells with other components/cell types of the tumour microenvironment. Thus while it is now established in the majority of pre-clinical models that the tumour microenvironment regulates metastatic progression the actual basis of these effects still largely remain to be explained. This project aims to determine the molecular basis for these tumour-stroma interactions using genetic and imaging methods, which will indicate targets that may lead the development of novel therapeutics directed to the tumour microenvironment and may ultimately be used in humans. The goal of this animal based research therefore is to develop an understanding of the cellular mechanisms responsible for the development and spread of cancer in an attempt to define possible therapeutic strategies 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)?**

Through these studies, we will be able to reveal the role of tumour surrounding normal cells (stromal cells) in cancer metastasis and therapy resistance. This project also allows us to identify factors that mediate tumour-stroma interaction and affect cancer cell behaviour and efficiency of anti-cancer therapies. Such information will provide novel strategies for targeting stromal cells to treat cancer metastasis and therapy resistance. Comparison of stromal cell functions between healthy tissue and tumour tissue gives us essential information to generate more specific and therefore less toxic therapeutic strategies. Investigation of pro-tumorigenic functions of stromal cells will also provide important information for cancer researchers to develop novel prognostic markers to predict disease outcomes, diagnostic markers to follow disease progression and select optimal therapy to prevent cancer progression to the more deadly forms. The potential benefit of this research is to provide the basis for cure of metastatic disease and therapy resistance in a wide range of cancers.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mouse 126,000 over 5 years

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

Both experimental and genetically engineered mouse (GEM) models of cancer develop benign to malignant tumours. This may cause moderate discomfort and can become severe in rare cases given extended time. However, their development is tightly controlled to minimize the suffering while allow scientific information to be collected. Furthermore, the tumour-bearing animals are monitored for their clinical signs, and are euthanized upon humane endpoint. All surgery and injection methods are well established and we have enough experience to minimize infection and health problems that may arise from these methods. So far, intravital imaging is the

only method to reveal tumour cell behaviour and dynamic interaction with stromal cells in the complex environment. To obtain clear images, we will insert imaging window over the tumours, which will cause pain to the mice and possibly to induce post surgical inflammation but only in rare cases. However, pain is controlled by general anaesthesia and analgesics, and risk of infection is minimized by good surgical and aseptic techniques. The insertion site is monitored for signs of inflammation and infection, and antibiotics will be given if necessary. All the animals used in the experiments will be euthanized at the end point.

## Application of the 3Rs

### Replacement

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

#### Replacement

We use animal models in these experiments because tumour progression involves progressive genetic changes that cannot be modelled adequately by cells in culture. Furthermore, the complex tumour environment contains many different cell types whose interactions can only be found *in vivo*. For these reasons only animal models can be used. We chose mice as an experimental animal because cancer in these animals has been studied for many years giving us a good foundation for our experiments. Furthermore our group and others have carefully documented tumour progression in this species for several different tumour types. We have some mice in which human cancer cells or tissue fragments can be grown, which allows us to study them in a fashion that is not possible in culture. We have developed and are using a few *in vitro* assays to study certain aspects of the cell-cell interaction *in vivo*. We will review and incorporate alternatives throughout the project duration whenever possible.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

To understand molecular mechanism and identify potential therapeutic targets, our study requires various single and compound genetically engineered (GE) strains, which requires complicated breeding strategies. To minimize the number of animals involved we have now identified the most efficient breeding crosses to generate mice of the correct genotype. To reduce the required numbers of GE animals, we will transplant bone marrow or mammary gland from GE animals to wild type recipients because it can provide enough animals with same genetic mutations in blood cells or other cells without further breeding. We will also use repeated non-invasive *in vivo*

imaging and intra-vital imaging to monitor tumours and stromal interaction, which will greatly reduce the number of mice to be required.

We ensure the experimental plans are designed using statistical principles to test our specific hypotheses and thereby only use the numbers of mice necessary. Group size will be determined based on our experience and published studies and power analysis based on preliminary data generated using minimal number of mice whenever not certain.

### **Refinement**

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

### **Refinement**

We use mouse tumour models that have been developed to mimic the progression of the disease in humans as much as possible to understand the disease mechanism and identify potential effective therapeutics to treat metastatic cancers. The welfare cost is metastatic tumour in these mice. Our research group has developed many of these models and thus have extensive experience of the clinical signs. This allows us to effectively use humane end points to collect necessary scientific data while avoid severe health problems in the animals during the experiments. We will ensure that all animals receive the highest standard of care, and animal suffering is kept to a minimum by close monitoring, in consultation with NACWO and NVS, of health status and clinical signs using a scoring system agreed by NVS and Home Office Inspector. We will use our refined genetic modification techniques to improve the welfare of our animals. We also have developed methods to examine tumours repeatedly using non-invasive and non-terminal methods that minimise the numbers of mice used. Furthermore, we continue to monitor technical advances and to innovate novel techniques in an attempt to reduce the impact of experimental cancer on the animals. If suitable models occur, we will test and adopt in consultation with NVS and Home Office Inspector.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 248. Interaction between cell cycle, cell death, development, and diseases</b>
<b>Key Words</b>	Cell cycle, Development, Human disease, Cancer, Retinal disorders
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Many human diseases result from defects in cell cycle regulation, development, and their interaction. However, little is known about the mechanisms of their interaction. Also, not much is known about the molecular mechanisms which cause human diseases. This project will investigate the mechanisms, aiming to understand human diseases such as cancer and eye 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)?**

This project will help to elucidate the molecular pathology of human disease such as cancer and eye disorders. This understanding is essential for development of new diagnosis and treatments. This project will try to develop novel diagnosis of cancer and eye disorders, it also aims to develop novel treatments for cancer and eye disorders.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Xenopus: 400 frogs over five years Mouse: 3,000 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 levels of severity? What will happen to the animals at the end?**

Xenopus: We maintain Xenopus in order to obtain eggs and testes. To induce a female to mature and then release eggs, she is injected with hormones and then, a few days later, eggs extruded by gentle pressure on the abdomen. She is then rested for at least three months, usually at least four, before the process is repeated. Typically this process very little stress or damage to the frog. The injections use a

short, fine needle which causes only a short lived prick of pain. Extrusion of eggs uses only mild pressure and is not painful. Complications such as infection at the site of injection can occur, but very rarely (I have never seen this in many years of work with these animals). If painful complications do occur the frogs will be culled by a schedule 1 method. Mouse: We will maintain genetically modified mice. Typically, the mice are maintained for their whole life with no adverse effects. 400 mice are maintained in protocol 4, a moderate procedure, because some of these 400 are expected to develop cancers. The mice will be carefully monitored and culled as soon as they show any signs of distress or other signs of cancer.

## Application of the 3Rs

### Replacement

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

#### Replacement

We currently use in vitro cell culture. However, the research we are planning is complex developmental/cancer biology, with multiple interacting tissues. Processes of embryogenesis or retinogenesis cannot generally be reproduced by cells in culture, so we have to perform studies on whole embryos. Also, this project aims to evaluate roles of genes that regulate co-ordination between cell cycle regulation, determination, and differentiation in systematic human diseases. Although we use in vitro systems, such in vitro systems are not sufficient to evaluate the roles in human diseases. Therefore, combination of in vitro and in vivo system is essential.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Through the combination of Xenopus and mice we will reduce the number of the higher organisms, mice. Also, we will re-use the female Xenopus to obtain eggs, which significantly reduce the number of Xenopus. Also, we will design experiments to minimise the number of animals.

### Refinement

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

#### Refinement

My lab. works in parallel on the amphibian *Xenopus* and on mice. It is necessary to use a mammalian model animal some of the time since pathways and drug responses may well be different in non-mammalian species. I choose to work on mice since they are small and readily breed in captivity, and since many mutant lines are available that model human diseases.

However, wherever possible I use the amphibian *Xenopus*. Because a single female frog produces 2000 – 3000 eggs at one time, it is possible to perform a screen of many potential drugs in one go. The equivalent experiments using mice would require many individual mice. The procedures necessary to harvest *Xenopus* eggs cause only brief discomfort to the animal. The animal house has all facilities necessary to maintain the frogs in health and comfort, free from disease. A single female can be used many times, removing the need to buy new animals and transport them , a stressful procedure for the frogs.

In our experiments on mice, I will for the majority of the time use genetically modified mice whose genetic modification causes no detectable harmful phenotype. For a relatively small number of experiments we will use mutant mice with a moderate harmful phenotype as models for human cancers. These mice will be monitored carefully and killed before any tumours become uncomfortable. Analgesia will be used wherever appropriate to reduce pain and irritation in experimental mice.

I am developing a technique in which each of over 100 individual cells dissociated from a single mouse or human donor is used to replace a whole animal model of disease progression and treatment. Once this is proved it will allow me to screen many potential drugs and other chemicals in one go, eliminating the need for many individual animal experiments.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 249. Neuroimmune mechanisms of CNS degeneration and regeneration</b>
<b>Key Words</b>	Neurodegeneration, Brain repair, Immune system, Infection, Microglia
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The immune system is essential for protecting us from infection but there is also growing evidence that it can have both harmful and helpful functions in the brain that can influence the risk of brain disease and the response to injury. Brain tissue is particularly sensitive to some forms of inflammation but conversely, some types of inflammatory activity are needed to help the brain heal after injury. The molecular and cellular mechanisms which influence the balance between harmful and helpful actions of the immune system in brain disease and injury are not well understood. It will be vital to better understand these so that the damaging effects of the immune system can be targeted to treat brain disorders without affecting the helpful functions, such as protection from life-threatening infection.

The aims of this project are to:

1. Identify key cells and molecules that control and cause the harmful and helpful effects of the immune system on brain injury and disease
2. Test if manipulating these cells and molecules can minimise the damage and complications caused by brain injury and disease and enhance brain repair and recovery

**What 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 the project will lead to a major step forward in knowledge about how the immune system positively and negatively influences brain injury and disease. This new knowledge may include the identification of new therapeutic targets, that with further development beyond this project, could have the potential to reduce death and disability caused by brain injury and disease. For example, the information

produced in this project could inform and lead to future testing of therapeutic agents in human trials. The new knowledge we create will be important for the progression of our own studies but will also help other scientists and medical professionals to develop their own work thus ensuring that progress towards treatments of brain injury and disease can be made as quickly as possible. We will share our data where appropriate so that this is possible. It is likely that the work we do in the project will develop and refine existing methods and techniques and potentially produce new approaches. These could benefit the scientific community by providing better ways to gain insight to how the brain is damaged. It is also possible these developments will help to further reduce the risk of adverse effects on animals used in research.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use around 4000 mice overall and around 50 larger animals (e.g. pigs) 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 levels of severity? What will happen to the animals at the end?**

Adult animals will be used and will be housed in spacious and well-maintained cages within a dedicated animal facility and provided with free access to food and water at all times. Trained animal care staff will ensure that best practices of animal husbandry are applied. Our project is aiming to understand processes that influence brain injury and disease therefore animals will undergo procedures that are designed to replicate aspects of the human conditions e.g. stroke, Alzheimer's disease. Some experiments will involve the surgical blocking/narrowing of blood vessels supplying the brain or the precise injection into the brain of substances that cause cell death or inflammation. Experimental infection may be achieved by injection of bacteria into the nasal cavity. Mice may be irradiated to deplete bone marrow cells and then receive bone marrow transplants from other mice. Brain scans will be performed on mice and pigs. The above procedures will be performed under general anaesthesia. Other procedures will include blood sampling from superficial vessels, administration of drugs or fluids by injection (usually into the abdominal cavity or under the skin) or into the food or drinking water. We will also assess the memory and other thinking skills of mice by placing them in experimental mazes. For many procedures, there is likely to be only a transient impact on the animal and a rapid return to normal behaviour without any intervention e.g. blood sampling, drug injections, brain scanning. For surgical procedures and those causing brain injury/disease there is the potential for the following: • Change in eating and drinking habits • Weight loss • Signs of neurological injury e.g. limb weakness, memory problems • Impaired movement The duration and frequency of these will depend on the individual experiment but it is expected that weight loss and eating/drinking habits will be commonly observed and recover within a few days. Signs of neurological damage may persist permanently (as they often do in humans). Death is not an expected

outcome and animals that are not able to perform normal functions will be humanely killed.

## Application of the 3Rs

### Replacement

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

#### Replacement

Many experiments will not require animals because we can use cells cultured in the laboratory, computer simulations and analyse samples taken harmlessly from patients. However, we need to use live animals for some experiments because it is not possible with current knowledge to recreate the complex interactions between the brain and immune system in isolated cells or computer models.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Each animal will be used for a procedure or set of related procedures only once.

We will design experiments in a way that ensures the minimum number are used – this will include using statistical methods that can accurately predict the number of animals necessary to meet experimental objectives based on previous data collected. Experiments will be conducted and data collected in ways that minimise the introduction of confounding variation

### Refinement

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

#### Refinement

Almost all studies will use adult mice. Although not identical, mice have many things in common with humans in the way that their brain and immune cells function that means information from mice can be used to predict how the same processes work in humans. Experimental reagents and methods for data collection are also most advanced for mice.

We will use a small number of larger animal species (pigs) for selected experiments because they provide a bridge between the anatomy/physiology of rodents and humans thus further improving predictions from animals to humans. The larger brain

can also enable study of certain aspects of neuroimmune function not possible in rodents. We predict that using a small number of pigs may enable many fewer mice to be used.

Brain injuries and diseases in humans can be devastating conditions therefore to accurately mimic aspects of these conditions it is necessary to use animal models that cause brain damage and changes to brain and body function resulting from this. However, we will do this in the most refined way possible with the minimum severity and follow well established care protocols to minimise the frequency, duration and severity of adverse effects. This will include the use of anaesthesia, regular monitoring and recording of animal health by trained staff, routine administration of fluids to maintain hydration, administration of drugs to provide pain relief in consultation with vets, enrichment of cage environments to encourage eating and drinking, and the use of defined humane limits that will not be exceeded. Throughout the duration of the project, we will be responsive to further developments within the scientific and animal husbandry communities that could be applied to further refine experiments.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 250. Investigating lysosomal and mitochondrial dysfunction in Parkinson disease</b>
<b>Key Words</b>	Parkinson, mitochondria, lysosome, aging, therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Parkinson disease (PD) is the second most common form of neurodegeneration. Functional genetic studies support that mitochondrial and lysosomal abnormalities represent key molecular causes. Yet further research, particularly at the systemic biology level is required in order to advance our understanding and enable us to design targeted and safe cures. The aim of this project is to model for PD in mice by modifying the lysosomal-associated glucocerebrosidase (GBA) and alpha-synuclein (SNCA) proteins mimicking the gene mutations found in PD patients, and that of the mitochondrial-associated PINK1 and MPV17. Aging these animals may further model the late onset of PD. These animal models will allow us 1) to gain knowledge on how GBA and SNCA-dependent lysosomal impairments, and PINK1 and MPV17-originated mitochondrial dysfunction, contribute to the development of PD; 2) to disseminate and apply the knowledge to design and test in these animal models therapeutic substances for a cure. We hypothesise that the class of molecular chaperones for GBA can reverse the pathogenic effect of GBA mutations, whereas the class of nucleotide supplement can compensate for the MPV17-dependent mitochondrial DNA deficiency.

**What 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 from analysing the proposed disease models, to provide insight into how alterations of the lysosomal and mitochondrial function, together with aging, contribute to PD. Moreover, new data will enable us to identify target pathways

suitable for drug targeting to slow PD progression. The results will form the basis for consideration of translation to clinical trial.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We wish to use mice in our study, as they share many similarities with human both in terms of physiology and anatomy. Upon careful calculation we expect to use up to 7500 mice.

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

Three protocols are described in this application. The first protocol involving breeding and maintenance of genetically altered (GA) animals is expected to be mild severity. Known adverse effects are that offspring carrying homozygous GBA mutations die shortly after birth, and homozygous MPV17 knockout and PINK1 knockin mice develop mild metabolic and neurological disorders after 18 months old. Earlier onset and/or stronger adverse effects are expected upon crossing 2 strains of mice in order to ameliorate the pathogenic effect. Aging the animals is also expected to promote symptom development. Under no circumstance will the animal be left beyond moderate severity. The described step on biopsy collection for genotyping in this protocol are all mild. 2 separated protocols are written to continued use the GA animals – one for younger another for older than 12 months respectively. In each protocol, mild behavioural tests, blood sampling and post-mortem tissue collection may be carried out to determine the neurological and biochemical abnormalities. The step describing intracerebral injection is of moderate severity; it shall only be performed in a small number of animals with strong scientific justification. It requires an hour surgery under general anaesthesia to make a very small window in the skull, in order to gain access to the brain and inject into specific locations pathogenic or therapeutic substance then seal with dental cement and wait for recovery.

Pathogenic substance e.g. SNCA aggregates is expected to produce an advanced PD model of SNCA accumulation with earlier onset and/or more adverse symptoms mainly movement disorder. Administration of therapeutic substance, expected to halt/invert the disease, directly into the brain shall only be carried out on substances not penetrating the blood brain barrier (BBB). Operated animals shall be monitored for surgical complications and disease symptoms - no animal will be allowed to progress beyond moderate severity. A non-surgical treatment step of moderate severity is described for administering therapeutic substances by parenteral and non-parenteral routes, which may produce a mild local irritation and discomfort and deemed mild severity. In case of oral gavage, severity becomes moderate in unlikely cases of inadvertent administration to the lungs. Should this happen the animal shall be killed by a Schedule 1 method. In case toxicity of the therapeutic substance is not known, in order to reduce the number of animals reaching moderate severity, tests shall be performed on a small number of animals at a low dose then progress in a

limited scale until an appropriate pharmacological dose level is reached. If the initial dose produces evident toxicity, doses will be reduced by a similar stepped, minimal numbers approach. In following disease progression upon ageing, animals will be maintained older than 12 months until they reach 21 months of age, and rarely to 24 months of age, under the ageing protocol that is of moderate severity. At the end of the study, animals will be killed by Schedule 1 method or terminal anaesthesia.

## Application of the 3Rs

### Replacement

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

#### Replacement

We want to investigate how lysosomal and mitochondrial dysfunction lead to PD, and evaluate the efficacy and dosing of therapeutic substances designed to intervene these pathogenic pathways. Relevant studies at biochemical and cellular levels have already been carried out; our progress is at the stage necessary to further our works in animals in order to obtain physiological in vivo data in a higher organism.

Data generated from non-protected animal alternatives is limited in translating into clinical studies especially for PD.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will use the same outcome variable in all the in vivo studies. These are a continuous numerical data, in each study we will use genetically defined inbred stocks. In the analysis of our results we will use a 5% significance level and a statistical power of 80%. We will search an advice of a statistician on a number of animals that should be used to obtain statistically relevant results from behavioural tests.

### Refinement

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

## Refinement

We consider the choice of animal models and number of animals used as the most refined but sufficient to achieve our objectives. Specifically, modification of GBA, SNCA, PINK1 and MPV17 will be by GA that produces stable and uniform models. A minimal number of GA mice shall be bred to maintain the strains; additional breeding shall only be carried out to generate mice sufficient for experiments.

In limited cases when a more pronounced model of SNCA accumulation are needed, SNCA aggregates shall be injected into the brain.

The majority of targeted therapeutic substances shall be administered via non-surgical means. Injection directly into the brain shall only be considered in cases where the substance does not penetrate BBB, and there is a strong scientific ground to test that substance.

Only if absolutely necessary, animals will only be aged beyond 12 months, and rarely to 24 months.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 251. Breeding and maintenance of animals for the study of liver diseases</b>
<b>Key Words</b>	Genetically Modified, Liver Disease, Model
<b>Expected duration of the project</b>	1 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to successfully breed and maintain animal model strains specifically for the study of liver diseases and the complications thereof.

The strains to be maintained have specific genetic modifications in ammonia metabolism, immune signalling and albumin production. All of which are key processes in the development of both primary liver disease and secondary organ 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 benefits are that the future use of these animal model strains will contribute to the understanding of disease processes and provide information leading to the development of new therapeutic methods. Previous work with these, and similar lines, have directly led to new drugs and medical devices currently in clinical trials.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over a one year period it is expected that up to 500 mice and 100 rats could be produced, though with good husbandry and careful genetic testing it is likely that far fewer animals will be bred to maintain the model strains.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels 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 level is mild. Animals not required for future

breeding will be humanely killed, the majority of which will occur pre-weaning. Some animals will be allowed to develop to adulthood, before being killed humanely to permit study of, blood, organs and body tissues.

## Application of the 3Rs

### Replacement

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

#### Replacement

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 maintained under this project provide insights into how the cross-talk between the various organ systems works.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

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

Biopsies will be taken between day 10-14 after birth, so that genotype can be established and unrequired animals humanely killed pre-weaning.

### Refinement

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

#### Refinement

The animals to be bred under this licence have direct relevance to the study of liver diseases and their complications. Where possible, blood and tissues from humanely killed ex-breeding animals will be used for experimental studies in cell culture and other laboratory studies. In this way we will maximise the utility of all animals created during the project.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 252. Interneuron function in the developing mammalian brain</b>
<b>Key Words</b>	Brain development, Autism, Schizophrenia, Nerve cells, Network function
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our brains are fundamental to whom we are - governing processes such as learning, memory and language, and central to these actions is a huge array of cells whose diversity has proven to be an obstacle to our understanding of brain function and conversely dysfunction. One approach to resolving this conundrum is to investigate the rules that govern the developing brain as this lays the foundation for the amazing processing power of our brains. At present we define two categories of nerve cell in the forebrain: excitatory pyramidal cells and local, inhibitory nerve cells termed interneurons. Although the latter are only a minor component of the total number of cells in the brain, they are critical to normal function. Our research relies on the power of genetics to interrogate the contribution of interneurons to emergent brain activity and dissect when and how these cells go wrong in models of autism and schizophrenia. Our purpose: to gain an understanding of the early brain that will (1) establish a set of rules for the more complex adult brain; (2) provide the foundation for a better understanding of these psychiatric conditions. This approach has proven hard to pursue in the past due to the dynamic nature of the developing brain and the difficulty in targeting specific cells. To overcome this, we will make use of genes crucial for cell identity to probe the contribution of specific cell types to emergent perception. Our recent findings have revealed that a population of nerve cell act as a scaffold to direct the development of a 'normal' brain. Parts of this scaffold are altered in a mouse model of schizophrenia so now we want to go on and identify when and how interneuron deficits trigger abnormal brain activity and mis-route development in mouse models of both schizophrenia and autism; another developmental disorder in which these cells have been implicated.

**What 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 identify when and how interneuron deficits trigger abnormal brain development in mouse models of autism and schizophrenia. This will significantly advance our understanding of these conditions and enable researchers, including ourselves, to design targeted therapeutic interventions that will have the capability to restore to some degree normal brain function in these model systems. Ultimately it is hoped that we can then translate these approaches to human developmental psychiatric disorders.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The project is reliant on a number of genetically modified mouse lines. These are currently the preferred model system for a number of groups across the world as they represent the lowest and best vertebrate group in which to study the cellular and network properties of defined neuronal populations. The nature of our research – tracking the emergence of the brain networks over a prolonged period of development means that we need to breed and use large numbers than would be expected when studying adult neurological conditions. We predict that we will use up to 25,000 animals for breeding during the 5 year period of this project and 4,800 for surgical techniques and tissue biopsies.

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

To achieve our scientific goals we need to breed and maintain a number of genetically modified mouse lines that replicate human neuro-developmental psychiatric conditions. In the majority of cases (~80%) these lines show no adverse effects and can be maintained on a mild breeding protocol. However a minority exhibit some moderate adverse effects – for example mouse models of autism display behavioural abnormalities similar to those observed in humans (e.g. withdrawn, antisocial behaviour), whilst others on occasion exhibit transient neurological deficits in behaviour such as epileptic seizures or schizophrenic-like episodes. To probe these conditions we need to be able to alter the genetic make-up of these animals either to track/manipulate the cells of interest or trigger a potential therapeutic intervention. Beyond our normal breeding paradigms we also use a variety of interventions (moderate severity) to more effectively target the neurons of interest. This can be achieved by either injecting embryos with biological material via a surgical procedure or switching on genes by injecting pregnant dams with tamoxifen that results in a genetic modification in the offspring. Both of these approaches can lead to complications in pregnancy in a small number of cases. An alternative is to perform surgery on neonates using anaesthetic regimes and analgesia appropriate for these early ages. Finally to judge the efficacy of our intervention we will need to perform non-recovery protocols that encapsulate a

battery of tests on the brain of both normal and mutant mice. This requires us to take tissue samples and record from the animals under terminal anaesthesia.

## Application of the 3Rs

### Replacement

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

#### Replacement

We cannot yet replicate the complexity of brain development using other alternatives. We are exploring and working with other groups to develop both cell culture systems and computer models that can replicate and be used to investigate some aspects of development; the aim being to replicate and better predict facets of this highly dynamic and intricate process. Ultimately these models could be used to interpret data obtained from re-sectioned human tissue – available from patients that have undergone neurosurgery – thereby validating and improving our understanding of this incredibly complex process.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The hope is that our development of cell culture and computer models will enable us to more effectively target our research and thereby greatly reduce the number of animal used in experiments. For effective experimental design we use power analyses based on the following assumptions: normal data; 2-sided t-test; significance level, 5%; statistical power, 80-90%; an effect size of biological interest and standard deviation derived from previous experiments. This allows us to derive meaningful scientific conclusions from *minimum* sample sizes of 6 – 14 recordings for our standard electrophysiology protocols for any given developmental age; relatively low numbers made possible through our adoption of developmental genetic strategies to enable targeting of select neuronal subtypes in fewer animals than would be necessary using traditional approaches.

### Refinement

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

#### Refinement

The project is entirely reliant on a number of genetically modified mouse lines. These are currently the preferred model system for a number of groups across the world as they represent the lowest and best vertebrate group in which to study the cellular and network properties of defined neuronal populations. Our use of developmental genetics allows us to very efficiently target the cell types we believe are responsible for autism and schizophrenia.

For all of our protocols we have defined our anaesthetic regime, possible outcomes and humane end points. The majority (~80%) of our genetically-modified mouse lines exhibit no significant adverse effect. For the remaining 20% of animals we have established end points that have been scrutinised by the veterinary and research communities, to avoid the occurrence of unnecessary pain and suffering to our mice. We continue to refine our surgical approaches in the light of our observations as well as new developments in anaesthesiology, analgesia and surgery. All our remaining work – investigating the neural circuitry of the developing brain – is performed under non-recovery protocols.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 253. Disease resistance in farmed fish</b>
<b>Key Words</b>	Fish, genome editing, disease resistance
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Infectious disease outbreaks present an enormous threat to food production through aquaculture. Genome editing can help us understand disease resistance in fish, and has potential to produce fish with enhanced resistance. Subject to an appropriate regulatory environment, this technology may enhance aquaculture production and animal welfare significantly in the future. The overall aim of the project is to identify genes and mutations in farmed fish that affect resistance to viral disease in salmon via the use of genome editing. The closely related applied aim of this research is to create disease resistant fish using genome editing. The specific disease under study is the problematic viral disease Infectious Pancreatic Necrosis Virus (IPNV).

**What 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 two main categories of benefits of this research are (i) improved knowledge of disease resistance and host-pathogen interaction in fish via knowledge of the functional mechanisms underlying genetic resistance, and (ii) a new breeding tool to help tackle the problem of infectious disease in aquaculture. The potential long term benefit has positive implications for both fish welfare and for global food security and human health.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The animals used will be juvenile Atlantic salmon from commercial breeding program strains that have different susceptibility to this infection. Newly fertilised fish eggs will be microinjected, and disease challenged during the first few months post hatching, timed according to knowledge of the host response to the specific pathogen under a separate license at REDACTED. A disease challenge experiment will typically last for approximately 3 weeks, and the entire process of microinjection to disease challenge will last approximately 4 months. It is anticipated that up to three viral challenge experiments will be performed, each with up to 1,000 animals (five

crosses, 100 edited fish and 100 unedited control fish per cross). To achieve this number of surviving edited embryos, a greater number will be microinjected (up to 3,000 animals per experiment; 2000 of which are expected to die or be killed prior to when the hatchlings become capable of feeding, which is when they reach protected animal status under the law). Therefore, the total number of protected animals anticipated to be used under this license is approximately 3,000.

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

All animals will be humanely killed at the end of the experiment using schedule 1 methods. The possible adverse effects of micro-injection of gene editors are infection or developmental abnormality of the embryo in the egg, which will be minimised by use of best-practice and pilot studies on non-regulated embryos. If this happens, the embryo would be unlikely to develop, and would die at a very early stage of development. Genome editing could induce more subtle unexpected effects which would be identified by close monitoring, with fish showing symptoms of disease or abnormal behaviour removed and humanely killed. During the IPNV challenge experiments, animals may experience adverse effects because they will develop clinical signs of infection, at which stage they will be humanely killed by a schedule 1 method.

## Application of the 3Rs

### Replacement

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

### Replacement

The objectives of the project cannot be achieved without using animals because the traits we are interested in studying, and those of relevance to aquaculture production, are most effectively measured on the animals. Cell culture models will be applied to test the gene edits and investigate their effect on (e.g.) viral replication in vitro, and this will inform the choice of edits to be used for the animal experiments. Further, extensive computer analyses will be performed to guide the choice of gene edit to perform. It should be noted that the expected outcome is a reduction in the need for animal disease challenges in commercial aquaculture production, via the use of genetic tests and genome editing for disease resistance.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The applicant's experience with experimental design is extensive, and professional statisticians will also be consulted to ensure that the animal experiment has adequate statistical power to detect impacts of the edit on the target trait (e.g. disease resistance). The edited animals will be compared to unedited controls from the same family, which will control for background genetic effects. These measures will ensure that the project maximises the chances of a successful result while only using the minimum number of animals necessary to achieve that result.

### **Refinement**

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

### **Refinement**

The research will focus initially on Atlantic salmon - the primary species used in UK aquaculture - for which the outcomes of the research are ultimately targeted. It is plausible that other species will be used in the future, for example rainbow trout or Nile tilapia. Since it is possible to work with large numbers of young fish directly of the species of interest, and there are adequate tools to study the DNA and immune system in those species, there is no need for prior testing in other model species. Suffering will be minimised by (i) daily checking and monitoring of both the water and the health of the fish / embryos. Fish or embryos showing signs of disease or distress will be killed, taking advice from the named veterinary surgeon. (ii) In the disease challenge, fish will be killed prior to mortality, as far as possible. This will be at the first signs of clinical symptoms that show that the fish have not been able to successfully fight off the infection.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 254. Physiological and neurobiological functions of the murine Gnas locus.</b>
<b>Key Words</b>	postnatal development, leanness, circadian rhythm, hypothalamus, genomic imprinting
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We are analysing genetically modified (GM) mice as a model for human inherited diseases called 'Albright's Hereditary Osteodystrophy' and 'Pseudohypoparathyroidism'. The human genetic disease affects the brain and the hormone system. It causes postnatal growth abnormalities, disturbance of body weight and energy expenditure, as well as unresponsiveness to various hormones. Using our mouse model, we intend to find out more about the biological pathways and molecular mechanisms that are disrupted in this genetic disease. We want to find out

- a) whether the postnatal growth and feeding problems can be improved by giving a subcutaneous dose of the hormone oxytocin to increase the suckling activity of pups.
- b) which hormones and molecules in the brains of adult GM mice are not working properly, thus causing leanness and increased energy expenditure.
- c) how and why the body clock and daily rhythm of activity, which are regulated by light/dark periods, are changed.

**What 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 basic research will increase our knowledge and understanding of the mechanisms of disease causing 'Albright's Hereditary Osteodystrophy / Pseudohypoparathyroidism'. Although these genetic diseases are not very common, more than 300 patient families are observed and treated by clinicians. By analysing our mouse models, we will describe in more detail, which brain functions, hormonal

pathways and molecular mechanisms are not working correctly. Our data might show whether certain hormone treatments could potentially be helpful for improving symptoms, or whether unresponsive hormone systems could be bypassed by activating alternative mechanisms. Since our mouse model shows a lean body composition and increased energy expenditure, our investigations might also provide valuable insights for understanding obesity, which has become a significant health burden in our society.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We are using genetically modified mice, which includes genetically normal littermates for comparison. We will need to undertake maintenance breeding of the mouse strains over the duration of this project licence (5 years), as well as breeding to generate experimental cohorts of mice. For the maintenance breeding we estimate a maximal number of 2500 mice might be used over 5 years when a new generation is produced 2-3 times a year. For the various experiments, we estimate an overall maximal number of 900 mice might be produced over 5 years. Most likely, the actual numbers used will be lower, depending on the outcomes of preliminary experiments.

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

Most procedures in this project will consist of injections (subcutaneous or intraperitoneal) of hormones or related substances. The analysis of daily rhythms and activity requires changing the light/dark periods for the mice (including constant darkness for up to six weeks). Some genetically modified mice, which lost the function of one particular gene, show postnatal feeding difficulties, growth retardation and weakness. This results in the loss of some pups during the first postnatal week, but those that survive to weaning age develop into healthy and fertile, but lean, adults. Pups that show postnatal symptoms will be monitored three times a day for substantial weight loss, lack of milk in their stomachs or lack of response to touch. We will try to improve their condition via hormone injections, but if they show a worsening of symptoms beyond a critical point, they will be killed humanely. All other mice, when they are not required anymore for maintenance breeding or experiments, or when tissues are needed for molecular and histological analyses, will be killed humanely in accordance with Home Office regulations at our local animal facility. Some experimental mice might be anaesthetised deeply and terminally, and tissues collected for specific types of analysis.

## Application of the 3Rs

### Replacement

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

## Replacement

The neurobiological and hormonal disease symptoms observed in the human inherited disorders 'Albright's Hereditary Osteodystrophy' and 'Pseudohypoparathyroidism' involve several organs and tissues of the body, and how they regulate each other. For example, the brain controls a number of hormones and the functions of many peripheral tissues. Such complex interactions cannot be modelled in non-mammalian organisms (e.g. *Drosophila* fly or *Caenorhabditis* worm), since their organ system is not similar enough to human. The mouse provides a more suitable model with greater similarity to human biology. Also, many genetic aspects are conserved between human and mice. The gene under investigation in this project is only found in mammals like mouse or human, but is very different in the fly or worm, such that the above human diseases cannot be reproduced in those animals.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

We will reduce the number of mice produced for the maintenance of the genetically modified mouse strains by only setting up breeding pairs occasionally to generate a new generation 2-3 times a year.

For experimental groups of mice, we will discuss appropriate experimental designs and statistical tests before starting the work. We will estimate the required number of mice for each experiment beforehand and seek advice from a dedicated Experimental Design Consultant and the Biostatistics Department of our University. We will also make use of the online tool provided by the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs), i.e. the Experimental Design Assistant ([www.nc3rs.org.uk/experimental-design-assistant-eda](http://www.nc3rs.org.uk/experimental-design-assistant-eda)). For explorative experiments, where initial estimates of required animal numbers are not possible, we will follow specific statistical rules to assess step by step from small numbers of test experiments whether results are likely to become significant.

## Refinement

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

### **Refinement**

We are using mice, since they are the preferred species for modelling human inherited diseases. We aim to extend our knowledge about the functions of disease-relevant mammalian genes. The gene we are investigating is only found in mammals, which makes species of lower sentience, e.g. flies or worms, unsuitable as model organisms.

One of our aims is to find out more about the postnatal feeding and growth problems observed in some of the genetically modified mice, and whether hormone treatment (e.g. oxytocin) can rescue these symptoms. It would be a refinement, if we can improve the symptoms and obtain an increased survival rate through regular treatment of the mice.

In the unlikely case of a surgical procedure, appropriate anaesthetics and analgesics will be used. Post-operative surveillance and care will be closely coordinated between members of my laboratory and expert staff in the animal facility.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 255. Understanding the regulation of brain monoamine neurotransmission in health and disease</b>
<b>Key Words</b>	Neurotransmission, Dopamine, Basal ganglia, Parkinson's disease, Acetylcholine
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

**Overall objective:**

Brain cells, or neurons, that use the transmitter dopamine, carry out key functions in our everyday motivated actions as well as our learned habits. We think these neurons tell us about things in our environment that have some motivational value that help us to detect them, and then respond optimally to benefit from them. These neurons die in the neurodegenerative disease Parkinson's disease. There is therefore a need to understand the workings of these cells better so that we can not only advance biological knowledge, but also improve our understanding and treatment of Parkinson's and related diseases.

The work we propose will promote our understanding of how dopamine regulates our everyday behaviours, and it will also allow us to explore at a subcellular level how these neurons communicate from synapses.

We will work towards these goals through a program of work that will identify how neurotransmission by dopamine (and related transmitters) is regulated by neural circuits with other neurotransmitters, neurotransmitter receptors, cellular signalling pathways, regulatory genes, and related mechanisms. We will also examine how dopamine release governs behaviour.

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We will work towards these goals through a program of work that will identify how neurotransmission by dopamine (and related transmitters) is regulated by neural circuits with other neurotransmitters, neurotransmitter receptors, cellular signalling pathways, regulatory genes, and related mechanisms. We will also examine how dopamine release governs 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)?**

This work should therefore advance basic biological knowledge and understanding of many brain functions relevant to our everyday motivations and actions. It shed also light on mechanisms relevant to key brain disorders. In turn, we hope to gain insight into potential future therapies for these disorders for which there are currently still very few effective treatments.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over 5 years, we estimate that we may use up to 2,600 mice in procedures other than simply breeding and maintenance. We may breed and/or maintain up to 12,000 mice, some of which will be the same ones used in the additional procedures. Mice will be the species used because they are the lowest vertebrates in the phylogenetic tree for which brain dopamine systems are suitably well characterised and comparable to that of humans, as well as there being models for neurodegenerative disease. The mouse is also currently the most tractable mammal for use in genetic studies.

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

We will raise genetically altered mice that allow us to explore the functions of key molecules in these mechanisms. In some animals we will insert genes into the brain during general anaesthesia which allow animals to express proteins that can be targeted with flashes of light or designer drug tools to activate the neural circuit we want to explore. Some animals might instead receive a toxin or will be genetically altered to make the animals begin to develop a Parkinsonian condition so that we can understand the disease better, and explore some options that might treat it. Some animals might be given drugs regularly when awake over a few weeks to enable us to understand better the processes which become disturbed, or how we

might treat them. And a small number group of animals will have small microelectrodes implanted in their brains and then be allowed to roam freely so that we can understand how neural circuits are important to behaviour. The adverse effects that some animals might experience might include the effects of brain surgery under general anaesthesia which might include transient pain and bleeding, some disturbances to normal movement particularly some slowness or other slight difficulties in initiating movement, which might compromise their ability or a failure to thrive. Animals will be well supported during these times. At the end of the experiments, the animals will be humanely culled.

## Application of the 3Rs

### Replacement

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

#### Replacement

This work is an exploration of fundamental mechanisms of operation of the brain and also studies the adaptive mechanisms and/or the impact of drugs in neurodegenerative disease. Use of live animals and real brains is therefore needed to provide tissue with synaptic circuitry that resembles the in vivo scenario. We are not aware of any alternative which does not use animals that would allow progress to be made towards the objectives. No cell or culture alternative can adequately provide this. We will use virtual neuron computer models in the limited experiments for which this is appropriate.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will keep animal numbers to a minimum by using power calculations and pilot studies where appropriate. We will also use experimental designs and powerful techniques that are high yield, by allowing multiple refined measurements per sample, or per brain or per animal.

### Refinement

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

#### Refinement

We have chosen mice because their dopamine systems are very similar to those of humans and because they are highly suited to using genetic manipulations that will help us understand how the dopamine system works in health and disease. The genetic tools we can use allow experiments and manipulations to be highly targeted to the cells we are studying, and therefore very refined.

We will select for each experiment the most refined mouse brain preparation. Sometimes brain slices are ideal because they have a good balance between containing substantial normal circuitry, unlike isolated cell preparations, whilst also allowing good access to the neurons we want to visualize and study. Sometimes, we need to use whole animals, when they are the only means to understand brain cell function in relation to behaviour.

The mouse models of disease we will use are the best available, and each one has been chosen because it closely mimics key aspects of the disease and with minimal suffering, and so is very refined.

We will use the lowest severity models applicable to each of our aims. Our general measures to minimise suffering in interventional experiments include appropriate use of anaesthesia, aseptic surgeries, close post-operative care, analgesia, and support. In all cases where an intervention is applied *in vivo*, monitoring systems and humane endpoints will be in place.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 256. Drug discovery for parasitic helminths</b>
<b>Key Words</b>	Drugs, Parasites
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Gut dwelling parasitic worms are extraordinarily common impacting on the health and wellbeing of around one quarter of the world's population. Drugs currently used to treat these types of parasite are losing their effectiveness therefore new drugs are urgently needed.

Aims: The project aims to discover new drugs and assess the possibility of using existing drugs, currently used for the treatment of other diseases, as anti-parasite 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)?**

In the short terms we will publish our work and discuss our data at scientific conferences. This will facilitate sharing of our compounds with other researchers who will be able to test them against other types of infection. In the long term, the discovery of new drugs to kill parasites will significantly help in controlling parasitic worm infections in developing countries. Within 5-10 years it is highly likely that we will have discovered and optimised, through chemical modification, new anti-parasitic drugs suitable for clinical trials in man and/or use in veterinary applications.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will work with adult mice and expect to use 2740 over 5 years. A total of 2740 mice gives us the potential to identify up to 17 new chemicals for use as new anti-parasitic drugs.

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

The procedures employed under this licence are classified as moderate. There are two main possible harmful effects, the first relating to administration of the new chemicals and the second to removing of blood samples. Both are expected to be

rare events (<0.1%). Protocol 1. Mice will be treated with new chemicals orally, or by an injection in to the abdominal cavity or into the blood stream, or under the skin. As these are new chemicals it is possible that, occasionally, the mice will react badly to these chemicals. We expect these to be very rare events (<0.1%) as any chemical administered to mice will have gone through rigorous testing in vitro to show that they do not kill cells. Mice will be checked daily after treatment with the new chemical. We will look for signs that the animal is beginning to suffer and any mouse affected will be humanely killed. Protocol 2. Chemicals which are well tolerated by mice in protocol 1 will then be used in protocol 2 which will enable us to establish how long the chemicals last in the bloodstream and the ability of the chemicals to eliminate the parasite from the mouse. In order to do this, mice will be infected with parasites using standard procedures, chemicals administered and blood repeatedly withdrawn from a tail vein. The main risk of harm to the mouse in protocol 2 is excessive blood loss. We expect loss of too much blood to be very rare (<0.1%) as we will carefully control the frequency and size of the blood sample taken. We will ensure bleeding has stopped after each sampling. If the bleeding cannot be controlled, resulting in a blood loss greater than defined limits, the mouse will be humanely killed.

## Application of the 3Rs

### Replacement

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

#### Replacement

Chemicals will be tested in vitro first, and only those chemicals which kill the parasite in vitro will be tested in vivo. Thus the work involving animals will be considerably reduced. However it is necessary to determine whether the chemicals show anti-parasitic activity in vivo

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will not only test new chemicals but also test to see if we can use existing drugs, developed for different purposes, to kill parasites. In these situations where possible we will use existing data which will tell us (a) if the chemical has caused harmful effects in mice before and at what dose, and (b) how the drug behaves in the body. This will reduce animal usage.

One of our key goals is minimisation of variation; this is pivotal in determining the number of animals required to demonstrate a real reduction in the number of parasites after chemical treatment. For example we will:

- Use the same strain of mice, purchased from a single supplier with minimum variance in weight
- Use the same personnel, familiar with each step of the model
- Study multiple compounds within one experiment, minimising the number of untreated control groups.
- Calculate sample size based on available data before the experiment.

### **Refinement**

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

### **Refinement**

Our studies focus on the infection of laboratory mice with the parasite *Trichuris muris*. *Trichuris trichiura* is the equivalent parasite that infects man and it is virtually identical to *Trichuris muris*. Thus the mouse model enables us to develop new therapies to treat *Trichuris trichiura* in humans.

The protocols employed are well established in our lab and designed not to induce suffering in animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 257. Mechanisms of axon and synapse loss</b>
<b>Key Words</b>	axon, synapse, degeneration, Alzheimer's, neuroprotection
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Axons are the long wires that conduct electrical signals around our brains and bodies from one nerve cell to another, or from our nerves to our muscles. Their extreme length makes axons vulnerable in disease. Our research group has pioneered the understanding of a widespread, and preventable mechanism of axon degeneration involving specific proteins in our nerve cells. Consequently, four proteins are known to regulate axon survival and degeneration, there is clear evidence that this mechanism is relevant to human diseases. The Pharma industry is now targetting this pathway for drug development. The next steps that we address here are (a) to determine how the proteins regulating this pathway interact with one another as a clue for how to block the pathway with drugs (b) to identify the human diseases in which it will be most effective to block this pathway, partly using animal models (c) to find ways to preserve synapses at the ends of axons, which are essential for sending chemical signals to the next cell.

**What 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 axon degeneration pathway we study regulates axon survival in many diseases. These include nerve disorders arising from diabetes and cancer chemotherapy, Parkinson's disease, glaucoma, multiple sclerosis, traumatic brain injury, motor neuron disease, and stroke. It also seems to cause some types of abnormal pain and some cases of stillbirth in humans. A role in nerve disorders, associated with HIV, polio and other viruses, and in Alzheimer's disease (the most common cause of dementia) is also possible. Any or all of these disorders could be alleviated if we could develop drugs to block this pathway. In a new Alzheimer's (3Rs) model we have developed, we find synapse loss similar to that in patient brains and have made

substantial progress in understanding and preventing the mechanism. By extending this research further we can open new ways to target Alzheimer's disease and we can extend this 3Rs method to studies of other neurodegenerative disorders.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to 27,000 mice or rats (over 95% mice) and 4,000 zebrafish may be used during the five year period. The vast majority of our work (ca. 80% of the rodents and all the zebrafish) involves the breeding and brief maintenance of genetically altered animals with no adverse effects. This is essential to produce sufficient nervous system tissue for the 3Rs methods we are increasingly using, such as cell culture and working with zebrafish embryos before they are able to feed freely. Up to 1,000 mice may develop disease signs so we can study how to alleviate disease.

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

Most animals will be completely healthy during life and will be humanely killed by an authorised method to obtain nerve tissue for cell culture. Similarly, zebrafish will be only used for breeding to obtain embryos for nonregulated procedures before reaching free-feeding stage. Animals with signs of disease may develop varying degrees of tremor or hindlimb weakness, ranging from unimpaired mobility around the cage to early stages of hindlimb paralysis. Food and water will be provided in accessible form and if limb weakness becomes more advanced, and animals will be killed by an authorised humane method before losing any significant forelimb function. Other interventions include lesioning a leg nerve in mouse or rat on one side to study nerve degeneration. This is carried with the highest surgical and aseptic standards under general anaesthesia, alleviated with analgaesic for pain relief and closely monitored subsequently. Animals recover quickly and movement around the home cage is near normal. A small minority of animals will be used in studies of transient pain, but this has no lasting effect, and some may be used for dosing with potentially neuroprotective drugs to determine their efficacy.

## Application of the 3Rs

### Replacement

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

### Replacement

Many replacement methods are used in our work. We do a lot of work in primary neuronal cultures to study nerve injury, and we culture slices of brain tissue to study Alzheimer's disease mechanisms. We work with nerves removed from humanely killed animals in a dish and zebrafish embryos to study events within nerve cells.

However, to understand the functioning and degeneration of the human nervous system we ultimately have to confirm findings from this work in a live mammal. Mice are the species for which genetic methods and analysis are most advanced so this is why they are chosen over other species here. Rats are sometimes needed for a greater supply of tissue or for confirmation of findings in a second mammalian species.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

The best way to reduce numbers is to do well-designed experiments limited to novel and important questions. Our research group has the highest standards of research with a strong track record of publications and we are world-leading in the fields of axon degeneration and synapse loss. Careful experimental planning is also key, so we reduce the numbers needed by minimising variability within groups, correct use of controls, and the use of pilot experiments and power analysis for new methods where the outcome is unpredictable.

### **Refinement**

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

#### **Refinement**

We refine our use of animals by using the highest standards of aseptic surgery to avoid infections (we have never had a single case of infection), by maintaining strains of animals as carriers rather than breeding parents affected by a disease, and by applying strict humane endpoints. We house animals in groups wherever possible and we use pre- and post-operative analgesia. Our pain studies are refined wherever possible by giving animals a choice between a potentially painful experience and a non-painful one and monitoring their choice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 258. A service to enable the cryopreservation and rederivation of genetically modified mice</b>
<b>Key Words</b>	Cryopreservation, Rederivation, Genetically Altered
<b>Expected duration of the project</b>	3 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

A genetically altered mouse (GA mouse) is a mouse that has had its genome altered through the use of genetic engineering techniques in the laboratory or by a naturally-occurring event. A genome is an organism's complete set of genetic instructions. Each genome contains all of the information needed to build an organism and allow it to grow and develop. The instructions in our genome are made up of DNA. Single strands of DNA are coiled up into structures called chromosomes. Within our chromosomes, sections of DNA are "read" together to form genes. Sometimes genes get damaged, or the building blocks of DNA called nucleotides get mixed up, and this is known as a mutation. Mutations may cause illnesses and disease.

Genetically altered mice are commonly used for research as animal models of human diseases as we can't use humans themselves, and they are also used for research on genes and how they contribute to disease. With this, in time, will come a better understanding of diseases and potential treatments. In order to make sure that any data that is gained from these animal models is interpreted correctly, they must be "clean". That is, they should not have been infected with contaminants known as pathogens (viruses, bacteria) which may cause disease. In this project we aim to take around 700 'lines' of mice from another establishment and "clean" them by a process called rederivation. This process will use fewer animals than if we were to remake all the GA lines from the beginning again. In addition, mice with pathogens are more likely to die or develop disease, which in turn may infect other mice. We want to avoid this so we can use as few animals

as possible to answer the scientific questions these mice will ultimately be used for. In addition, we will cryopreserve (freeze) the GA lines to safeguard them for future 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)?**

When mice have pathogens it is possible that you might interpret some results you see from an infected mouse as being due to the genetic alteration you have engineered, when in fact it is caused by these pathogens. Additionally, mice may unexpectedly die or develop disease, or infect other mice within a colony. We therefore aim to 'clean up' some specific mouse 'lines'. These clean mice can then be used to answer scientific questions about genes and their role in disease, without the potentially confounding presence of pathogens.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use adult mice for this project, as well as sperm and eggs, and early embryos. We estimate to use a maximum of 27,080 adult mice over a 3 year period.

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

We will use established and refined techniques which have the least adverse effects. This licence is being used to provide a service to "clean" animals so they can be moved to a brand new animal facility. This will mean initially cryopreserving (freezing down) sperm and embryos from the genetically altered mice. This will use a technique called superovulation to generate a large number of eggs in the female mouse, which can then be fertilised by mating to males, or in a dish with sperm called in vitro fertilisation (IVF). Superovulation involves two injections of hormone into the mouse's abdomen and will cause only momentary pain. We do not anticipate any adverse effects from this technique. The females will then be humanely killed, and embryos or eggs harvested. The next stage in the process will be to re-animate the mice at a planned time. We will thaw the sperm and embryos, and perform an IVF using the sperm with eggs from superovulated females. Resulting embryos which have been thawed and those produced by IVF are placed into a pseudopregnant female mouse, either surgically or non-surgically, and allowed to develop to term. The mice are made pseudopregnant by mating the female to a male that has previously been surgically vasectomised. This means the female mouse is producing all the correct hormones to maintain a pregnancy without actually being pregnant. Whilst in this condition, any embryos transferred to the female will be taken to term and birth. All surgeries use a general anaesthesia and pain-killers, followed by post-operative monitoring from trained and skilled technicians. After mice have been born they will be identified individually within a cage by taking a small ear biopsy on a particular location of the ear, causing

momentary pain. This 'ear clip' as a bi-product of identification, will also be used for genotyping to ensure the gene of interest is present. Selected mice are then bred to generate more mice for moving to their new accommodation. The vast majority of mice will show no adverse effects, with less than 5% showing some harmful effects caused by the genetic change. The harmful effects caused will be addressed where possible with husbandry and veterinary support. Where this will not help, the mice will be humanely killed. During the course of the project, if at any stage an animal experiences adverse effects that cannot be ameliorated, it will be killed humanely and in a timely manner. All animals that have reached the end of their study will be humanely killed.

## Application of the 3Rs

### Replacement

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

#### Replacement

This project will involve taking genetically altered mice that are already established and their use justified in other project licences, in order to 'clean' them up for future research. We therefore cannot replace them with non-protected alternatives.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The other establishment's genetically altered mice have a number of pathogens associated with them. In order for the other establishment to gain the most meaningful scientific data from these animals, the mice need to 'cleaned' and freed of these pathogens before being sent to a new facility. One way to do this, where materials are available, would be to re-make the genetically altered mice from the beginning. Another way would be to 're-derive' the existing lines. This is done by transferring embryos obtained from the original 'dirty' mice and washing them to free them of any pathogens, and subsequently transferring them into 'clean' female mice to establish them as clean 'lines'. By adopting the rederivation method, we can save a substantial number of animals by avoiding the initial production and breeding phase, as well as, in cases where mice carry genes with multiple changes, avoid the long and complex breeding required to generate such mice. The saving by adopting the rederivation route is likely to be several thousand animals.

The type of cryopreservation used will be chosen on the criteria which results in the use of the least possible animals to secure the genetically altered animals. To this

end, we will use sperm cryopreservation after the animal is killed wherever feasible. If the strain is amenable, superovulation may be used to maximise the number of eggs obtained for IVF. IVF will also be exploited as frequently as possible to produce large numbers of embryos, which can then be frozen in order to archive stocks. This procedure can lead to a substantial reduction in the number of superovulated females required to archive stocks.

Monitoring of efficiency rates will be performed and any technological advances embraced to ensure that the minimum number of mice are used in this process. For superovulation, doses and ages will be optimal to reduce the number of females required for embryo generation.

At all times the minimum numbers of embryo recipient females will be used to re-establish a mouse line to match the anticipated number of genetically altered animals required. We will keep the number of females used as egg donors required in each IVF session to a minimum number to avoid wastage.

We will aim to breed only when the numbers of animals recovered directly from embryo transfers fail to give the number required for re-housing. Well-established breeding calculations will be used.

Where a mouse colony is to be used by more than one group of researchers, stock will be shared making the most efficient use of the mice bred.

A genetic 'contaminant' in the in-bred stocks could have far-reaching effects on the integrity of genetic research, potentially leading to the culling of mice. Routine genetic testing of mouse colonies can go some way to avoiding this by providing defined genetic backgrounds.

## **Refinement**

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

## **Refinement**

Husbandry and health monitoring of all animals under this licence is performed by a team of highly competent Animal and Scientific Technicians that are assessed under the Institutes competency assessment program. Cleaning regimes are minimised to ensure stress and disturbance to breeding and stock animals is reduced.

Environmental enrichment is provided to account for the individual needs of the animals e.g. nestlets for nest making by pregnant or lactating females. All animals will be group housed where possible.

All surgical techniques will look to adopt the principles of aseptic techniques as described in the LASA Guiding Principles for Preparing and Undertaking Aseptic

## Surgery

[http://www.lasa.co.uk/pdf/lasa\\_guiding\\_principles\\_aseptic\\_surgery\\_2010.2.pdf](http://www.lasa.co.uk/pdf/lasa_guiding_principles_aseptic_surgery_2010.2.pdf)

All animals may experience some post-operative pain or discomfort following surgery. Pain-killers will be given and maintained after surgery for as long as is necessary to alleviate pain. We will monitor for pain by observation of the mouse's behaviour and general appearance, and this will guide the administration of appropriate levels of pain-killers.

For genotyping, we will use tissue from ear-clips taken primarily for husbandry purposes to provide tissue for genotyping. If a second tissue sample by tail-tipping is required for technical reasons, authority from the Project licence holder and Head of the animal facility will be required.

We will ensure that when we have to transport any mice to another establishment, we will use ways which meet the highest welfare standard, for laboratory animal transport. Mice will be contained within secure and appropriate containers. The containers will allow adequate ventilation, be escape-proof, leak-proof, and capable of being handled without the animals posing a risk to handlers, and be of such a design and finish that an animals will not damage themselves during loading, transport or removal from the container. Where possible, animals will be grouped as socially harmonious pairs or groups

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 259. Morphogenetic mechanisms underpinning congenital heart defects</b>
<b>Key Words</b>	mouse, congenital heart defect, genetics, environment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Congenital heart defects are very common, affecting almost 1 in 100 babies born in the UK. Many of these are life threatening and require surgery, sometimes multiple times, in order to correct them. These surgical procedures are not a cure, however, and in many cases the patient will have lifelong health problems and often face early death. It is now recognised that many congenital heart defects seem to be genetic, as they run in families. Despite this awareness, few genes have been shown to directly cause heart problems in babies. This is because we are only now able to identify faulty genes in human patients and work out in animal models, why they cause the problems. It is the aim of this Project to identify specific genes that cause heart problems in human babies, and work out why they cause them. The goal is that this will lead to prevention of these devastating malformations.

**What 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 could identify more genes that cause congenital heart defects, and show how they cause the abnormalities, that would be a major step forward to preventing them. Once it is known that a specific gene is responsible for a heart problem in a baby, then their family can be counselled about the likelihood of the same thing happening again in another baby. It can also lead to the identification of more minor problems in the parents or other family members, that can help protect them from developing symptoms and becoming ill themselves. In the long run we hope that we will be able to develop treatments that would reduce the risk or the severity of heart problems in babies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We use transgenic mice throughout this project. These are usually analysed as embryos, before they have a well-developed nervous system and before it is thought that they are able to experience pain or distress. Throughout the 5 years of the project we will use up to 16,000 adult animals, although most of these will be used only for breeding purposes.

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

The transgenic mice will be bred together and then embryos collected at key time points during their development in order to collect their tissues for analysis. In no situations should the adult animals develop obvious disease or distress. Sometimes we will expose the animals to drugs or specific diets to see if this makes the congenital heart defects worse or better. In all cases, the expected severity is mild-moderate. At the end of all the experiments the animals are killed by a humane method.

## Application of the 3Rs

### Replacement

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

### Replacement

Our studies require us to understand how the complex mammalian heart forms and matures. These processes cannot be modelled in the two-dimensional environment of the petri dish. Although lower organisms such as insects have a heart, it is so different to ours that it cannot be used to understand the causes of specific human congenital heart defects.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We carry out statistical tests before we start our studies, and at the end, to make sure that we use the minimum number of animals to give us a reliable and meaningful result. We seek advice from expert statisticians where this is necessary.

### Refinement

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

### **Refinement**

In order to study the causes of congenital heart defects it is essential to work with an animal model that has a heart with a very similar structure to the human heart. The mouse is perfect for this as it is almost identical at embryonic stages. In addition, it is now easy to alter the genes of mouse embryos so we can see which genes are important and why they cause heart problems. In all cases, we try and use embryonic stages before they have a well-developed nervous system and can feel pain and distress. Where this is not possible, we focus on the earliest stages of disease, before they cause significant discomfort to the animal. Animals are checked regularly for any signs of distress or disease and any that show signs are humanely killed.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 260. Novel Agents for Molecular Imaging and Therapy of Cancer</b>
<b>Key Words</b>	Cancer, Molecular Imaging, DNA damage repair, radionuclide therapy, radiation therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The main objective of our work is to develop novel molecular imaging agents for visualising the biology of cancer tissue. Novel imaging agents, labelled with radioisotopes to allow imaging with dedicated PET or SPECT scanners, will allow us to better understand the influence of DNA damage repair and the interactions between cells during tumour formation and tumour therapy. Translation of these imaging agents, targeting as of yet underexplored hallmarks of cancer will allow earlier detection of tumour tissue, and early assessment of cancer therapy success or failure, allowing faster decision making and avoiding unnecessary and potentially harmful treatment. We will also investigate the relationship between vascular structure and delivery of larger, antibody-based imaging agents. In the coming 5 years, we aim to translate two of our imaging agents to the clinic in first-in-human phase 1 studies.

Because imaging tumours delivers radioisotopes to tumour tissue, by increasing the amount or type of radioisotope that is delivered to the tumour, it can be used as a therapy, irradiating the tumour from within. We will also develop novel radionuclide therapy agents, and evaluate their ability to cause radiation-induced DNA damage and tumour cell death. We aim to investigate the efficacy of these therapeutic agents, and visualise their biological effects, such as the induction of DNA damage. Once translated to the clinic, this will enable optimisation of patient dosing, depending on their individual tumour's response to the treatment. We will also investigate the combination of radionuclide therapy with other anticancer therapies, to improve its therapeutic effect.

We will focus our efforts for developing novel imaging and therapy agents around pancreatic and brain cancer, two cancer types of unmet need, with very low survival rates, especially pancreatic adenocarcinoma.

**What 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 developing novel tool for studying biological processes during cancer formation and cancer therapy, which will help cancer researchers. The imaging agents may, if successfully translated to the clinic, help with predicting whether chemo- or radiotherapy will be effective in an individual patient. Because DNA damage is increased in very early tumours, these imaging agents will also allow very early diagnosis of tumour tissue, well before they could be detected with now commonly used techniques such as MRI or CT imaging. Novel radiotherapy agents may result in novel methods for treating chemotherapy-resistant cancers. Although we provide proof-of-principle of our imaging and therapy agents mainly in pancreatic, brain and breast tumour models, all of our methods are applicable to a wide variety of tumour types, because we are targeting general hallmarks of cancer. We will patent any potentially commercially applicable technology, so it may be taken forward for further development and translation to the clinic for use in cancer patients.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the next five years, we expect to use up to 8500 mice, including genetically engineered mice.

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

To study radiolabelled proteins that visualise tumour biology, mice bearing tumours are needed. Tumours will be introduced by injection of human cancer cell cultures into the flanks of mice. Genetically altered mice with specific cancer-inducing mutations also develop tumours. This results in moderate levels of harm to the animals. To study potential false-positive uptake of imaging agents in inflamed tissue. Therefore, we will also employ a limited number of animals in which inflammation of the pancreas is induced, or a sterile inflammation of the muscle is caused. This also results in moderate levels of harm to the animals. Animals will be euthanized before tumour burden or inflammation becomes a hindrance to their natural behaviour. The measurements of tumour uptake of the radiolabelled

compounds we will develop are acquired using dedicated small-animal scanners, and any surgical therapy are performed under general anaesthesia. The radiolabelled compounds themselves are not toxic. Mice may also be treated with chemotherapy or radiotherapy, but at levels which have been proven not to cause any side-effects in mice. In all surgical cases it is also expected that animals will experience a degree of pain. For these reasons we monitor the animals very closely and always seek advice from the veterinary staff. We use analgesia where appropriate and all surgical procedures are performed under general anaesthesia. The likely incidence of other adverse effects resulting from the majority of procedures used is low, and may include haematoma following blood sampling, and incomplete wound healing or infection following surgery. For the brain tumour models (maximum 11% of mice), there is a greater chance of neurological symptoms or compromised health owing to the nature of the models. In all surgical cases it is also expected that animals will experience a degree of pain. For these reasons we monitor the animals very closely and always seek advice from the veterinary staff within the University. We use analgesia where appropriate and all surgical procedures are performed under general anaesthesia. The likely level of severity in most cases is moderate. Where non-tumour-bearing animals are used, this is reduced to mild. All animals will be killed by an approved method at the end of any study.

## Application of the 3Rs

### Replacement

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

#### Replacement

At this point there is no tissue culture or modelling system than can predict the behaviour of a radiolabelled molecule in a living animal. Although we will first evaluate all novel imaging agents extensively in cell culture systems, no replacement for using tumour-bearing animals exists to date. Since our final goal is to develop radiolabelled model systems that can be translated to the clinic to aid cancer patient diagnosis and treatment evaluation, pre-clinical testing in animal models is essential and unavoidable. The use of lower, less sentient, smaller animal species is precluded by the limited spatial resolution of preclinical PET and SPECT imaging (0.8 mm)

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Imaging (PET and SPECT imaging) is by its very nature non-invasive, and therefore allows repeated measurement of the same animal, and therefore represents a significant reduction. We have recently piloted the use of two imaging agents in the same imaging study, presenting a 2-fold reduction in the total number of animals, without any observable effect on the animals. This also allows us to perform control experiments in the same animal. The number of animals in this licence has been chosen to be sufficient for statistically reliable data, based on previous results, the intrinsic variability of imaging data, and the magnitude of the expected changes. We consult extensively with the departmental statisticians as new studies begin to ensure that the optimal number of animals is used to obtain meaningful results and also kept to a minimum. Appropriate control groups are included and specified in each protocol and will be essential for proper statistical analysis and evaluation of observed effects. In all cases brain tissue will be used following the *in vivo* experiments, for immunohistochemical and molecular analysis, in an attempt to use the minimum number of animals possible. For all experiments, we optimise the experimental design, including binding and randomisation where possible, to give us the most meaningful data using the least number of animals.

For all experiments, we optimise the experimental design, including binding and randomisation where possible, to give us the most meaningful data using the least number of animals.

## **Refinement**

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

## **Refinement**

All procedures used in this project will be the least invasive and least harmful possible.

- For all tumour models, we use the minimum size tumour that will provide us with meaningful data.
- Another examples includes the use of micro-irradiation techniques (using instruments like SARRP), which allow much more precise delivery of radiation dose to target tumour tissue, while sparing normal tissues, thus sharply reducing normal tissue toxicity.
- After every procedure, animals will not be returned to their housing until fully recovered from any procedure.
- Any surgery will include the use of aseptic technique according to LASA guidelines and the appropriate level of analgesia.

- Where general anaesthesia is required, this will be performed using inhalation anaesthetics, where possible. Over the last three years, no injectable anaesthesia agents have been used. The impact of anaesthesia is minimised by the use of heating and physiological monitoring. At minimum, the breathing rate of the animal will be monitored.
- We will use the most optimal experimental design for each study. Where possible, we will use blinded randomisation of animal groups.
- We will pilot the use of less invasive regimens for tamoxifen administration in oestrogen-receptor-activated genetically engineered mouse models
- The impact of all procedures is evaluated by daily visual assessment of animals. Humane endpoints are indicated in all protocols, and animals will be killed by a Schedule I method if certain symptoms are observed, as indicated in the protocols. Animals will be monitored visually (daily) and weighed regularly (at least weekly).
- Animals showing mild adverse effects, before becoming limiting, will also be provided with wet mash, jelly, and food pellets, to increase access to food and water.
- We are optimising the way we detect pancreatic tumours: ultrasound imaging and palpation was replaced by FDG-PET imaging, a technique routinely used in the clinic, with much better tumour detection rates. This meant that animals with smaller tumours could be used in imaging experiments using novel imaging agents, and unnecessary adverse effects from large tumour growth could be avoided.
- Injection volumes of administered substances will be kept to the necessary minimum, and are limited per administration route.
- We work closely with the NACWOs and vets, to establish relevant behavioural scoring sheets for each individual model used, where appropriate. In this way we ensure that all relevant clinical symptoms and signs are assessed and humane endpoints applied appropriately. All surgical and anaesthetic methods will be reviewed regularly as new methods become available

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 261. Initiation and regulation of chronic inflammatory responses in kidney disease associated pathologies</b>
<b>Key Words</b>	Sterile inflammation, Peritoneal dialysis, Fibrosis, Cardiovascular disease
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overall aim of this project is to develop therapies to treat or manage human pathologies linked to chronic inflammation. Initially, we will focus on two issues experienced by patients with kidney failure on peritoneal dialysis (PD): development of fibrosis on the peritoneal membrane and increased incidence of cardiovascular diseases.

To achieve this, the work will need to address the following objectives:

- 1- The identification of the immune receptors or pathways involved in mediating peritoneal sterile inflammation and membrane fibrosis development in PD
- 2- The identification of the PD-associated triggers of the pathways identified in 1.
- 3- The evaluation of the capacity of inhibitors of the pathways and triggers identified in 1 and 2 to prevent or reverse PD-induced membrane fibrosis.
- 4- The investigation of the involvement of the immune pathways and triggers induced by PD in the development of systemic inflammation and vascular damage.
- 5- The evaluation of the capacity of inhibitors of the pathways and triggers identified in 4 to prevent systemic inflammation and vascular damage.

**What 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 provide novel information on the molecular processes underlying sterile chronic inflammation and the onset of fibrosis. The immediate applications are

in the management of peritoneal dialysis (PD)-associated complications in patients with severe kidney dysfunction, but we expect that our work will also benefit other researchers in the field of sterile inflammation-related pathologies (e.g. atherosclerosis, arthritis, tissue fibrosis) by revealing potential avenues of investigation and new targets for therapies. The ultimate aim of our project is to improve clinical outcome and quality of life for patients with chronic inflammation, in particular patients on PD treatment. We will test several treatment options to prevent or reverse peritoneal membrane damage and fibrosis due to the routine exposure to PD solutions. A successful strategy could ensure PD treatment works better for longer and increase quality of life for patients. In addition, chronic inflammation of one organ often leads to inflammation and damage to other organs. This is also true for end-stage renal failure patients on PD, who experience an almost 10 times higher risk of cardiovascular disease than the general population. The reason for this is still unclear and this work will evaluate the impact of PD-induced chronic inflammation on the onset of cardiovascular disease. We expect that our findings will help determining whether the choice of PD as treatment modality for end-stage renal failure may aggravate the risk of cardiovascular disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to 2500 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 levels of severity? What will happen to the animals at the end?**

To complete the aim and objectives of the work under this licence, animals will need to be repeatedly administered substances with low inflammatory potential, to mimic the low grade inflammation that is observed in patients with kidney failure on peritoneal dialysis. Injections of these compounds will be done either in the peritoneal cavity or intravenously. In both cases, we expect that, due to the low inflammatory nature of the compounds, these injections will only induce a short lasting, mild discomfort to the vast majority of the animals (85-90%) However, as our work identifies new potential compounds, optimal dosage will need to be determined and it is possible that in some cases this will lead to increased discomfort, such as moderate abdominal inflammation and discomfort. However, we do not expect that these effects will last more than 24h. In order to study the role of particular immune pathways in the inflammatory processes observed, genetically altered animals, lacking part of these pathways, may need to be bred and used under this licence. These genetic modifications are not expected to induce any adverse effects to the animals. At the end of the experiment, animals will be humanely killed sacrificed in order to obtain the tissues and samples necessary to our study.

## Application of the 3Rs

## Replacement

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

### Replacement

Inflammation is a complex process involving the local tissues as well as recruited cells from the circulatory system. It is impossible to fully reconstitute this system *in vitro* and investigate multi-factorial changes leading from sterile chronic inflammation to tissue fibrosis. Non-protected animal species' immune systems are typically very different from ours, so their use in that context would be unlikely to generate relevant findings.

Each step of the proposed work also encompasses *ex vivo* and *in vitro* experiments, often using samples from patients. But the use of a dynamic *in vivo* model remains necessary to address the precise role of specific pathways in the genesis of peritoneal fibrosis and investigate the therapeutic potential of targeting these pathways.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will endeavour to use the minimum number of animals to achieve clear and reliable results. In order to achieve this, the following measures are being/will be taken:

- We are getting as much information as possible from the literature to determine the range of doses and frequency of injections likely to be necessary for unambiguous results. This will help to reduce the number of animals used to determine the optimal dosage for novel compounds.
- Small size pilot experiments will be run, with a reduced number of animals per experimental group and only the minimum number of conditions for interpretation of the findings. Although these experiment will likely be too small to lead to reliable final results, they will be sufficient to determine whether the avenue of investigation being followed is promising. For example, if a treatment is being investigated, we will first check that it is efficient at treating diseased animals before checking that it has no effect on normal animals. If the potential treatment is found to have no effect on the disease, no further investigations will be made.

## Refinement

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

### **Refinement**

Peritoneal dialysis solutions are used several times a day by PD patients and do not lead to any adverse effects other than asymptomatic peritoneal inflammation, therefore we do not expect that they will lead to pain or discomfort in the animals. Due to their sterile nature, we expect that the other compounds to be injected will similarly have only a very mild inflammatory capacity, but this will be confirmed in *in vitro* experiments when new compounds are used.

The optimal frequency and number of injections will be carefully determined and kept to what is strictly necessary to achieve robust, reliable and significant results.

Insulin needles will be used for the intra-peritoneal injections to reduce the potential discomfort to the animals. Injections will alternately be done on the right and left side of the animal to reduce the risk of skin irritation or damage.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 262. Calcium signalling in smooth muscle function</b>
<b>Key Words</b>	Mouse, genetically modified, physiology, smooth muscle, contractility
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Finely tuned control of smooth muscle contraction is essential for the normal functioning of bodily systems. For example, contraction/relaxation of the smooth muscle lining arteries and veins determines blood flow through organs and tissues and contributes to the control of blood pressure, while lung airway smooth muscle influences lung airflow and breathing. Similarly, contraction of smooth muscle propels the contents of hollow organs such as the intestines, bladder and uterus. Incorrectly functioning smooth muscle therefore contributes to several common diseases, such as high blood pressure and asthma.

Smooth muscle contraction is regulated by a wide range of internal and external stimuli and all of these act partly through the control of intracellular  $Ca^{2+}$  concentration. This in turn occurs through the opening and closing of channels in the cell surface membrane or deep inside the cell where  $Ca^{2+}$  is released from internal stores. There is much that still needs to be learnt about how these channels operate, how they are regulated and how they contribute to normal and abnormal smooth muscle function.

The aim of this project is to use genetically modified animals to identify links between genes that express membrane channels or related molecules and altered smooth muscle contractile 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)?**

In general, the main benefits of this project will be publication of data and dissemination of knowledge. In the medium-term, this data will add to the wider understanding of smooth muscle function in animals and humans and in the longer

term, it may inform public health policy with regard to new treatment strategies for the control of abnormal smooth muscle function.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice. Approximately 500 mice during the first 12 months. Subsequently, similar numbers per year, pending favourable results obtained during the first 12 months and provision of additional research funding.

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

The only regulated procedures outlined in this licence are the breeding of genetically modified mice and the taking of biopsy by ear-punch for essential genetic identification. All experiments will be performed *in vitro* on tissues extracted from adult mice after they have been killed. Therefore the level of harm done to the animals will not extend beyond that caused by the genetic modification itself and the associated ear-punch. The overall level of harm is 'mild' because the types of genetic modification specified in the licence do not result in an adverse phenotype in terms of physical appearance or behaviour.

## Application of the 3Rs

### Replacement

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

### Replacement

This project requires the use of genetically modified mice because it is the most effective way of examining the importance of specific genes or alterations to those genes. Other animal species cannot be used because in each mouse line, a specific gene modification will be examined.

For ethical reasons, the majority of the planned *in vitro* experiments cannot be performed in humans.

Depending on the nature of the data obtained from these studies, further experiments in mice (*in vivo*, *ex vivo* or *in vitro*) may be warranted, or the data may be used to plan *in vivo* experiments with human volunteers.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The projected number of animals to be used per year is based on the number of different experimental protocols required to fully address the aims multiplied by the expected sample size (number of repeats) required for robust statistical analysis. Generally, with experiments of this type, to achieve a reliable significance level of <5% for a 25% difference between groups would require 8 - 12 repeats. Larger differences of >50% usually require 4 – 6 repeats. Total numbers of animals used will be minimised by using tissues from each mouse in multiple different types of experiment simultaneously.

The mouse breeding programme will be carefully managed, including estimates of the number of matings required, to ensure that the optimum number of mice are produced at the required times. We will ensure that only colonies being actively used will be mated to produce new animals. Colonies that are no longer required or will not be required for long periods will be closed and cryopreserved until they may be needed again.

### **Refinement**

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

### **Refinement**

As explained above, we will be examining the effects of loss or modification of specific genes. Genetically modified mice are ideal for this purpose and such modifications are not available using other species.

Furthermore, as also explained in the replacement section, all experiments will initially be performed in isolated tissues following killing of the animal, so animal suffering will be as minimal as possible.

In general, such an approach is the most refined possible without completely replacing the animal model, since the level of harm (mild) is due solely to the genetic modification itself (and associated ear-punch) as explained above.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 263. Provision of Biologicals Materials</b>
<b>Key Words</b>	Blood, tissues, service, biological materials
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to provide blood and biological products (organs including brains, lungs and kidneys) from a range of animal species (mice, rats, rabbits, chickens, turkeys and dogs) to support in research, diagnostic and regulatory work. This can include ensuring new medicines are safe before release for use and checking the calibration of diagnostic devices used in treatment of both humans and animals.

To do this we produce fresh bloods, plasmas and serums after assessing individual customer requests looking at the purpose of the work to be carried out by the customer and the benefits it may provide.

By storing frozen plasma, serum and organs we can then ship them internationally to customers, giving a consistent timely service across different end users working on similar work. This allows researchers to purchase the specific product required as opposed to animals having to travel.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The benefits from conducting this work are dependent on the research projects of the customers ordering: a large proportion of products produced under the previous licence supported regulatory work, which is required under government guidelines and ensures the safety of drugs. Other products go to support calibration of assays and equipment to ensure results from work conducted are validated and that drugs produced are free of viral contamination. Regulations which guide the choice of species selected by customers to perform this testing include: Food and drug administration, world health organisation and the ICH (International council of harmonisation). The data from the assays performed will be used in regulatory submissions to the appropriate regulatory authorities or is used to help form a picture of the potential of putative new drugs to be more efficacious with a better side effect profile than existing therapies in a wide variety of human and animal health

indications. These data may not always be positive, and hence, some of these tests may prevent the further development of such entities, preventing the un-necessary use of animals in efficacy and regulatory testing prior to testing in human or animal clinical trials. The scientific benefits directly linked to this licence are dependent on the research projects of our customers; but under previous licences the tissues have contributed to the knowledge of disease processes in man, animals and food crops, understanding of the development of the immune system and its regulation, and extension of the knowledge of neurobiology and associated neurological disease. By offering the different products and species from one location we can give consistency across the samples, allowing direct comparisons in the end work performed, even if this is at different locations by different customers. We are able to reduce the movement of animals by shipping blood products to end users across Europe who would otherwise have to transport animals increased distances to produce products themselves. We also can take organs after the death of the animal (for example brains and lungs) and store these until needed. The customers we supply have a preference to outsource this work so they can benefit from the high levels of specific experience and knowledge we provide.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5 year term of the PPL we expect to use 160,000 mice, 60,000 rats, 6250 rabbits, 180 dogs and 1060 birds (chickens and turkeys). The majority of animals used will undergo non-recovery procedures (collection of blood or organs and tissues); i.e. carried out under terminal general anaesthesia. However, approximately 250 rabbits, 80 dogs and 160 birds will be used for the repetitive collection of small blood samples. Dogs, birds and a small proportion of the rabbits (5%) would have blood withdrawn from a superficial vein at approximately fortnightly intervals resulting in each animal having approximately 24 samples taken per year. Most rabbits would only have one blood sample taken.

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

The majority of the animals under this licence will only undergo procedures under non-recovery anaesthetic; these animals will only experience mild discomfort, due to being held still as the anaesthetic is introduced, such as experienced by human patients undergoing surgery. The only difference is that they will not awaken from the anaesthetic and will have death confirmed or be humanely killed at the end of the procedure. Anaesthetic will be introduced either by injection into the veins or by inhalation of gas. For rabbits and dogs sedation may be used before hand to reduce the need for longer periods of restraint. Chickens, Turkeys, Dogs and Rabbits will be kept as blood donors, and will have approximately 2 blood samples taken a month. These are small volumes that are under 10% of blood circulating volume and will be collected from superficial veins, similar to human blood donations. These animals will

only experience minimal restraint during the period of sampling and it is not expected to cause any adverse effects. Where appropriate topical local anaesthetic will be applied to the area before sampling.

## Application of the 3Rs

### Replacement

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

#### Replacement

The work performed, that uses products produced under this licence, is required under safety and regulatory guidelines; these include testing of drugs (both medical and veterinary) prior to their release to market, as well as ongoing calibration and quality checks of equipment and processes to ensure accuracy of the results that are published.

Currently there are no methods to generate animal specific blood products (cells, plasma, serum) without the use of animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

By keeping donor colonies of dogs, rabbits and birds we are able to take small blood samples across a period of time from the same animals. This reduces the number of animals needed overall and provides a consistent product decreasing the need for retesting.

By collecting blood under non-recovery anaesthetic we are able to collect a higher volume of blood per animal compared to collection after the death of the animal. This reduces the numbers of animals used overall.

Our customer services department provides a central point to order blood and other biological products from, for a range of customers from small university groups to large contract research companies. This means we can collect different products (blood and organs, including brains, heart, liver and lungs) from the same animal and provide to multiple end users. This is frequently done with blood products from birds. All tissues are collected after death from all species.

### Refinement

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

### **Refinement**

The choice of animal is determined by the customers' and regulatory requirements, with species such as dogs only used where non-rodent species are required and it is the best model, due to either the research being related to dogs, or due to the similarities in systems that they share with humans.

The methods used for the collection of blood samples are based on guidelines of volume and frequency that will cause the least harm to the animals. The processing after collection is designed to get the highest quality and quantity of product so sample sizes can be kept as small as possible; we consider storage methods from across multiple fields including human transfusion services to ensure that we can maintain the quality of stored product.

Dogs and birds kept as donor animals are held in group living conditions, with dogs having access to both inside and outside areas as part of their housing; all donors are assessed individually and both their behavioural and physiological condition is monitored throughout the time they are a donor. Rabbits are only kept as repeat donors if the end use requires it, for example we work with a customer who uses fresh rabbit blood cells in human medical diagnostic work and before using the cells from any rabbit they have to validate it in line with ISO 15189 (International standards for medical laboratories). By keeping a donor rabbit they can complete the validation once and then only take small volumes thereafter.

For donor dogs, chickens and turkeys the jugular vein is used for collection of blood samples, this is a superficial, easily accessible, larger vein which means the time the animal is held for the procedure can be kept to a minimum and adverse effects, even for larger samples are rarely seen. For rabbits the marginal ear vein or artery will be used, with the vein mostly used as the samples taken are small and the vein has less chance of bruising. This is an accessible blood vessel that means the rabbit can be held in a natural position for the duration of the sample. For all the animals used as blood donors the sample time and experience of feeling is similar to a human blood donation or blood test performed medically.

Dogs will only be used when the product is required for work that cannot be done without using dog specific materials, currently there is a requirement under EU legislation that drugs are tested in a non-rodent species before release into the medical and veterinary markets. Dogs are used in cases where they have similarities with humans in how they deal with the drugs at a cellular level.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 264. Understanding and helping central nervous system white matter disease</b>
<b>Key Words</b>	Multiple Sclerosis, Remyelination, Small Vessel disease, Oligodendrocyte
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We aim to understand why the brain repairs poorly in diseases such as multiple sclerosis and other similar diseases, so we can design therapies to try and improve repair. This is important as there are no therapies which are effective for stopping the dying back of nerves in the brain in neurodegenerative diseases. So, patients with progressive multiple sclerosis or similar diseases become more disabled, and we cannot even yet slow this process down, let alone stop or reverse it. Nerves are covered with a substance called myelin, which has a similar role to the insulation on wires, protecting the underlying nerve. This is damaged in diseases such as multiple sclerosis, and there is evidence that if this is repaired, nerves are protected from dying back, reducing disability. This project aims to understand this repair of myelin and improve it.

Another disease with brain changes similar to multiple sclerosis, but occurring mostly in older people is called “small vessel disease”, and it is not understood why this happens. Without this understanding, it is difficult to design therapies to treat it. This project aims to increase understanding of this disease in order to find ways to improve it.

Therefore, our project is to try and understand the processes of brain damage and repair in both multiple sclerosis and small vessel disease, and to design therapies to combat them.

In this project, we aim to:

1) understand how repairing cells recognise damaged parts of the brain, how they travel to these areas and how they then replace the protective covering of nerves (myelin).

2) manipulate some of the molecules that we already know are involved in the repair pathway to try and improve how repairing cells reach areas of damaged brain, with the idea of developing drugs and therapies to do this better.

3) discover new signals involved in the pathway, especially in the recruitment of repairing cells to areas of damage.

4) understand how interactions between nerves, repairing cells, immune cells and blood vessels cause a type of neurodegenerative disease called small vessel 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)?**

There are currently no therapies that are effective for neurodegenerative diseases such as the progressive stages of multiple sclerosis. By increasing understanding of the way that nerves die back in these diseases, and finding ways to repair the damage, we will start to fill a completely unmet need. Therapies to aid repair in the brain aim to reduce disability from these diseases. Work such as this from our lab in the last 5 years has produced two new targets for drugs to help repair, and these are now in the process of being developed in collaboration with pharmaceutical companies to make drugs suitable for testing in humans.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

To do this work, we will use about 7000 mice and rats per year. We are careful to use as few as possible to answer our questions, and aim to get the maximum amount of information from each animal to help us in our research. We do this by asking simple questions with cells/sections of animal tissue initially, and only progressing to live animal work for the most promising possible therapies for disease and most vital research questions. Rodents are the smallest mammals that are used to model human disease, and many drugs/therapies now in use in humans were first tested in rodents, giving more confidence that if they work in these animals, they may work in humans.

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

We will use animal tissue to obtain cells and sections of brain to grow in dishes to study how repairing cells of the brain function, and how we can improve how efficiently they can carry out repair. To understand neurodegenerative diseases better, we will also use live models of these diseases, to try and understand what

causes the damage and how it is repaired, and to test possible therapies to improve the diseases. This involves using genetically modified rats and mice, and causing small areas of damage to parts of the brain by neurosurgical operations to mimic the problems found in these diseases in humans. These animals are closely monitored for signs of harm, as they sometimes develop symptoms such as difficulty moving a limb or tremor. We will use strict humane endpoints, and we aim to improve repair, and reduce the disease severity. All animals will be culled at the end of the experiments.

## Application of the 3Rs

### Replacement

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

#### Replacement

We try and use human tissue where possible to study brain repair – using donated post mortem tissue and also generating brain cells from stem cells in a dish. This technology is new but will be very powerful to test research questions and possible therapies. However, the brain is a three dimensional structure with many interacting cells, and we cannot model this yet in a dish, and so have to turn to animals, and in particular, mammals, to help with this complexity.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We use less live animals by using cells or brain slices grown in a dish for many initial experiments. We calculate the correct number of animals required for an experiment to show an effect using statistics, so we use enough but not too many.

### Refinement

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

#### Refinement

Research experiments do not produce good, reliable and repeatable results if the animals involved are ill or suffering. Therefore, in order to answer important research questions, as well as for personal reasons, we are highly motivated to provide excellent care for our animals. Our experiments use much specialised equipment to

ensure small areas of damage in the brain, fast operations and excellent post-operative care including pain relief.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 265. Hepatitis E virus in Pigs: Viral Distribution and Transmission Studies.</b>
<b>Key Words</b>	HEV, Virus, Distribution, Transmission
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Hepatitis E virus (HEV) is an emerging viral disease in humans in the both the UK and across Europe. Infections in humans have traditionally been associated with travel outside the UK and Europe but recently significant increases in the number of non-travel related cases have been documented indicating a change in how people are infected There is mounting evidence of an association with pork/pork products, and pigs have been shown to act as a carrier of HEV without showing signs of infection or disease. To understand and address this transfer of HEV into the pork food chain we aim to develop an infection model that mimics the field situation in pigs. This will allow us to investigate the distribution of HEV in edible tissues and look at how HEV spreads pig to pig. To help understand the epidemiological picture, two different age groups of pigs will be studied, reflecting two different stages of pig farming.

**What 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 is designed to address knowledge gaps that hinder the effective control of HEV in animals and the spread to humans. 1) Establishment of a reproducible field equivalent infection model for pigs that can be used subsequently for techniques such as transmission investigations and vaccine testing. 2) Understanding HEV distribution in edible tissues to help protect the human food chain. 3) Investigation into HEV shedding from infected pigs to understand how it spreads and improve epidemiological understanding and risk management of the disease in both animals and humans. 4) As part of the above, observation of whether and how the virus changes during transition through the host and to in-contacts and what impact this has. 5) To understand the differences in the dynamics of HEV

infection for different age groups of pigs to help understand the epidemiology and risk management of the disease. 6) Understanding the effects of co-infections of HEV with other common viral pathogens of pigs, again for epidemiology and risk management.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Three hundred and sixty six pigs over the five years 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 levels of severity? What will happen to the animals at the end?**

Unlike for humans, HEV is not known to cause clinical disease in pigs so the severity level is mild for the HEV infection studies, and raised to moderate for the studies involving co-infection with other pig pathogens that are prevalent in the field and have the potential for clinical signs. These could also be exacerbated by co-infection with HEV. By analysing samples taken in a timely fashion during the experiment it is anticipated that the number of samplings may be reduced. Ultimately all of the studied animals will be euthanized as part of the disease control requirements.

## Application of the 3Rs

### Replacement

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

**Replacement**

There are currently no alternative techniques available to replace the use of pigs, as the work is studying the interaction of HEV infections with the pig's various organ systems including its immune system. Therefore only the use of pigs allows the achievement of the projects key aims which are to understand viral distribution, shedding and transmission with regards to the pork food chain.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

**Reduction**

The numbers are selected at the minimum that will enable a robust experimental design compatible with obtaining reliable and meaningful results, for which the advice of statisticians from the Biomathematics and Risk Research team has been sought

### Refinement

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

### **Refinement**

HEV does not cause clinical symptoms in pigs and so the adverse effects are limited to the sampling techniques, the worst of which is blood sampling. When co-infection studies are done with two other common viruses of pigs to study what impact this has on HEV infection, the two other viruses used may cause temporary fever and loss of appetite.

The staff working under the auspices of this project licence have extensive experience along with access to appropriate facilities to deal with this type of work. Refinement has been practised with an emphasis on animal welfare reflected in the protocols and clinical observation regimen, to minimise possible suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 266. Mechanisms involved in obesity and associated metabolic diseases</b>
<b>Key Words</b>	Adipose, Thermogenesis, Obesity, Human stem cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The project has an overarching theme of understanding why people become obese and then become sick (particularly why they get diabetes). Moreover we want to identify new genes/molecules that we could target with specific treatment to treat obesity and diabetes.

We have two specific objectives: The first is to determine if the negative effect of obesity can be counteracted by activating a specialised kind of fat called brown adipose tissue (BAT) Unlike the well known white fat (WAT), BAT burns fat instead of storing it. Activating BAT could be used to reverse obesity itself, or by preventing fat to go in wrong locations, diabetes. The second is to investigate how WAT function connects obesity and diabetes. Doing so we aim to identify genes/molecules that could be used as markers to predict the risk of diabetes and that could be targeted with new treatments to prevent its 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)?**

We expect the main benefit from work carried out under this license to be in terms of scientific advancement. Our work will provide information from which other scientists and drug companies can build on to perform human studies and design new therapies. We hope to identify new genes that can be manipulated to treat obesity. Moreover, the in vivo transplant system of human stem cell derived-cell types in mice developed in this license could be valuable to other scientists interested in organ development in human context and regenerative medicine (making new organs to replace faulty ones). 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 types and approximate numbers of animals do you expect to use and over what period of time?**

We will use exclusively mice. We expect to use in the region of 10400 animals.

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

We will perform experiments where mice will be transplanted with fat cells derived from human stem cells. Stem cells can turn into all cell types of the body. We have developed ways to turn stem cells into fat cells in petri dishes. In the human body, fat cells are present in different locations, such as under the skin and in the abdomen. We plan to transplant fat cells generated from the stem cells into mice and study how they will form adipose tissue. At first we will determine the best conditions for the transplant using normal cells (pilot study). Then once the methodology is set up and validated, we will investigate the impact of the transplantation of normal cells and mutated cells and the tissues that they will form on the development of obesity or diabetes in the mice. Because we will use mice having a partially compromised immune system, to perform the transplant, these mice will be maintained in a clean environment where pathogens such as bacteria and viruses are absent. According to the type of gene that we want to study, the transplanted mice could be fed diets high in fat to make them obese and/or insulin resistant (insulin resistance leads to diabetes). Some very insulin resistant models may become diabetic and drink a lot of water and produce a lot of urine. These mice will require extra care (more frequent cage changes) to prevent the development of ulcers. We perform a range of procedures that are classified as mild. These include glucose and lipid tolerance tests where mice receive a large amount of sugar or fat and we take blood samples to determine how well they can cope with it. Mice and humans with diabetes cannot deal with sugar or fat well. Other procedures that we will perform are classified as moderate, such as the transplant itself and the administration of insulin to study how specific organs become insulin resistant. Very rarely animals respond badly to these protocols and may have to be killed for welfare reasons. With the transplant of cells types derived from stem cells there is a small risk of developing tumours called teratomas. That will be detected by monitoring the speed of formation of the fat pad, weight loss and levels of markers of teratomas in the blood of the mice. The mice that present sign of teratomas formation will be killed. All mice will be killed at the end of the experimental procedures.

## Application of the 3Rs

### Replacement

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

## Replacement

Complex diseases such as obesity and diabetes and that we want to study and understand in this project involve the interaction and crosstalk between different organs (i.e. muscle, brain, adipose tissue, etc.). This degree of complexity cannot be recreated in a dish using current methods, and for this reason the mouse remains the best model organism to answer the metabolic questions addressed in this project.

However, we are developing a 3D culture system for the generation of organoids (mini tissues), to try to reproduce more closely the environment of the adipocytes, and even study the interaction among different cell types. Even these 3D culture systems so far cannot fully substitute for the use of animal models where the impact of mutations can be investigated at the level of the whole organism. However we are confident these 3D cultures will help us to prioritise, reduce and refine our *in vivo* experiments.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

The use of our human adipocyte cells model will allow us to reduce the number of mice used in our project because in principle the genes showing an effect in culture will be chosen and put forward for the animal work. Moreover, the way our project plan has been conceived will allow the use of the same animals for different aims, reducing the total number of mice to be used.

On top of that, if appropriate, both control and mutant cells may be injected into the same mice, allowing us to obtain paired comparison, increasing sensitivity and reducing the number of mice used.

Another method to reduce animal usage will be through experimental design. By using the correct number of animals for each experiment we avoid wasting animals by obtaining either false positive or false negative results. We will determine how many animals to use based on data we already have and then use a statistical tool called a power calculation to work out the smallest number of animals we need to get a meaningful result. It is clearly important that we do not use too many animals, as then animals will have gone through the procedures unnecessarily, however using too few animals in an experiment can be even worse as we can get results which

look promising, but do not provide strong enough evidence to be confident that the result we have obtained is real. In cases where too few animals are used the whole experiment has to be repeated again, wasting the animals that were used in the first experiment.

## **Refinement**

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

## **Refinement**

Mice are a suitable species for this project because they have similar organ systems to humans which have similar metabolic functions, which are absent in more simple animals. Many mouse strains can develop the symptoms of metabolic disorders found in humans, including obesity and elevated insulin levels. The use of immunocompromised mice in this license is mandatory because of the human nature of the cells to be transplanted. The grafted mice, because of their immunocompromised nature will be handled in dedicated laminar flow cabinets and maintained in pathogen free environment. As a commitment to the 3Rs, we intend to use experimental variations which minimise the stress the mouse is subjected to. These methods are well established both in humans and in mice, and provide a way of being able to compare results between species.

Mice are social animals, so in this project they are always maintained in groups in the cage, except for specific periods and specific scientific reasons such as being able to accurately measure food intake from a single mouse. In most cases being alone in a cage will be well tolerated, however some mice respond to that by losing weight. To address this question the following refinement measures will be considered: where possible mice will be regrouped between periods of being alone. However, prolonged time as single mouse in cage could result in subsequent regrouping intolerance between males of more aggressive strains, as males begin to behave like exclusive territory owners. In this case regrouping the mice may not be possible. In less aggressive strains regrouping the mice will be attempted. Mice will be monitored for fighting. If fighting occurs to the extent that mice are injured, the aggressive mouse or mice will be removed to a separate cage. The mice are provided with environmental enrichment such as cardboard tunnels and nesting materials to facilitate normal behaviours and when they are being regrouped, some nesting material will be transferred at cage change.

Moreover, to reduce harms to the animals we employ a dedicated staff of animal technicians with specific expertise in working with mice with compromised immune

system and performing metabolic analysis. They will also guarantee to the mice the required husbandry, care and welfare. The facility uses bespoke animal tracking software that help keeping good records of what is done to the mice and their health status for each project licence.

Moreover the techniques and procedures used for the metabolic analysis of the mice described in this PPL have been REDACTED.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 267. Gene therapy in the cardiovascular system</b>
<b>Key Words</b>	Angiogenesis, VEGF (Vascular Endothelial Growth Factor), Cardiovascular Development
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project aims to identify molecules and mechanisms that play important roles in blood vessel formation and the development of cardiovascular diseases, and to develop approaches such as gene or cell therapy that can effectively target these molecules to achieve a therapeutic effect in human cardiovascular disease.

Cardiovascular disease is one of the major causes of death in the developed world and is rapidly increasing in developing countries. However, the mechanisms that cause, or protect against, this disease are poorly defined, and there is a continued need for new therapeutic approaches. We will identify molecules and mechanisms with important roles in heart disease, and disease-related angiogenesis and thereby identify new therapies or therapeutic targets.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

This work will improve understanding of mechanisms and the key molecules involved that maintain cardiovascular health. VEGF-linked signalling pathways, which are the focus of this application, are already known to be important for human cardiovascular health and in human diseases such as atherosclerosis. Since many of these mechanisms and molecules are conserved between vertebrate species, the work proposed here will have direct relevance for analogous process and disease states in humans. This work will therefore advance knowledge and understanding of important processes underlying human health and disease. Furthermore, by identifying key novel molecules in these processes we will be able to identify novel targets for the development of therapeutic drugs, which may lead to the development of novel therapies for heart disease and vascular disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats (200) Mice (11,250) Zebrafish (22,000) These are the approximate maximum numbers we anticipate to use over the course of the 5 year PPL.

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

Our protocols are based on well-established procedures that have already gone through a considerable amount of refinement. Most animals will not undergo procedures that will inflict harm. Instead these animals will be used for phenotyping the effects of mutations using minimally invasive imaging or analysis. Some animals will undergo procedures that include minor damage to the lining of a small region of a single artery in a mouse or rat, or ligation of an artery in the mouse or rat that will restrict blood flow to the hindlimb, or in the zebrafish, injury to a small region of the heart. Based on our experience, adverse effects are anticipated to be very limited in all our protocols and where they do occur to be very brief in duration. Adverse effects that may occur in rodents include lethargy, hunched posture, loss of appetite, weight loss, and in fish, difficulty breathing, abnormal colouration, abnormal swimming, feeding or schooling behaviour. All our protocols, have a severity level of mild or moderate. All animals will be humanely killed at the end of the relevant protocol, and/or when signs of discomfort or pain are manifested. All animals undergoing surgical procedures are expected to recover quickly and will be given appropriate painkillers and post-operative care. At the end of a procedure, animals will be killed by a humane method and tissues taken for analysis after death.

## Application of the 3Rs

### Replacement

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

### Replacement

While cell culture models have been helpful and we continue to use them extensively, there are no computer, tissue or cell culture models that successfully mimic human cardiovascular disease or angiogenesis. Two major reasons for this are: these diseases develop in complex multi-tissue environments in living animals, which cannot be mimicked by non-animal models; they occur over long time periods which make it difficult to perform similar studies in non-animal models.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

Where necessary, pilot studies involving small numbers of animals will be performed to establish the proof-of-concept, and only if these small studies are encouraging, will we proceed to larger studies. Since protocols are already well-established in the chosen species, the minimum numbers of animals needed can be determined more accurately, and unnecessary pilot work can be avoided. Studies will be performed only using animal numbers sufficient to produce statistically robust results.

## **Refinement**

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

## **Refinement**

Animal species were chosen mainly because protocols were established in those species, avoiding unnecessary pilot work. Small rodents (rats and mice) were chosen, as these are the simplest appropriate mammalian organisms. The choice of mouse and fish is determined by the unique ability to genetically alter these species. Use of zebrafish allows us to perform studies wherever possible in simpler vertebrate organisms.

Protocols will be performed by experimenters who have experience in the models chosen. Measures will be taken at all appropriate stages of each protocol in order to prevent pain, discomfort or other adverse effects, and to promptly treat such signs. Experiments will be of sufficient duration to achieve our objectives, and persistence of adverse effects will be avoided by immediate humane killing.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 268. Study of environmental contamination from an experimental flock with naturally occurring scrapie</b>
<b>Key Words</b>	TSE, Scrapie, Sheep, Decontamination, Efficacy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Classical scrapie is a fatal neurodegenerative brain disease and one of the naturally occurring transmissible spongiform encephalopathies (TSEs) in sheep and goat. It can be transmitted to these animals by exposure to pasture, buildings and objects contaminated with the scrapie agent, a prion, which we studied in the past on a farm with naturally occurring scrapie. The recommended disinfection protocol for scrapie agents in case of a scrapie outbreak is exposure of all surfaces that cannot be removed with 2% hypochlorite solution for at least an hour. Under the previous project licence, we tested the efficacy of this protocol using an exposure time of 1 hour, repeated three times, in a barn previously occupied by scrapie sheep: sheep highly susceptible to scrapie were moved in the barn and infection monitored by examination of rectal biopsy for prion protein and – in parallel – a sensitive prion detection method was used to study environmental contamination. This study demonstrated that the building was still contaminated or contamination had re-occurred from the outside. The study was repeated after a similar decontamination session and all sheep are currently alive with no evidence of infection based on rectal biopsy examination. Dust samples from the barn were collected at regular intervals, which will be tested for prions.

As the previous licence expires, the objective of this licence is to continue keeping these sheep to monitor infection and to determine whether decontamination has now reduced the level of infectivity to a level that is not detectable in sheep (no infection occurs in the number of sheep used) and in sensitive prion detection tests.

**What 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 result will inform on the efficacy of our decontamination protocol to inactivate prions in a scrapie contaminated environment, which is important for policy makers and risk assessors, in cases of outbreaks of scrapie where the farmer wants to re-stock with susceptible animals.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The project will continue to use 46 sheep for up to 5 years.

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

All sheep are housed in a barn that is potentially contaminated with the scrapie agent and thus potentially exposed to scrapie. Infection will be monitored by collecting a rectal biopsy under local anaesthesia every 6 months and examining it for prion protein. Animals will also be monitored daily by animal care staff for signs of scrapie and examined in more detail if signs of scrapie are suspected. It is expected that infected animals will present with prions in the rectal biopsy before they show clinical signs so that they can be humanely euthanased but regular monitoring for signs of scrapie will ensure that clinical signs are identified as early as possible and animals are euthanased before displaying more severe neurological signs. Any animals surviving until 36 months post exposure will be euthanased as it is expected that prions would be detected by then if infection had occurred.

## Application of the 3Rs

### Replacement

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

### Replacement

Animals are used because they are the most sensitive model to assess whether the scrapie agent is still active to cause disease. Prion detection tests will be used in parallel to see whether they can be used as alternative to live animals in future, even though the absence of prions may not always mean that the area is not infectious.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The number of animals is restricted by size of the barn and the availability of animals with a desirable prion protein genotype that makes them susceptible and prion detectable in the rectal biopsy.

Statisticians have been consulted to determine optimal numbers.

### **Refinement**

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

### **Refinement**

Sheep are used because they are the natural host and this study aims to tell us whether the decontamination protocol can be applied to field situations where farms are restocked with sheep.

The study does not require animals to develop clinical signs since only proof of infection is required, which can be done by examining rectal biopsies that are taken under local anaesthesia. Clearly defined clinical end-points have been established in case of an animal developing signs of scrapie despite a negative rectal biopsy.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 269. Novel therapeutics for metabolic disorders</b>
<b>Key Words</b>	Obesity, diabetes, drugs
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The objective of this Project License is to provide information on the complex interactions responsible for the regulation of food intake and energy balance that occur between the gastrointestinal tract and the brain. This work could lead to new anti-obesity drugs, and treatments for diabetes and other metabolic disorders such as anorexia nervosa and eating disturbances associated with cancer. There is a huge clinical need for this research because of the global epidemic of obesity and diabetes.

**What 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 England, 62% of adults are overweight or obese, and in the UK 800 deaths per week are caused by obesity and its complications. Obesity increases mortality because of associated diseases such as diabetes, coronary heart disease and an increased risk of cancer. There is a clear unmet clinical need in the treatment of obesity. Although obesity and type 2 diabetes can be treated by a weight reduction diet, this is predictably ineffective as most patients are unable to maintain a reduced calorie diet long term.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

9000 rats and 5000 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 levels 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 reduced appetite, hunger and weight loss which will be carefully monitored. 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

### Replacement

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

#### Replacement

Energy homeostasis involves the interactions of multiple body organs and systems. Its 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 tissue from animals rather than whole animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

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. However, to ensure the minimum number of animals are used which will provide reliable data all animal experiments will be carefully planned and where appropriate advice from the statistical advice will be sought to assist with the experimental design and methods of analysis. 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 number of animals required.

### **Refinement**

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

### **Refinement**

Mice and rats are the species most widely used to study energy 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.

We will take a number of steps to minimise harm to the animals. These include using excellent and aseptic surgical technique. We also have a number of strategies to minimise pain, and these will be constantly updated on the advice of the named veterinary surgeon. For example, wherever possible, we will conduct experiments on terminally anaesthetised animals. We will also ensure the general welfare of the animals by regular monitoring.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 270. Improving therapies for diabetes</b>
<b>Key Words</b>	Diabetes, Islet, Transplantation, Therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The prevalence of diabetes is on the increase and diabetes has been identified as “one of the main threats to human health in the 21st century”. It has been estimated (by the charity Diabetes UK) that up to 2% of the population of the UK have diabetes, and the costs for treating diabetes and associated complications account for up to 10% of the total healthcare budget. Type 1 diabetes is caused by a complete loss of the insulin producing cells ( $\beta$ -cells) in the pancreas, and treatment is generally by insulin replacement by regular injection. Type 2 diabetes is also associated with a failure of insulin secretion and a reduction in the number of functional  $\beta$ -cells. The aim of this project is to use animal models to explore ways to improve therapies for diabetes by (i) improving current cell replacement therapies, (ii) developing new orally active insulin formulations, and (iii) maintaining and/or increasing the numbers and function of existing  $\beta$ -cells.

Clinical studies have demonstrated that transplantation of  $\beta$ -cells may offer a cure for type 1 diabetes, but the application of this therapy is restricted by the very limited supply of transplant material from human donors. One way of extending current transplantation therapy is to improve  $\beta$ -cell survival and function after transplantation so that fewer donor cells are required per treatment. Our group has developed in vitro models of  $\beta$ -cell function, survival and re-vascularisation but we also require in vivo models of diabetes to ensure that our treatments improve the ability of the transplanted  $\beta$ -cells to regulate blood glucose. We also have access to human islets and these will be used to confirm that these treatments are effective in human tissue and potentially translational.

There is also considerable interest in developing new strategies for increasing  $\beta$ -cell numbers and insulin secretion in people with Type 2 diabetes. This is dependent on understanding the mechanisms involved in regulating islet function. We currently apply in vitro methods for assessing the effects of experimental treatments on  $\beta$ -cell growth, survival, and insulin secretion so it is essential to determine whether these effects are relevant in vivo. Similarly, we need to know what effects different

hormonal and metabolic states have on islet function - hormones and metabolic changes affect a wide range of different tissues throughout the body, and an in vivo model is often the only viable way to study the interactions between pancreatic islets and other tissues.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Overall, the studies in this project will be designed to validate prior in vitro studies in the context of a hormone-deficiency disorder that involves multiple organs and so requires the use of a whole animal model in vivo. The studies will generate information that will be of direct clinical relevance in improving the outcome of transplantation therapy for Type 1 diabetes, and in the development of new therapies for Type 2 diabetes.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We estimate that the studies described will use approximately 9000 mice over a five year time-frame, although a large number of these will be used in establishing breeding colonies, rather than in direct experimental use. Approximately 500 rats may also be used for specific experiments where necessary.

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

Transplantation studies will primarily use a well-defined mouse model of diabetes, in which the mouse's own  $\beta$ -cells are selectively destroyed by administration of a chemical called streptozotocin. Regular monitoring of blood glucose allows us to identify diabetes, and to avoid any adverse effects of hyperglycaemia by administering insulin. We then transplant islets and assess the ability of the graft to cure diabetes. Subsequent removal of the graft can confirm the recurrence of diabetes, thus each animal acts as its own control, minimising the numbers of animals required. The surgery involved in the islet transplantation is of moderate severity, and animals will be monitored and administered with analgesics to ensure recovery. In some instances our in vitro studies implicate specific targets in  $\beta$ -cell function, and one way to validate such studies is through the use of genetically-modified mice. This type of animal model will be used to validate specific targets involved in islet function, as described above. The models that we anticipate using have a mild severity phenotype. For example, mice may exhibit some symptoms of Type 2 diabetes (e.g. glucose intolerance, resting hyperglycaemia) if maintained on a high-fat diet, or when they become older (6 months and beyond). Development of diabetes will be monitored by regular measurements of blood glucose, body weight and food/water intake and, where necessary, adverse effects of hyperglycaemia will be avoided by giving insulin. Some animals will undergo bariatric surgery which is classified as a severe procedure. These animals are monitored carefully after

surgery and administered analgesics and palatable food to optimise their recovery. All animals will be killed by a schedule 1 method at the end of a given experiment and tissue or blood samples taken for further study.

## Application of the 3Rs

### Replacement

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

#### Replacement

Islets contain several cell types which interact with each other, individual cell lines do not secrete insulin in response to glucose as islets do. Additionally the pathology of diabetes involves multiple organs, and strategies developed *in vitro* require validation *in vivo*. For example, we can test the insulin-secreting properties of islets pre-transplantation using *in vitro* methods, but *in vivo* studies are required to demonstrate efficacy in maintaining normoglycaemia. Similarly, we can use *in vitro* methodologies to screen agents that enhance  $\beta$ -cell proliferative or anti-apoptotic responses, but we then require animal studies to demonstrate that these modifications result in enhanced  $\beta$ -cell survival/function *in vivo*.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

In all studies we aim to reduce animal numbers to a minimum by applying the guidelines outlined by the NC3Rs concerning experimental design. This includes using statistical calculations to plan how many animals to use and where possible using inbred animals and thus minimising variation. In addition, where possible individual animals can act as their own control (e.g. in telemetry studies) and therefore fewer animals are required.

### Refinement

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

#### Refinement

The majority of the experiments will be conducted in mice, and they provide the simplest, most ethical and cost-effective model. Experiments may also be conducted in rats, primarily where blood samples are required at higher volume or frequency than is possible in mice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 271. Characterising the role of ubiquitylation and phosphorylation in innate immunity</b>
<b>Key Words</b>	immunology, phagosome, signalling, infection
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

In the next five years, we plan to use wild-type and genetically altered mice to investigate the role of particular genes in cells of the immune system – specifically, to understand how the immune system recognises “foreign particles” within the body and responds appropriately to them. For example, discriminating between whether a particle is dangerous (such as bacteria) or harmless (such as a dead cell or some debris) must be carefully regulated. When these processes fail, it can lead to infectious diseases (e.g. tuberculosis) or to “autoimmune disease” such as Crohn’s. These mice will provide valuable information to increase our understanding of these, and similar, conditions.

Mostly, we will use the mice for breeding and to generate primary immune cells and tissue after culling through schedule 1. However, a small percentage of the animals will also be challenged the mice with infectious microbes such as Salmonella, Listeria or Staphylococcus and test their ability to clear the microbes and test the roles of the genes altered. Moreover, we have recent evidence that phagocytosis and signalling from the phagosome plays an important role in inflammatory signalling in obesity models. We will therefore further test the roles of altered genes in animals challenged with high-fat diet.

**What 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 contribute to our understanding of infectious diseases and autoimmune disorders. Infectious diseases such as tuberculosis have in recent years become a severe problem with ~1.6M death yearly for tuberculosis alone. Surprisingly little is understood about the host defence to intracellular bacteria and this proposal is targeted to increase our understanding in this area. Moreover, autoimmune disorders, which include rheumatoid arthritis, multiple sclerosis, lupus as well as neurodegenerative diseases, are chronic and severely debilitating diseases. Together, autoimmune disorders affect a similar number of people as cardiovascular disease and cancer and represent a major healthcare issue. Despite advances, autoimmunity remains difficult to treat. Current therapies only arrest or slow disease progression and do not provide a cure to the underlying cause. As a result long-term or life-long treatment is required, which carries a major risk of the development of adverse side effects. There is therefore a pressing need to develop better drugs for these conditions. This project aims to use genetically modified mice to understand how specific proteins in the immune system control its function. Through doing this we hope to identify new targets that can be used to develop novel drugs to treat infectious disease and/or autoimmune conditions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will only use mice. Most of the mice will be used either for the breeding of gene targeted mouse lines or the provision of mice for the isolation of cells or tissue for further study. Up to 15000 mice will be used for this over 5 years of the project. A subset of these, in the region of 1000 to 1500, will be used in experimental protocols to examine their immune function.

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

The majority of the gene-targeted lines to be used do not exhibit adverse welfare effects. A small number of the lines to be used may develop autoimmune disorders or neurodegenerative disorders. The number of mice exhibiting these symptoms is likely to be less than 5% of the total number of animals used. Typically these adverse effects will occur in older animals and to minimise this mice will be used at a young age as possible. Where possible, in conjunction with the named vet, treatment programs will be used to further minimise and adverse welfare effects in these lines. Animals will be humanely killed by an approved method at the end of their use. The proposed infections are unlikely to result in significant adverse effects and will not exceed moderate severity levels

## Application of the 3Rs

### Replacement

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

### **Replacement**

For the majority of this work we will study cells isolated from the immune system of these mice, as this will allow us to complete much of our work without the need for experiments of the live animals. Due to the complex nature of the immune system it will be necessary to test some of the predictions made from these studies in live animals (mice).

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Breeding programs will be kept to the minimum required to maintain the line and provide mice for experiments and cell isolation. Cryopreservation will be used to archive lines that are not required for on-going research.

Whenever possible we will use studies on isolated cells or tissue. For experimental models accepted statistical methods will be used to establish the minimum group sizes necessary for the work. In this way we will minimise the numbers of animals in which a direct experimental intervention is required.

### **Refinement**

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

### **Refinement**

Mice will be used, as the ability to use genetically targeted mice to study the function of specific genes is essential for this work. The majority of the mouse lines used for this project do not have apparent adverse effects on the animal's welfare. For the small number of lines where this does occur, protocols will be put in place in conjunction with the named vet in order to minimise any adverse effects. For in vivo experiments, end points with the lowest severity possible to answer the scientific questions will be selected.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 272. The multitasking roles of Ghrelin</b>
<b>Key Words</b>	Feeding Patterns, Ghrelin, Multisystem Effects
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The physiological impact of temporal feeding patterns is very poorly understood. We will use our unique automated feeding station to address this unknown and determine the role of one of the likely hormones, ghrelin, in mediating the multiple physiological consequences of feeding pattern modification.

We will also develop and test a novel hormone sensor.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Establishing the impact of feeding patterns will open up a whole new area of scientific investigation, ultimately leading to best practice guidelines on feeding strategies being issued to the public, especially to those with responsibility for feeding children. Successful development of a hormone sensor will have an immediate impact on hormone quantification in rodents (hormones will be measured in real time in vivo without the need for blood sample withdrawal) and will lead to the development of multiplex hormones (measuring many hormones at the same time) and have applications in human and veterinary medicine, science, agriculture and sport (anti-doping).

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will use wild-type and genetically-modified rats and mice. The maximum number of animals used for generating and maintaining colonies of genetically-modified rodents will be 7710 rats and 10,710 mice, of which some will be used in experimental protocols (maximum 5250 rats and 3350 mice), with the maximum duration of individual feeding studies being 12 weeks.

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

Given the wide range of experimental protocols described the potential adverse effects are diverse, but extremely rare, with the severity level never exceeding moderate. Interventions described will include: • Injections • Diet manipulations (both time and content) • Anaesthesia/repeated anaesthesia for non-invasive procedures (e.g. MRI imaging) • Minor surgical procedures (e.g. implantation of a delivery device, vascular cannulation, excitotoxic lesion) Animals housed in CLAMS or metabolic cages may experience social harms associated with single housing and depleted environmental enrichment. A proportion of animals exposed to diet variation may develop early signs of obesity (e.g. accelerated weight gain and fat deposition). A proportion of the animals undergoing vascular cannulation surgery may experience weight loss. A proportion of animals receiving excitotoxic lesions will experience signs of Parkinson's Disease (e.g. reduced motor function) not exceeding a moderate severity level. Rodents with Prader-Willi Syndrome may experience overeating, obesity and reduced skeletal growth. All animals will be killed at the end of each protocol.

## Application of the 3Rs

### Replacement

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

### Replacement

Given that the impact of feeding patterns on end points such as adiposity, growth, fertility and neurodegeneration involves complex interactions between multiple organs and systems, such complex temporal interactions can only be properly understood in the context of the whole organism. Since these investigations involve time-dependent interventions (e.g. of diet and circulating hormone levels) and multiple system outcome measures, technical and practical consideration preclude the use of less sentient species (e.g. zebra fish and *C. elegans*) and are most suited to study in laboratory rodents.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The experiments performed under the authority of this licence will be designed and conducted in accordance with the NC3Rs ARRIVE guidelines.

The number of animals used will be minimised by:

- Using the correct combination of experience and power analysis determine group sizes
- Analysing multiple systems in single experiments
- Quantifying multiple hormones in single samples
- Using MRI (where appropriate to chart the progression of time-dependent variables in the same animals)

## Refinement

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

## Refinement

Rats are the most appropriate species for hormone/nutrient profiling and behavioural assessment because they are large enough for serial blood sampling and behavioural protocols are the most robust in this species. Rats or mice will be used where appropriate genetically-modified strains are available or can be generated *de novo* for either the generation of novel models of human diseases (eg. Prader-Willi syndrome mice) or for experimental approaches to understanding physiological/pathophysiological mechanisms (eg. ghrelin-null mice). The novel PWS rat offers the advantage that the mechanisms underlying the impairment of GH secretion can be determined in a way that cannot be achieved in mice.

Similarly, the techniques for the delivery of specific feeding patterns and the characterisation of hormone profiles are the most refined and advanced approaches available. However, since we are committed to seeking further refinements, we are currently seeking to develop a novel system for measuring hormone profiles that does not require the collection and analysis of multiple blood samples.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 273. Mechanisms of Particle Toxicity</b>
<b>Key Words</b>	Mesothelioma, Tumour Suppressor Genes, Carcinogenesis, Nanoparticles, Nanotoxicity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

During our lifetime we are exposed to nanomaterials. Due to their useful properties, nanoparticle manufacture is an area of fast industrial growth. In the past decade, both academic communities and the public worldwide have become highly concerned with the adverse effects of nanomaterials and their potential hazard to humans. Recent studies showed that nanoparticles may drive toxic effects leading to disease. The potential for human exposure, both occupational and public, and subsequent disease development is of serious concern.

It is not known how, in molecular terms, certain types of nanomaterials affect the body and how fibres of different types compare in their potential hazard.

The Objectives of this project are:

Objective 1 To obtain a detailed adverse outcome pathway for MNP-induced toxicity

Objective 2 To compare the toxicity of different types of MNP using molecular readouts with the focus on Tumour Suppressor Genes

Objective 3 To identify biomarkers of exposure to pathogenic MNP, prior to tumour development.

Objective 4 To gain new insights into therapeutic approaches targeting the Tumour Suppressor Genes and related pathways in the tissues exposed to MNP.

What 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 no explicit legislation concerning the respiratory exposure limits for nanomaterials and there are gaps in regulation of exposure for people involved in manufacturing or disposal. A better understanding of particle toxicity will help to develop safety regulations and thereby prevent the harmful effects of nanomaterials. Mechanistic data obtained through this research may also serve to improve existing therapies for patients exposed to asbestos in the past. The data determining toxicity of different types of nanofibres will identify less harmful nanomaterials and thereby promote the “safe by design” approach to manufacturers. In general, any strategy that can reduce the adverse effects of particles would have the potential to benefit people who had been exposed in their lifetime.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Our previous in vivo work has been successful and showed a similarity in the hazard posed by asbestos and certain types of nanomaterials. We now wish to take the experimental work further and fully describe, in molecular terms, the mechanism of nanofibre toxicity. We are planning to use approximately 5500 animals over 5 years of the project. Mice and rats are proposed for the studies because there is an advantage of using transgenic mouse models; and the rat model of inflammation is considered to be the gold standard for in vivo work and also has higher susceptibility than mouse to mesothelioma.

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

Our main model of fibre toxicity is based on delivery of low doses of particles into mice. The adverse effects related to the routes of delivery (typically injections into the pleural or peritoneal cavity) have a minor impact on animals. This is followed by the exposure period without any symptoms for many months, mimicking a long latency period of disease development in humans. As signs of disease start presenting themselves, animals are monitored for their health and humanely killed when disease is manifested (e.g. respiratory distress, weight loss). The severity level expected is moderate. In the tumour initiation experiments mice are injected with tumour cells; that is tolerated very well. Animals are monitored after injection and killed before tumour has greater than a minor impact on mice. The severity level expected is moderate. All the animals are killed by Schedule 1 at the end of the experiments.

## Application of the 3Rs

### Replacement

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

## Replacement

To address the mechanistic questions we will utilize *in-vitro* based approaches as well as analysis of whole tissues and systems from mice. The pleura is the main target for fibre-shaped particles and it produces a complicated response to fibres. Exposure-induced toxic effects are generated *via* participation of different cell types. Additionally, these effects differ during the time-course of disease development. *In vitro* assays can't possibly mimic such complexity. Broad and accurate evaluation of fibre toxicity requires using tissues which can accurately model toxic response. Further, for translational outcomes there is no substitute for animal experimentation. We have generated a substantial collection of primary mesothelioma cells, both human and mouse, that provide a valuable tool for replacing animals. 80% of our work is done *ex vivo* and all current mechanistic studies are conducted in cellular systems before we attempt modulation *in vivo*. Where possible we will use primary cell lines and 3D explants for evaluating potential biomarkers and modulators. However, the clinical validity of these and their relevance to human disease requires validation in animal models.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

All study designs will be based on 3Rs principles and minimum number of mice will be calculated using Power Analysis. Experiments will be designed utilising Experimental Design Assistant software that facilitates using a minimum number of animals consistent with predicted statistical significance. Through our strong collaboration with the surgeons we have access to patient mesothelioma tissues and have established a substantial collection of primary mesothelioma cell lines. We use patient-derived samples for *in vitro* studies to narrow down the research options required to be studied *in vivo*. We developed a method of mesothelioma cell isolation from the mice with fibre-induced tumours and now have numerous cell lines to utilize in mechanistic studies. This approach allows us to use earlier experimental endpoints and reduce the number of animals in intervention studies. The colonies of mice will only be continued until the consequence of experiments has been confirmed and will be kept to the minimum size, consistent with good practice on breeding genetically altered mice. In addition, mechanistic and morphological testing will be conducted in human and mouse cell lines before modulation in animals. This not only refines, reduces and replaces animal work, but ensures that animals are only used in a targeted way to verify the role of molecules shown to be significant *in vitro*.

## Refinement

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

### **Refinement**

The mouse and rat have been chosen as an animal model because of the similarities between their physiology and human physiology. Biochemically and physiologically many of the mechanisms of toxicity are very similar to those in humans. The fibre toxicity models, based on injections into the pleural and peritoneal cavities, have an advantage of more accurate dosing compared to inhalation studies, where there is a concern about what proportion of particles is reaching the pleura. The doses we use are relevant to potential human exposure. Mice have a great flexibility for targeted genetic modifications, particularly for molecular mechanistic studies. Use of genetically modified animals will accelerate experiments and facilitate in-depth mechanistic studies. We use a model-specific scoring system that helps to assess the health of experimental animals. Rats are known to be susceptible to fibre toxicity and specifically to mesothelioma development in the pleural and peritoneal cavity. The incidence of mesothelioma in rats exposed to fibres via intrapleural or intraperitoneal injection is higher than in mice and develops after ~1 year of exposure. Intratracheal instillation of fibres in rats provides a good model for studying the lung response to fibre toxicity.

We use published NC3Rs and LASA guidelines for maximum volumes of injections and blood samples. We excluded the intra-muscular delivery route from our experiments due to painful effect of these injections on animals.

To avoid/minimise single-housed animals at the end of prolonged study we design experiments in such way that 5 mice are group-housed in the beginning of the study so very few animals end up being singly housed. In an unlikely scenario of having two mice on their own, we will use mirrors to re-introduce singly-housed males.

We also introduce additional refining factors specific to the strains (e.g. for animals that have skin sensitive to infection, we optimised husbandry by introducing paper-based bedding, which is autoclaved in the cage and the cages changed frequently, as well as feeding their diet on the cage floor to prevent any trauma to the nose).

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 274. Environmental programming of phenotype during pregnancy and early life</b>
<b>Key Words</b>	Pregnancy, Development, Environment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Observations in human populations and experimental studies on animals have shown that poor environmental conditions during pregnancy and early development are related to degenerative conditions later in life that shorten lifespan, like high blood pressure and diabetes. The overall purpose of the project is to identify the mechanisms by which environmental conditions before and during pregnancy, and in early life, alter the mother and her offspring with the ultimate goal of improving pregnancy outcome and long term health. In particular, the study will investigate environmental challenges, such as low oxygen levels seen in pregnancy at high altitude or with poor placental development, inadequate or inappropriate nutrition and raised levels of stress hormones that are common features of human pregnancies with complications needing clinical monitoring. The project is designed to answer the following key questions.

1. What are the mechanisms by which environmental conditions during pregnancy and early life alter the mother and her offspring from the level of the gene to the whole living animal?
2. What are the consequences of these environmentally induced changes for the disease risk of the mother and offspring in later life?
3. Do environmental challenges before pregnancy alter the maternal responses to subsequent environmental conditions and the ensuing risk of maternal and offspring ill health?
4. Are there therapeutic interventions either during pregnancy or after birth that can prevent the detrimental outcomes for the mother and offspring of a poor environment during pregnancy and early 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)?**

The output of the programme of work will largely be in the form of published scientific data, which will be used by researchers, clinicians (doctors and veterinary surgeons), health professionals in the field (eg midwives, nurses, health visitors) and, potentially, in the long-term by government and professional agencies. More specifically, the data are likely to be used to • Design experiments by the scientific community to discover fundamental biological principles, to further optimise human health and animal welfare, to diminish the burden of disease and to reduce and refine the use of animals in experimental procedures. • Identify environmental conditions during early life with and without potential health risks for the mother and offspring. • Design new methods of recording data which improve animal welfare and reduce animal use. • Initiate epidemiological studies in human populations on the basis of the results of the experimental studies. • Advise clinicians and health professionals on parameters to monitor, treatments to give or avoid and on potential clinical trials. Overall, the results will advance understanding of the basic biological processes governing mammalian development with benefits in reducing health care costs, increasing livestock productivity and more generally in raising awareness of the early life origins of adult health and disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will be using sheep, rats and mice in the pregnant and non-pregnant state with studies of the mother and fetus during pregnancy and of newborns, juveniles and adults at different stages of development. Mice may be genetically altered to study the effect of specific genes on the response to environmental challenges. Numbers of animals likely to be used over the 5 years of the licence are 880 sheep, 800 rats and 4000 mice.

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

Mothers and their offspring will be exposed to environmental challenges before and after birth, with and without therapeutic interventions. The challenges will involve overnutrition to achieve obesity, low oxygen levels to mimic conditions at high altitude and with poor placental development, reduced food intake and altered dietary composition to reflect the diversity of human nutrition worldwide and overexposure to stress hormones as seen with transport, isolation, emotional distress or certain clinical conditions and treatments. The interventions will involve, for example, administration of nutritional supplements, drugs and anti-oxidants. The consequences of these challenges and interventions for key physiological systems will be determined in the mother and her offspring before and after birth and, in

rodents, across generations after mating. Physiological systems to be monitored include the cardiovascular, metabolic, hormone, respiratory and nervous systems together with the placenta. Measurements of the physiological variables (eg. blood pressure and flow, use of nutrients such as sugar and fat and the secretion of hormones) will be made in normal and challenged offspring and their mothers using measuring devices and indwelling catheters inserted surgically under general anaesthesia. Data collection may involve blood sampling and giving substances by different routes including under the skin, into the blood stream or via devices implanted surgically as well as confining the animals in sampling or metabolism pens for variable periods of time. Adverse effects are rare, even in surgically instrumented animals, and any discomfort associated with surgery is minimised by use of pain relief, close monitoring and appropriate veterinary care. All animals are inspected regularly. Most of animals including those pregnant and their unborn young will be killed at the end of the experiment by humane methods to collect tissues such as the heart, liver, lungs and brain for biochemical and other analyses to explain the changes observed in the living animal induced by the environmental challenges. Those animals allowed to give birth as part of the study do so uneventfully but if problems arise during delivery veterinary assistance is sought.

## Application of the 3Rs

### Replacement

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

#### Replacement

As interactions between mother, placenta and offspring involve many factors that change frequently during the course of pregnancy, environmental control of development can only be determined by using living animals, which coupled with analyses of tissues and organs after death allows a comprehensive, integrated assessment of development from the gene to the level of whole living animal.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We minimise animal use in a number of ways. First, all protocols start with the least invasive procedures on small numbers of animals and, then, progress to additional numbers or more extensive investigations only on the basis of positive results. Secondly, using advice from expert statisticians and preliminary studies, advanced statistical calculations are used to determine minimum numbers required for statistical significance for any given study. In farm species, 4-7 animals are used per treatment group, whereas, in rodents the number is 8-10 to allow for variations in

litter size and sex ratios. Thirdly, when animals cannot be studied as originally intended due for example to catheter blockage, they are not wasted as the animal will still provide tissues for further analyses. Finally, in addition to the requirements of the planned study, a wide range of tissues is collected following death to provide material for later use by the group and other researchers, thus minimising the unnecessary use of further animals for experimental purposes. Collectively, these approaches maximise the data gained from each animal while minimising animal usage in the long term by providing archival tissue for additional collaborative studies and evaluation of new analytical techniques.

## **Refinement**

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

## **Refinement**

The project uses three species (sheep, rats and mice), each of which provides a unique element while collectively allowing identification of unifying mechanisms relevant to health and disease across different species that are also likely to be relevant to human populations. Farm animals, unlike rodents, are large enough to be studied in detail in the uterus and subsequently across multiple physiological systems, whereas rats and mice with their short lifespans are good for identifying critical periods of development and intergenerational consequences of environmental change. Mice can also be manipulated genetically, allowing identification of specific gene effects and targets for therapeutic intervention. These three species also differ in litter size, placental structure, nutrition, metabolic constraints and mechanisms of pregnancy maintenance. These differences means that we can identify common and unique mechanisms by which the environment affects development across species with direct relevance to the human population and to the general welfare of each species.

Using veterinary advice, we ensure that we use best practise for all surgical, anaesthetic and experimental techniques. This reduces surgery and post-operative recovery times and leads to the collection of better data. All animals are monitored daily and particularly closely post-surgery for food and water intake, demeanour and wellbeing determined by blood parameters where possible. During the course of the last project licence we have implemented remote wireless recording of a wider range of maternal and fetal physiological parameters eg blood and other fluid pressures, blood flow, which allows continuous measurements to be made in the unrestrained

state while the ewe has free movement within a pen. Previously animals would have been confined to a metabolism cage which restricted an animal's free movement for longer periods during data collection. When the study requires blood samples on a regular basis during physiological assessment, the ewes are held for several hours in a sampling pen which allows the animal to stand, sit and turn around with access to food and water before return to their larger home pen which allows greater freedom of movement. All animals are acclimatised to the sampling procedures and experimental environment for several days before experiments begin.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 275. Cartilage Resurfacing</b>
<b>Key Words</b>	cartilage, resurfacing, OA, stifle joint, cartilage repair
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Cartilage defects are a source of pain, immobility, and reduced quality of life for patients who have acquired these defects in their joints through injury, wear, or disease. In particular, Osteoarthritis (OA) has significant impact on the health care system with prevalence rising with age. The prevalence of painful, disabling OA in the UK populations over 55 has been estimated at approximately 10% with an estimated 6 million people in the UK having painful osteoarthritis in one or both knees. Of those aged 75 years and over, 49% of women and 42% of men seek treatment for OA. Cartilage also has limited healing capacity and as a result injury of the articular surface may lead to further OA. Focal trauma causing defects in the cartilage surface is repaired with tissue (fibrocartilage), which is commonly inferior to the original cartilage. In addition, aging, obesity, and physical activity exacerbate articular cartilage defects in the knee. While manifesting symptomatically as extreme pain in the knee, these defects eventually lead to the immobility of the patient and ultimately a reduction in their quality of life.

Untreated lesions most commonly eventuate in the need for total knee replacement and there are approximately 100,000 total knee replacement procedures performed in the UK every year. This figure is indicative of the significance of developing viable and effective cartilage defect treatments. Current treatment options include microfracture surgery, autografting, and allografting; however, these options have drawbacks such as prolonged healing times, donor site morbidity, and availability and compatibility issues of available tissue.

Consequently, the project is structured to develop new resurfacing treatments to repair large cartilage defects in damaged hips and knees caused by injury, wear, or disease. The clinical need is for a reliable, patient-specific treatment for large cartilage defects (>1 cm<sup>3</sup>) that can induce repair or regeneration of stable cartilage 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)?**

Benefits: Currently, autologous chondrocyte implantation and osteochondral grafting bridge the gap between palliation of cartilage injury and resurfacing via arthroplasty. The new cartilage resurfacing treatments developed under this licence will seek to advance first generation techniques and accomplish several goals simultaneously. For example, predictable outcomes, cost-effectiveness, single-stage, less invasive procedures, relief of joint pain, faster recovery (shorter hospital stay), restoring function and protecting undamaged joint structures and creating durable repair tissue.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the five year duration of the license it is estimated that no more than 600 sheep will be used across the four 19b protocols.

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

Expected adverse events: Whilst much of the early research and development work can be carried out in a laboratory or using a computer, 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 and cartilage tissues will respond to the implant. 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 implants because pain-free joint repair is one of the key objectives of the project. The project will involve the surgical implantation of either donor cartilage plugs from a low loaded site to a recipient site (MOSAICPLASTY) or resurfacing of a large bone/cartilage defect with a material. This procedure may lead to some degree of discomfort following surgery, which will be addressed by making refinements to the surgical technique. . Any pain and discomfort will be minimised by the use of appropriate pain relief administered before, during and after surgery, as advised by the NVS. Any local or systemic reactions to the materials are very unlikely due to the known biocompatibility of the materials when implanted in bone/cartilage but if problems arise then the animal will be promptly and humanely killed. Significant surgical sepsis is unlikely but if the condition is suspected or if the implant is dislodged in such a way that it compromises the welfare of the animal, it will be promptly and humanely killed. regardless of the project requirements.. In addition any animal showing severe signs of suffering whilst on study will be humanely killed. The expected level of severity is moderate for all 19b protocols outlined above. The animals will be humanely terminated at the end-points of each study.

## Application of the 3Rs

### Replacement

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

#### Replacement

**Replacement:** The project consists of an extensive laboratory based testing regime to select the most appropriate cartilage resurfacing formulation(s) for development. While this testing will allow accurate selection of prototypes, cell culture and other non-animal types of testing cannot fully replicate the loading, physiological and anatomical conditions required by regulatory authorities to demonstrate safety and efficacy. This is because they cannot replicate the complex biological interactions at work within the body, thereby making performance testing in animals an essential part of the plan before human trials can commence. The sheep model selected for this project offers some unique advantages over other species that makes it particularly favourable for testing new cartilage resurfacing technologies in terms of anatomy, body weight, surgical technique/instrumentation, post-operative care, and the acceptability of this model to regulatory authorities.

#### (i) Anatomy:

- Body weight of adult sheep is similar to humans (approx. 70-80Kg) and their long bones have a similar surface to volume ratio.
- The histomorphological and anatomical characteristics of sheep bone are comparable to those of human.

#### (ii) Surgery:

- The mechanical characteristics of the implant and instrumentation are usually identical to those used in human patients.
- Surgical approaches and anatomical positioning of the cartilage resurfacing device are also similar between sheep and human given their similar bone dimensions.

#### (iii) Post-operative care:

- The physical activity levels of the animal can be quantified using Force Plates to determine when the operated limb reaches full weight bearing.
- **The activity/noncompliance of the animal can/may be controlled if necessary during the postoperative period by the use of a cast or Splint, which limits the peak forces exerted on the tissue surrounding the implant.**

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

**Reduction:** Prior to studies covered by this project license, extensive laboratory based testing will be used to screen the most promising technologies that will be put forward for testing in animals. However, animal studies will be necessary at some point in the development of these devices to show safety and efficacy. Consultation with a statistician at the planning stage will be used to optimise study design, minimise the number of animals required, and meet the study objectives. This will comprise setting clear study objectives, and ensuring appropriate output measures are collected and analysed using appropriate statistical methods. Historical data will be used wherever possible to determine the appropriate sample size to achieve the required study power.

### Refinement

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

### Refinement

**(i) Choice of Species:** After a thorough review of the literature, 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. It is also a requirement of regulatory authorities that cartilage resurfacing devices are tested in large animals before they are tried in human clinical trials. For this reason, we have built up an extensive amount of expertise in using sheep on previous projects for the evaluation of prototypes /devices intended to repair cartilage.

**(ii) Minimising suffering:** Animal suffering is minimised proactively using the following approaches: (a) consultation with people with expertise in either orthopaedic surgery, animal welfare or biostatistics, (b) thorough laboratory testing and refinement of the techniques and equipment through cadaver sessions before any surgeries take place, (c) small pilot studies to monitor animal behaviour to a particular surgery/implant design, (d) standard veterinary procedures performed aseptically under general anaesthesia, (e) administration of antibiotics, and pain-relief before, during and after surgery as advised by the NVS until there is no further requirement to control post-operative discomfort or infection, and (f) careful and close monitoring of animal behaviour before and after surgery.

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 276. Understanding Synaptic and Network Dysfunction in Neurodegenerative Diseases</b>
<b>Key Words</b>	Neuroscience, Alzheimer, Synapse, Network, Neurodegeneration.
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Dementia is one of the leading causes of death in the UK, and a growing problem for our society as we are living longer. Dementia is a devastating, progressive decline in mental function that is caused when the brain is damaged by diseases such as Alzheimer's disease (AD) and related neurodegenerative disorders. In the UK, we spend over £26 billion per year caring for people with dementia, and currently, we do not have any treatments that can stop the devastating progression of the underlying diseases. The symptoms of dementia result when the cells in the brain are damaged and can no longer communicate effectively. Currently, we do not fully understand these changes in the brain, which is why we do not have effective treatments. In this project, we aim to better understand the brain changes that cause Alzheimer's and related diseases in order to develop effective ways to prevent or treat these devastating 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)?**

We will use rodent models of dementias to study changes in the brain and to try and reverse damage. The short term and highly likely benefits of this project include: - The advancement of knowledge about dementias - Knowledge shared with other scientists and drug companies that they can then use for further advances - Scientific paper publications which are freely shared with everyone - Data about dementia which are freely shared on open web based systems for others to use The longer-term potential benefits include; - Development of medicines that will help people with dementia - Influences on government policy about how to help people with dementia and how to fund the best types of research.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice in this project over the 5 year programme. Many of these mice have genetic modifications to either reproduce the brain changes observed in dementias or changes in the brain called reporters that allow us to ask specific scientific questions. Most of these genetic modifications do not cause overt symptoms that affect the daily lives of the mice. Over the 5 years, we expect to use approximately 20,000 mice.

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

The vast majority of the mice on this licence will not undergo potentially harmful procedures as they will be used for breeding or post-mortem tissue collection after humane killing. Some of the genetic modifications can cause seizures, which are rare but could be severe. We also plan some moderate procedures including recovery surgeries to induce models of dementia and to observe brain changes and recovery with treatments over time. We plan some mild experiments to examine behavioural changes and treat mice with drugs that might help the dementia like symptoms and brain changes. We do not expect any common adverse effects from these procedures. Rarely, our procedures may have adverse effects such as infection after surgery or side effects from treatments. Any animals experiencing adverse effects will be examined by a veterinarian, and if the effects cannot be alleviated, the animals will be killed humanely. At the end of experiments, animals will be killed humanely. Some of our genetically modified mice may be provided to another Project Licence if appropriate.

## Application of the 3Rs

### Replacement

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

### Replacement

Animal experiments are essential to meet our goal of better understanding of brain changes in dementias and how to develop effective treatments. We are studying brain function and degeneration, which requires a system with an intact network with all of the types of cells that are needed to make a healthy brain. The diseases we study occur over many years and involve clumping of toxic proteins in the brain. Currently, there are not cell models that can make entire brain networks that develop age-related disease like our mouse models. Nor are computer models advanced enough to test the questions that we need to in order to help people with dementia. It will be impossible to develop effective dementia treatments without using experimental animals at this point, although we are continually evolving both

cell and computer models that we hope will replace even more animals in future. Mice are an ideal species as their brains share with humans the basic structures involved in memory. They are also amenable to genetic manipulation, which allowed the introduction of genes that cause human dementias into the mouse resulting in brain disease and memory impairments.

The mouse work in this programme is part of a wider effort incorporating experiments in human post-mortem brain and human stem cell derived neurons, which replace some mouse experiments. Only by taking multiple approaches will we be able to come to a better fundamental understanding of disease that will lead to effective treatments.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We have taken several measures to reduce animal numbers:

- Studying animals while they are alive with advanced techniques that let us look at the brain before and after dementia changes and before and after treatments will reduce animal numbers needed for each experiment. This is because the brain is assessed at the first time point in each animal allowing it to serve as its own baseline, lowering the numbers needed compared to needing large cohorts.
- Wherever possible, an individual animal will be used for investigating brain function when the animal is alive and also for looking at brain changes after it is killed. This practice will reduce numbers, and increase power of the data. We routinely share brain tissues from individual animals between multiple experimenters in order to maximise the data collected from a single animal.
- We will use best practice for designing experiments. This will avoid having either too few animals to answer the question (which wastes the whole group), or too many animals (which adds unnecessary mice).

## **Refinement**

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

### **Refinement**

The majority of experiments will be carried out in models of dementias. The mice exhibit many of the brain changes seen in dementia patients and so provide an appropriate model for these studies. We have chosen particular dementia models based on how well they mimic the aspects of the disease we are trying to study, and we participate in international scientific initiatives to refine our models.

We also refine the procedures we use on the mice in order to minimise harms, for example, administering the drug in jelly instead of other more stressful methods. The mice love receiving their jelly and get the entire needed daily dose in a stress-free manner.

We propose some surgical procedures, which will all be carried out using appropriate anaesthetic and analgesics. We continually interact with our veterinary team to be sure we use the most refined methods.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 277. Pharmacology of colistin in chicken</b>
<b>Key Words</b>	Antimicrobial, Colistin, Chicken/s, Pharmacology, E. coli
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Colistin is an antibiotic used in both human and veterinary medicine for the treatment of *E. coli* and other bacterial species. This old antibiotic has not been used much in human medicine due to toxicity until recently where it has become a last resort antibiotic in where it is used to treat multidrug resistant bacteria. Colistin, however, is routinely used to treat infections in the gut of poultry in veterinary practice. The discovery of colistin resistance in bacteria from animal species and, less commonly, in humans has led to concerns that there is a link between the use of colistin in animals and the spread of resistance across host species. To ensure that colistin can continue to be used in both human and animal medicine without increasing the prevalence of resistance, we need to acquire good quality scientific data to optimise dosage regimens based on measured effective concentrations. There are currently crucial knowledge gaps regarding how colistin use in chickens affects resistance in gut bacteria, and what effective dosage levels are most suitable to ensure efficacious treatment whilst also limiting the spread of resistance.

This project aims are:

1. To measure Colistin concentration within the gut to understand how the administered dose transits through the digestive system.
2. To describe how the movement of colistin through the gut impacts on bacterial flora.
3. To monitor the numbers of and species of bacteria within the gut and faeces. Including the prevalence (if any) of resistance.

4. To determine effective concentrations for colistin use that would increase its sustainability in both human and animal medicine.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The knowledge derived from this study will inform regulatory bodies, veterinary practitioners and veterinary drug companies regarding the optimal dosing of colistin to poultry. This will improve animal health and welfare, and potentially help reduce the spread of resistant bacteria. This will also have benefits for human medicine, ensuring the sustainability of colistin usage as a 'last resort' antibiotic to treat resistant infections.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will use broiler chickens sourced in the UK. Approx. 400 chickens will be used over the 4-year study period. Chickens will be used in separate study groups for each facet of the study with each group being study reared for around 40-45 days

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

The animals will be kept in conditions compliant with welfare guidelines, provided with enrichment, and trained to cope with human interaction. This will ensure that adverse effects are minimal and that severity remains mild. Dosing procedures may cause coughing or choking, although this is extremely rare, as all investigator will be trained to handle the chicken appropriately. Any animals showing considerably signs of distress or illness will be euthanized.

## Application of the 3Rs

### Replacement

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

### Replacement

Although models can be used that allow some aspects of drug dosing to be studied accurate determination of drug action relies on understanding the specifics in the target species.

Because of the administration of antibiotic through the feed, it is important to monitor how feeding affects dosage, how the antibiotic transits through the gut, what bacteria

are present and how they are effected, and how this influences gut infection which can only be accurately assessed in the target animal

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Computer simulation and modelling of previously published data coupled with laboratory based dosage experiments will reduce the number of animals required to explore multiple different doses

The pilot study will ensure that all methods are valid to reduce negative impact throughout the project.

### **Refinement**

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

### **Refinement**

Poultry farming is an important aspect of food production worldwide and with Colistin being one of the most commonly used antibiotics for their welfare it is important that chickens and studied to ensure optimal use and benefits

To minimise any harm to the animals taking part in the study, they will be trained and familiarised with the persons and procedures involved to prevent undue stress. They will be provided with enrichment materials including perches for roosting and litter for foraging behaviour. All animals will be observed on a daily basis to identify any illness so that it can be treated swiftly and appropriately.

All animals will be housed in a controlled environment where ventilation, humidity, temperature and light are controlled to ensure they are maintained in a comfortable and healthy environment.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 278. Functions of siglecs in the immune system</b>
<b>Key Words</b>	Innate immunity, macrophages, neutrophils, pathogen
<b>Expected duration of the project</b>	2 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The different cell types in the immune system have to communicate with each other if the response is to be effective in fighting off infections, without becoming dangerously over-active. Much of this communication involves molecules being presented by one cell and interacting with a “receptor” on the surface of another. By using strains of mice that have been bred to either lack individual receptors or express forms of the receptor that don’t work anymore, we can compare their responses to those of normal mice and work out the function of each receptor. Using this approach we have demonstrated that the receptors are important in controlling immune responses to infectious agents such as flu and in inflammatory responses such as asthma and septic shock. The proposed project will continue this work and will provide additional new information and knowledge that will allow us to understand how the immune system is regulated in health and disease. Not only will this research lead to better understanding of disease processes, but it is also expected to result in better treatments for these important human diseases in the future.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Regulation of inflammatory responses is crucial in human diseases such as acute lung injury, sepsis and recovery from influenza infection. Our work expected to give important insights into the signalling pathways involved which may lead to new therapeutic approaches to treating human disease. We also expect to reveal new insights into how the immune system defends itself against the influenza virus, especially during the first few hours after infection. Therefore this research could also lead to better treatments for infectious diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice will be used exclusively. We expect to use approximately 2000 mice over the 2 year period of this project. Only a small proportion of these will undergo any treatment. The majority are used in breeding programmes and humanely killed to produce the tissues such as bone marrow which are a vital source of specialised cell types for in vitro experiments.

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

Apart from breeding and maintenance, some of the mice will be treated with agents that induce mild inflammatory and immune responses. They will also be inoculated with influenza virus to study how the receptors of interest control the response to infection. In all of the studies of inflammation and flu infection, the majority of animals will only undergo short-lasting feelings of malaise and possibly mild fever. This is very similar to how we feel when we have a cold. For our scientific studies, we do not need the disease in mice to develop beyond this point. These treatments are not expected to lead to long-lasting harm or suffering of the animals. At the end of experiments or at the end of their useful breeding life, mice are humanely killed.

## Application of the 3Rs

### Replacement

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

### Replacement

The immune system is a highly complex, integrated network of cells, secreted molecules and tissues. Although individual cell types can be isolated and studied *in vitro*, in most cases it is not possible to extrapolate these *in vitro* findings to how the whole network responds to infectious agents and inflammation. Therefore, *in vivo* studies are essential if one is to obtain a complete understanding of the role of a given molecule(s) in the immune system. The mouse provides an excellent model system for understanding how the mammalian immune system works and the use of gene targeted mice has greatly increased our knowledge of the functions of specific proteins involved in host immunity. In this project, we propose to use mainly 'Knock-Out' and 'Knock-In' mice to continue our functional analysis of cell surface receptors involved in these important functions.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

We have developed cell culture methods for expanding large numbers of cells from the stem cells present in mouse bone marrow. These include bone marrow-derived macrophage cultures using CSF-1 and dendritic cell cultures using Flt-3 ligand. We are also currently refining expansion of other cell types such as T regulatory cells. These *in vitro* cultured cells mimic their natural counterparts very closely and are therefore an effective replacement for animals in biochemical studies. We will continue to exploit immortalised cell lines wherever possible to extend findings made with the *in vivo* mouse models of inflammation and infectious disease.

Animal numbers are also minimised by the use of good statistical tests. The number of mice used in this study will be calculated according to four components; 1) the nature of immune response expected in control groups of mice; 2) the anticipated effect of the loss of a particular immune cell/molecule on the immune response 3) the significance level; and 4) the error rate (acceptable false negative) that is judged to be reasonable.

## Refinement

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

## Refinement

The mouse provides an excellent model in which to study the relationship between the immune system and disease induction, since mice are well characterised immunologically, their immune systems closely resemble those of humans, and the majority of these models have been extensively studied and have pre-determined correlates of disease regulation. This allows us to reduce the number of unknown factors in any given experiment and increase the probability of obtaining interpretable and meaningful data.

In addition, multiple genetically modified mice lacking various immune molecules/cells have been generated, and can provide a very refined approach to performing detailed analyses of the role of receptors in immunological functions.

We aim to minimise welfare costs, yet at the same time maximise the output of data from animal experiments, by using sophisticated *in vitro* assays on tissues and cells in order to evaluate how the mice have responded to the various challenges used. We aim to stop experiments with inflammatory or infectious agents at the earliest possible point where scientific validity is reached, thus reducing or preventing unnecessary welfare problems. Animals that undergo challenge with infectious agents are supported, for example, with a high-energy diet, much as human 'flu patients would be.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 279. Organ Transplantation and Replacement</b>
<b>Key Words</b>	Organ transplantation, Organ replacement, Organ regeneration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project aims to advance our group's understanding of alternatives to conventional transplantation, to enable and inform further human clinical trials. Our particular objectives are to:

1. Assess in vivo safety, biocompatibility, durability, and function of organ replacements made from natural, synthetic and hybrid materials manufactured by our group;
2. Assess strategies for improving blood vessel ingrowth within these scaffolds and integration into surrounding host tissues;
3. Assess cell harvest, culture and scaffold seeding techniques to determine optimum cell type, combination and delivery systems;
4. Assess and refine optimal surgical strategies for deployment of personalised organ replacements.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Organ failure or dysfunction affects millions of people worldwide and frequently necessitates the replacement of such organs with new ones. Conventional organ transplantation is not possible for all organs, due to the poor quality of harvested donor organs. The need for new organs is also comparatively high amongst babies and children, where there is a low supply of donor organs of a suitably small size. Additionally, conventional transplantation come with a life-long need for immune system-lowering medication, which can have terrible side-effects including an

increased susceptibility to life-threatening infections. Great leaps have been made in the fields of organ regeneration and personalised therapies over the last decade. The prospect of being able to build individualised tissues and organs is a hugely attractive one and would get rid of the need for human tissue donors. However, although animal work has been pivotal in the previous refinement of our clinical programmes, many scientific and safety questions remain to be addressed before the technology can be universally accepted and widespread clinical trials can be set up. This project will allow us to build on our group's extensive in vitro and in vivo work, and preliminary clinical work, to focus on making second generation scaffolds with improved cell survival, as well as optimising which combinations of cell types, scaffolds, seeding techniques and surgical techniques are likely to be most successful clinically for maximal cell survival, tissue integration and functionality of grafts. It will also allow us to work out the best ways to ensure a new and functional blood supply connects the scaffolds to the recipient. We have collaborations with the leading clinical groups working in this area. This data will therefore form an integral part of the pre-clinical justification for full-scale clinical trials.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use the following approximate numbers of animals over the course of the 5-year licence: • Mice - 600 • Rats - 850 • Rabbit – 375. Actual numbers used in any individual year of the project will vary, with a progression from smaller animal trial of materials and/or cells to larger animal studies where organ scaffolds are tested in the correct anatomical (orthotopic) position.

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

Our animals will typically undergo a 60- to 90-minute procedure, where they have the organ of interest exposed. A portion of this is removed and replaced with a graft seeded with the animal's own cells (harvested at an earlier date) and the skin is re-sutured. Animals may have the grafts implanted many weeks before into a section of muscle, or abdominal lining (omentum), to start the process of growing a new blood supply before it is needed at the time of surgery – this procedure will also take around 60- to 90-minutes. In cases where human cells are to be tested, animals will have been given immune system-modifying medication to help the seeded cells to survive. Animals recover well with good painkillers. In the majority of surgeries, animals are often active, eating and drinking on the same day. Animals tend to remain active throughout the experiments, but their breathing can become laboured if grafts develop scarring. Animals are put under sedation regularly to check that the grafts are acceptable and they are treated using surgical instruments to stretch or remove scar tissue or secretion plugs. However, there may be times where these problems develop too suddenly for treatment under anaesthesia and animals may die as a result. Animals who have had digestive organ surgery will take longer to

feed on oral food (grafts are at risk of blockage in the early days) and so will be implanted with a feeding tube directly into the stomach to enable them to be given nutrition in the first week (they will always be allowed to drink water). These tubes will have to be kept clean and flushed regularly to avoid blockage. In addition, animals with digestive organ surgery may have to wear Elizabethan collars at the start to prevent grafts blocking with ingested fur and be kept in a barren environment without bedding, and so will be unable to groom themselves initially. Single housing may be required on a temporary basis to look after grafts under particularly close attention are for the immediate post-operative period. If grafts block despite these measures, instrumentation of the blockage will be done under general anaesthesia to see if the blockage can be cleared – if this is not possible, the animal will be killed by deepening the anaesthetic. At the end of the experiments, animals will be killed by a humane method and tissues taken for analysis after death.

## Application of the 3Rs

### Replacement

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

#### Replacement

Neither in vitro nor ex vivo systems model the in vivo milieu with sufficient complexity to evaluate complex tissue-engineered organs or the in vivo interplay of cells and scaffolds with the host's tissue and ensure such transplants can function effectively when in their desired position within the body. It is vital to ensure ongoing investigation into scaffolds and cells for continued improvement of the scientific understanding and safety profile of second and third generation implant candidates, the results of which form mandatory parts of a dossier to the MHRA to allow clinical trials.

Alternatives of testing cell-seeded scaffolds ex vivo in bioreactors will be performed prior to animal experiments to inform in vivo experimental conditions, as well as limit experimental groups and sizes to those that show sufficient in vitro and ex vivo promise.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Statistical analysis will be planned at the conception of each experiment to ensure minimum numbers of animals are used. Multifactorial randomized block designs will be employed to reduce group numbers as far as possible whilst maintaining statistical significance (at 90% power and significance of 5%). Paired grafts will be comparatively evaluated for statistical differences (e.g. using Student's paired T-test), whilst experiments with more than one group will be analysed using ANOVA analysis or similar. All animals will be included in statistical analysis to minimise attrition bias in survival data (analysed via Kaplan Meier survival curves). To account for 5% morbidity requiring early termination from causes related to immunosuppression/immunodeficiency (such as increased susceptibility to wound infections), this number might need to be increased in experiments where Immunosuppression/immunodeficiency is used.

To reduce bias, animals will be ordered in batches from the same suppliers according to a specified weight and sex. Pairing of experimental and control grafts in each animal is an example of how we plan to use multifactorial designs to minimise bias due to individual variability. Animals will be randomised to surgery arms at the start. Prior to surgery, grafts will be randomised to cell seeded/non-seeded groups and prepared for surgery by a separate researcher, such that surgeons do not know the seeded status of a given graft. Where possible following surgery, animals will be reassigned identifiers to blind surgeons during postoperative endoscopic follow-up and post-mortem analysis. Video footage will be taken during endoscopic procedures to enable repeat assessment by a second blinded surgeon, and to enable the same animal to receive longitudinal follow-up at multiple time points. Each animal will be analysed in as many ways as possible without increasing animal suffering to reduce the numbers needed for experiments (e.g. physiological monitoring, imaging in vivo, analysis of organs).

## **Refinement**

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

## **Refinement**

Mice and rats are appropriate species for the screening of combinations of scaffold materials, cells and growth factors, as they are economical and are the lowest animals on the evolutionary scale suitable for the modelling of some human conditions. We will use rodents for early phase experiments, whilst rabbits are the

appropriate species for organ replacement modelling in babies and young children as they are an excellent size and geometric match and have low individual variation in organ size. Longer follow-up is achievable in rabbits than in rat or mouse surgery, as commonly-available veterinary equipment can be used to reduce unnecessary mortality from graft scarring.

Animals will receive regular monitoring several times a day, particularly in the first month after surgery, and will be monitored continuously if/when problems arise. Painkillers will be given routinely following any painful procedure, and local anaesthetic will also be given generously to aid comfort in the immediate recovery period. Animals undergoing digestive organ surgery will be given supportive care with their nutrition via a tube directly into their stomach (implanted under the skin at the time of surgery) – our previous experiments have found this to be well-tolerated.

Even with frequent observations and supportive care, the risk of sudden severe breathing difficulties in some animals with respiratory grafts, or the potential risk of them being found dead in the morning despite appearing completely well at their evening checks, cannot be completely removed. The unpredictability of this secretion plugging is why we feel a 'severe' rated severity limit is justified on this protocol, in order to prevent premature termination of animals who are well at the time of assessment, or who may be improved following treatment. End points for animals who develop clinical signs of respiratory problems will be at a default of 36 hours following the first discovery of symptoms in animals who continued to decline, as we have seen that by 48 hours this moderate respiratory effort would usually either resolve, stabilise or worsen to the point of requiring early termination. Assessment of these animals under anaesthesia will help to further tailor endpoint timings to individual animals (i.e. less than 36 hours).

## NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 280. Cell signalling in the pancreas</b>
<b>Key Words</b>	Acute pancreatitis, chronic pancreatitis, pancreas, calcium signalling
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our general working hypothesis is that manipulation(s) of cell signalling signallingto reduce cellular stress and calcium toxicitycan be beneficial in preventing or treating pancreatitis and help to inhibit pancreatic cancer development.

Objectives of the project:

- 1) To further study calcium signalling in pancreatic cells during the development of acute pancreatitis.
- 2) To characterise the functions of poorly studied pancreatic cells (stellate and neurons) in pancreatitis and pancreatic cancer.
- 3) To study the effects of inhibitors and regulators of calcium signalling in pancreatic stellate and acinar cells on the development of acute pancreatitis
- 4) To study the potential protection against acute pancreatitis provided by adding nutrients, energy supplements and reducing fatty acid intake.
- 5) To studychronic pancreatitis in mice and progression of the disease to pancreatic 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)?**

These findings promise new opportunities for the development of preventive and therapeutic measures for both alcohol- and Asparaginase-induced acute pancreatitis. We have found ways in vitro to boost the protection system by applying

inhibitors and regulators of calcium signalling, adding nutrients and energy supplements, changing diet. Testing our findings using mouse models of acute pancreatitis are extremely important for developing new drug prototypes and relevant therapies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Breeding and maintenance of genetically modified animals – mice: 4000 per protocol  
Isolated cells, pancreatic tissue and whole diet/drink protocols – rat, mice: adult rats 100; adult mice 1200

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

This work involves inducing acute and chronic pancreatitis in mice and rats in order to mimic the human disease. We will also give the animals therapeutic treatments in an attempt to rescue the disease. Animals will mostly experience mild severity and in some cases moderate severity. Animals will be killed by a humane method at the end of experiments.

## Application of the 3Rs

### Replacement

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

### Replacement

Our research is essentially based on experimental work on rodents. Unfortunately, there is no substitute for animal experiments. Sharing cell preparations between experimenters allows us to reduce the number of animals to the absolute minimum. Pancreatic acinar cell culture and cell lines can only be used with severe limitations, because the structure and properties of freshly isolated pancreatic acinar cells are not retained in culture.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

As it is not feasible to produce an adequate model of pancreatitis *in vitro*, we are going to use already established models of experimental acute pancreatitis in living animal *in vivo*. Though most of acute and chronic pancreatitis studies are done initially *in vitro*, the research of initial and other stages of disease requires experiments using mouse models. The use of freshly isolated pancreatic acinar cells

provides a large amount of *in vitro* work from a very small number of donor animals, and the work using living animals will be carefully designed with the help of pilot studies to minimise the number of animals necessary to achieve the data we need. The design of individual experiments will involve a collective approach, which maximize the information obtained from the minimum resource. For most of our quantitative experiments, sample sizes will be set using power analysis, i.e., the significance level will be set at 5%, and the power 80%.

### **Refinement**

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

### **Refinement**

It is well known that acute and chronic pancreatitis are accompanied by severe pain, but that the initial stages are not painful, according to human studies. Therefore, during *in vivo* work mice should not experience significant pain or enduring discomfort. Pilot studies and protocols, therefore, will be limited to mild or moderate severity with appropriate analgesia used in collaboration with NVS and NACWO. Mice were chosen species for experiments work because a) appropriate knockout and mutated models are available in mice, and b) standard models of induced pancreatitis have been successfully developed in mice. The dosage and timing of any stimulus will be refined to minimise suffering and to ensure that the earliest events are captured. Some animals will receive pre-treatments prior to experimental intervention to investigate ways of protection against disease. To reduce the amount number of injections, pilot studies will be applied to determine a chemical cross-reaction and solubility of different substances. In all cases, pain-reducing measures will be implemented wherever possible in collaboration with the NVS. Animals will be killed by a Schedule 1 method whenever analgesia fails to control pain adequately.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 281. Molecular mechanisms of tolerance and immunosuppression</b>
<b>Key Words</b>	Immunity, Inflammation, Infection, Cancer
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The normal function of the immune system is required to fight infections by bacteria and viruses while its disordered function contributes to numerous disease processes including autoimmunity, allergy, chronic infection and cancer. The immune system is composed of diverse cell types that can either promote or inhibit immune activation. While inhibitory components of the immune system are required to suppress autoimmune and allergic inflammation in a process referred to as immunological tolerance, they can also suppress potentially beneficial responses against infections and cancer, in a process known as immunosuppression. Importantly, newly developed therapies targeting mechanisms of immunosuppression have shown promise in activating immune function in patients with cancer, but since the mechanisms of immunosuppression being targeted also contribute to immunological tolerance, a proportion of individuals treated develop inflammation which causes side effects and limits therapy. Our research aims to discover new mechanisms underlying immunological tolerance and immunosuppression, and define those mechanisms that have distinct rather than shared roles in these two processes. Discovery of such distinct mechanisms may enable more specific therapies to be developed, allowing, for example, disruption of immunosuppression without induction of inflammatory disease 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)?**

Therapies that work using the immune system have brought about significant health benefits on a global scale. For example, therapies targeting immunosuppressive mechanisms within the tumour are presently revolutionising the treatment of certain chronic diseases such as cancer. Our research will extend our fundamental

knowledge of how the function of the immune system is controlled not only to prevent otherwise deleterious autoimmune and allergic inflammation but also to limit effective immunity against chronic infections and cancer. The research will also provide a basis for development of new therapies aimed at controlling immune function in patients with a variety of disorders in which the immune system plays a critical role, including cancer, inflammatory diseases and infections. Our research will also be of benefit to researchers in related academic fields studying inflammatory diseases, infectious diseases, ways in which cells control their gene expression and tumour biology. Aside from its relevance to academic researchers, the work is relevant to researchers aiming to make new therapies for individuals with immune-mediated disorders, cancer and chronic infection, including pre-clinical researchers and the pharmaceutical industry, with whom we have established collaborations.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

About 50680 mice are expected to be used over a five year period. The immune system in mice is similar enough to the immune system in humans that valuable parallels can be drawn. The availability of different genetically modified mice and reagents that recognise mouse cells means that this species can be used more efficiently than any other species to ask questions about the role of particular genes in the immune system. The breeding of the mice will be planned and monitored carefully to ensure that we only produce the mice needed for experiments. The majority of the mice will be used to provide immune organs harvested for lab-based assays. Other mice will be immunised, infected or will be challenged with tumour cells, and the results immune response monitored. In each case, the lowest number of mice that produce robust reproducible (statistically significant) results 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 levels of severity? What will happen to the animals at the end?**

The majority of mice bred under this license will have genetic alterations that allow the function of molecules within the immune system to be tested. Animals are housed under tightly controlled conditions where their exposure to infections is limited. In many cases, animals with genetic alterations will not have any abnormalities and will be killed humanely and their tissues used for experiments in the laboratory. Where it is necessary to gain new knowledge about how the immune system works within living organisms, some mice will be subjected to immunological stimuli. For example, we will test the immune response of animals to tumours by implanting small numbers of tumour cells under the skin of animals and letting them grow. Tumour growth will be monitored regularly by trained staff and animals will not be allowed to suffer excessively with euthanasia of animals triggered by well-defined criteria. Euthanasia is often carried out by administering carbon dioxide gas to animals which causes them to become unconscious and die after five minutes of exposure. After this, a secondary method of killing, such as dislocation of the neck is

used to ensure that animals are dead. After euthanasia, tumours will be analysed in the laboratory to gain insights into how tumour immune responses are suppressed. In general, these experiments take around three weeks from tumour injection to euthanasia. Because some of the ways in which immunity to tumours is suppressed are similar to the ways by which the body prevents inflammation, we also need to use models of autoimmune and allergic inflammation. For example, animals will be administered a substance that causes gut inflammation in the drinking water. Animals will be routinely assessed for signs of illness and weight loss and animals likely to experience excessive suffering or weight loss will be euthanised prior to onset of such disease. Such experiments usually take two weeks from the administration of the colitis-causing substance to euthanasia. In each case, the exposures given to animals will be adjusted to elicit measurable and informative responses but to minimise pain, suffering, distress or lasting harm. Humane endpoints have been carefully considered to prevent excessive suffering, are clearly documented and available to trained staff who regularly monitor experimental animals. As a result, no animals are expected to die as a result of experimental procedures. Experimental protocols are continually refined to incorporate new knowledge and technologies that reduce harm.

## Application of the 3Rs

### Replacement

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

#### Replacement

Animals are necessary to understanding how the immune system works because the various interactions of immune cells with other cells and substances in the living animals cannot yet be generated in test tubes. Features such as the distribution of immune organs throughout the body and the ability of immune cells to migrate into almost all tissues of the body make investigations of the immune system in the whole animal context essential. Adaptive immunity (the type of immunity which remembers previous exposures), which is the subject of this research, evolved in vertebrate animals and is not present in less sentient organisms. Among vertebrates, many cellular and molecular features are highly conserved between mouse and man. Many useful tools and well-established experimental models for experiments in mice already exist, enabling us to perform research using mice in an efficient manner that minimises the number of animals we need to use. Therefore use of mice in this research is necessary.

However, where possible, before using animals in experiments, we use cell culture experiments using immune cells in petri dishes, to determine whether certain molecular pathways are likely to have an important role in controlling the immune system. These experiments replace the need to use animals at the early stage of

discovery. However, it is often necessary to validate findings made in the petri dish using experiments in mice.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We have implemented changes to reduce the number of animals we need to do this research. This includes improvements to the efficiency of generation of genetically modified mice as well as breeding and mouse colony management. We carefully calculate the size of the groups of mice used in experiments so that enough numbers are used, but not too many, to make statistically sound conclusions from our experiments. Finally, by generating and publishing so-called 'high-content' data, or data that contains a lot of information from single samples, we reduce the need to do repeated experiments to make single different measurements frequently.

We will seek to further improve experimental design with careful consideration applied to make sure we use the least number of animals required to test scientific questions.

### **Refinement**

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

#### **Refinement**

All experiments we will do are classified as causing only either mild or moderate suffering to animals. We have defined clear clinical endpoints that will trigger the end of an experiment for a particular mouse or a cohort and as a result, death of animals is not an endpoint in any experimental system used. However, careful monitoring of experimental animals by trained staff anticipates deterioration in health and animals such that experiments are ended before animals are subjected to suffering in excess of that defined by either mild or moderate severity limits.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 282. Murine models of cancer progression and therapy</b>
<b>Key Words</b>	Cancer, Melanoma, Precision Medicine, Metastasis, Therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our aim over the next five years is to continue to develop both our basic and translational research programmes in order to improve our understanding of cancer biology and implement precision medicine to improve outcomes for melanoma and other cancer patients.

Our specific aims are:

To understand the relative contributions of ultraviolet radiation, genetics, pigment and inflammation to the development and course of melanoma

To improve knowledge of, and develop new treatments for uveal (eye) melanoma, a form of melanoma with particularly poor prognosis upon metastasis, and for which notreatments are available.

To implement precision medicine for melanoma and other cancers, tailored to patients whose tumours carry particular genetic changes.

To improve knowledge of, and develop new treatments for different types of metastatic disease, such as metastatic melanoma, breast, pancreatic, and prostate cancers.

To evaluate and understand the biological mechanisms of new cancer drugs and assess their efficacy in faithful cancer 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)?**

This project will contribute to the understanding of tumour progression and metastasis mechanisms, and potentially to novel therapeutic approaches for cancer care; the mouse models characterised in this project will provide more powerful methods to identify the underlying mechanisms of tumour progression and spread, and to introduce drugs targeted to individual cancer patients. In addition, by assessing standard-of-care and new treatment approaches we aim to support therapeutic decisions in the clinic, based on the results from our studies. Finally, we expect to publish our work in peer reviewed journals thus sharing our findings with the scientific community.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice; 17,600; 5 years

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

The vast majority of mice in the project will carry some form of tumour – mostly superficial tumours that develop or are implanted in the skin, but in some studies internal tumours developing or implanted in such organs as the eye, breast, prostate and pancreas, or in organs where metastatic tumour cells spread to naturally. In our melanoma studies, animals will be exposed to short doses of UV radiation, and across our studies, animals will be treated with therapeutics (typically orally, or by injection) appropriate to the cancer types under study. Where possible, non-invasive imaging will be used to maximise our understanding of tumour progression and spread and to accurately monitor tumour growth. In approximately 75-80% of cases, mice would be expected to experience a “Moderate” or lower level of discomfort, as the tumours they carry would not make a significant impact on their general health and wellbeing, and the majority of other procedures (UV exposure, non-invasive imaging, injection of therapeutics), will generally result in no more than transient discomfort and no lasting harm. However it is sometimes difficult to predict the growth and behaviour of internal tumours and of metastatic spread, and thus in some models the tumours may have a more significant impact on the animal’s well-being. All the mice will be killed by humane methods at the end point of the experiments.

## Application of the 3Rs

### Replacement

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

### Replacement

The development of effective cancer therapeutics is an important goal of modern biomedical sciences. To identify potential cancer therapeutic targets, the processes

involved in tumorigenesis must be understood at all levels, which requires the development of model systems accurately mimicking tumour progression.

Cancer development is dependent not only on the changes occurring within the cancerous cells, but also on the interactions of the cells with their environment and surrounding cells. The majority of our current understanding of carcinogenesis comes from the laboratory analysis of late-stage tumour tissue removed from cancer patients. In our lab, we perform a collection of laboratory assays to understand important points of tumour biology. While this has revealed many changes experienced by cancer cells, it provides little information about the factors influencing early-stage cancer development in their tissue environment.

Also certain hallmarks of cancer, such as metastatic spread and blood supply changes, are impossible to study in the laboratory. Therefore, mouse models are important for studying the physiological aspects of human cancer development. Transgenic mouse models have been engineered to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. This cannot be replaced by laboratory studies or different live models such as zebrafish or insects.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Our use of laboratory-based methods limits the number of animals required for the live models investigation stage.

For our transgenic (genetically modified) models we will use standardised experimental approaches, so that control groups can be used across experiments.

We are also reducing the number of transgenic animals bred for our studies by propagating tumour cells obtained from these models and re-implanting them into unmodified mice.

By standardising our experimental methods, we are often able to compare data from new experiments to data from historical experiments, rather than set up new similar experimental groups to those used in previous experiments.

The proposed experimental designs and methods of analysis of the results are always in agreement with statistical guidelines and advice from our bioinformatics team to provide meaningful data from a minimum number of animals used per experiment. The design of individual experiments will generally involve factorial designs, which maximise the information obtained from the minimum resource.

## **Refinement**

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

### **Refinement**

We strive to review and improve husbandry and procedures, which minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable.

We ensure we provide the appropriate anaesthetic and analgesic regimes as well as appropriate humane methods of culling within the animal facility. We ensure procedures are undertaken out of view from other mice. Transport of live mice between facilities will be in appropriate containers.

By propagating tumour cells obtained from transgenic models and re-implanting them into unmodified mice, we are more easily able to control, and minimise tumour burden.

Introduction of viruses carrying the genetic material necessary to induce tumour growth also allows us to minimise transgenic mouse breeding and better control tumour development.

Our imaging capabilities are continually improving, allowing us to use imaging not only to acquire valuable scientific data, but better monitor tumour growth and spread.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 283. INTESTINAL INTRALUMINAL INJECTION (ILI) OF TEST AGENTS</b>
<b>Key Words</b>	Oral protein and peptide delivery
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We are examining the potential for rationally designed molecules to alter the permeability properties of the small intestine or to utilize pathways across intestinal cells that are used by certain pathogens to improve the uptake of drugs that currently must be administered by injection.

**What 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 these studies would be the identification of a method to manage diabetics and patients with other chronic diseases using oral dosage forms for drugs that currently must be given by subcutaneous injection. The information obtained from these studies may also provide an improved understanding of how the small intestine functions in health and disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats and mice will be used. We plan to use ~300 animals to determine the potential of several novel agents for their ability to modulate the uptake of biopharmaceuticals across the mucosa of the small intestine. If toxicity of a modulating agent is observed, we will use up to 50 additional animals to find non-toxic doses of these modulating agents for subsequent testing

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

All of the studies to be performed will maintain animals under anaesthesia throughout the study. While it is possible that some of the treatments might result in

hypoglycaemic events or some kind of unexpected toxicity, it is unlikely these unconscious animals will experience any pain, suffering or distress. At the termination of each study, the animal will be euthanized without regaining consciousness

## Application of the 3Rs

### Replacement

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

#### Replacement

We carry out extensive testing of compounds *in vitro* to select ideal candidates for therapeutic use but only mammals provide the complexity of the small intestinal barrier organized with portal and systemic circulations.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

All of the modulating agents and strategies to improve transport of biopharmaceuticals across intestinal epithelia that will be tested *in vivo* will have first gone through extensive *in vitro* studies that screens out those that are not effective or are cytotoxic. We have refined our methods so that we can extract valuable statistically robust information using only 2 or 3 animals for each experiment.

### Refinement

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

#### Refinement

Rats and mice are the smallest animal with similar anatomy and physiology to man that can be used to place catheters in the portal and jugular veins. In this way, we are able to monitor blood levels of a biopharmaceutical before and after passage through the liver once it has been absorbed across the intestinal mucosa. All studies

will be performed with animals under anaesthesia. At the termination of each study, the animal will be euthanized without regaining consciousness.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 284. Neurophysiological study of nociceptive transmission and pharmacological modulation</b>
<b>Key Words</b>	pain, analgesia, pain control mechanisms
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

To investigate the mechanisms by which the peripheral nerves and then the central nervous system (CNS) becomes altered following the induction of a number of pain states. To aid the development of novel analgesics and better use of existing agents. Using mostly in vivo electrophysiology, which is a procedure where we can measure the activity of nerve cells to different pain stimuli in fully anaesthetized animals, and behavioural measures we will study the consequences of how chemicals, nerve networks, drugs and genes play a role in pain transmission. We wish to understand the mechanisms mediating the pain associated with tissue damage, cancer, peripheral nerve injury and osteoarthritis. These are the main types of pain in patients and many people have insufficient pain control with the existing painkillers.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The aim, that has been achieved in particular areas in our previous work is to further improve the understanding and hence treatment of pain in veterinary practice and in patients. Here we wish to work towards personalized pain medicine by understanding how different types of pain respond to different drugs. We are interested in how pain changes our brain functions and how pathways from the brain to the spinal cord can modulate pain. These powerful systems can switch pain up or down and we aim to understand how they can be harnessed in patients. Two of our major discoveries are used in patients to assess their pain mechanisms and this is leading to using pain characteristics to predict effective treatments. We are also

working on novel targets that could lead to new pain drugs but also whether drugs, already used for other medical conditions, could be used for pain. We work and interact with many pain health care professionals to align our work with clinical issues that need solving. We make a major contribution to pain education since we are invited to speak on our work to audiences that include nurses, GPs, anaesthetists and pain specialists in the domains of palliative care, arthritis and pain from nerve injury.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

About 300 rats and mice per year in total for the 5-6 researchers in the group with each study lasting about 2-3 weeks. The licence will run 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 levels of severity? What will happen to the animals at the end?**

The endpoints in the large majority of studies are done in fully anaesthetised rats and mice and so there is no suffering (non-recovery). In these studies, we need to study pain models but there is a rapid recovery of the animals after surgery with minimal adverse events since we have worked very hard to optimise and refine our techniques (moderate). They gain weight and interact normally. We use minimal stimuli to assess pain (mild), searching only for the pain threshold and using very short time courses of no more than 3 weeks. At the end of the studies, typically euthanasia by overdose of general anaesthesia will be used.

## Application of the 3Rs

### Replacement

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

### Replacement

Our work involves studying the role of pain pathways in an integrated system from the site of pain in the body to the brain so cannot be replicated in a dish or modelled by computers.

We would use mainly rats as there is extensive knowledge regarding anatomy and physiology. Their larger structure permits more ready access to peripheral nerves to enable surgical manipulation and easier access to spinal cord neurones during *in vivo* electrophysiology. The widespread use of this species (and the fact we use models that are used world-wide) allows comparison with published studies.

Mice represent an ideal species that allows for genetic modification of identified targets, studies that are not possible in other mammals.

A number of our published electrophysiological studies in the rodent have been shown to be excellent predictors of analgesic effectiveness in man and have also guided us towards the understanding of human hereditary pain disorders.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The number of animals to be used in the studies is based on our extensive experience of *in vivo* physiological and pharmacological studies related to pain and ensures that the experiments are powered sufficiently to achieve the purposes of the experiment. The ability, in this species to produce extensive dose-response relationships of drug effects on up to 10 different responses within a single animal markedly minimises the overall number of animals. The study design allows obtain behavioural, neurophysiological and *ex vivo* immunohistochemical data from the same animal, thus minimising use of animals, and some cases the opposite non-injured side of the animal can be used as an internal control, thus reducing the need for separate control animals.

The neuronal activity is an objective unbiased measure but in the small number of behavioural pharmacological studies that we will carry out, we have always used blinding (eg drug or vehicle) and to condition and also randomisation and will continue to do so. We confirm that the design of the experiments conform to the NC3Rs ARRIVE guidelines so allowing for reproducibility and to minimise confounders and maximise experimental rigour.

## **Refinement**

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

### **Refinement**

Several altered pain processing systems we have described are widely used in clinical practice.

All models used have been refined and mimic human pain states. Where study design allows, we can compare behavioural, neurophysiological (using the same stimuli that are used to assess patients) and ex vivo immunohistochemical data from the same animal, again minimising use of animals, and some cases the opposite non-injured side of the animal can be used as an internal control, thus reducing the need for separate control animals. Minimal stimuli are used in sentient animals. Improved surgical techniques will aid the healing process and hence reduce duration of post-operative pain. Cage enrichments and soft diet will provide a comfortable environment for recovery.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 285. Genes regulating tumourigenesis and the response to cancer treatment.</b>
<b>Key Words</b>	Cancer, Genes, Therapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Despite the advances in cancer research and the treatment of cancer a number of critical questions still remain largely unanswered: (i) which genes drive the development of cancer, (ii) what genes determine whether a cancer patient responds well to therapy and (iii) how do we use genetic information to improve the way patients with cancer are treated? For example, DNA sequencing studies have shown that mutations in genes such as *ARID1A* are found in patients with breast, ovarian and oesophageal cancers; at present the reasons for why these mutations cause cancer are largely unknown. Perhaps more importantly, we do not know how to effectively treat cancer patients who have *ARID1A* mutations. Likewise mutations in the *BRCA1* and *BRCA2* genes are known to cause cancer but also cause sensitivity to drugs such as PARP inhibitors. Despite PARP inhibitor sensitivity, some patients with *BRCA1* or *BRCA2* gene mutations become resistant to their treatment. The work outlined in this application is aimed using mice to model human cancer that is caused by genes such as *ARID1A*, *BRCA1*, *BRCA2*, using these mice to identify better ways of treating cancer patients and understanding why some patients become resistant to treatment and others do not. Our work will focus on cancers that arise in the breast, ovary, bone, peritoneal cavity or oesophagus.

**What 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 aims are to design better ways of treating cancer patients, so that they live longer and so that their quality of life is improved. To do this, we need to identify better ways of targeting cancers without also having a harmful effect on normal cells. The work that we propose in this application is designed to identify these more effective treatment approaches and thus will have obvious benefit to patients (reducing tumour burden or prolonged life). Alongside this clear advance, our work is also aimed at increasing our understanding of how genes control cancer. This can be useful in deciding best line of treatment or understanding what causes resistance to treatment in cancers of patients who initially respond to treatment.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will only use mice in this project. Our estimates are that the work will involve the use of 14,900 mice over the next 5 years. In terms of reducing the number of animals, we will design each experiment so that we use only the number of animals that mathematically allows us to discriminate the response between, for example, drug treated and non-drug treated groups of mice.

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

The adverse effects we expect to occur are similar to those seen in human patients who develop and are treated for cancer. Most of the animals will develop tumours/cancer growth mimicking what happens in humans (moderate severity) which will then be treated to observe for any effects of drug treatment (moderate severity). For example, animals will undergo pain from surgery, may experience weight loss from treatment or redness in the skin caused by the effects of local, low dose radiotherapy. Any individual animal will experience no more than moderate severity. Harm classed as moderate will be as a result of surgery, tumour implantation, repeated treatment as would happen in cancer, handling of animals, measuring tumour growth as well anaesthesia where necessary for numbness and restraint. All the animals will be killed by a method recognised as suitable for the species and size by the ASPA(1986).

## Application of the 3Rs

### Replacement

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

### Replacement

The generation of accurate preclinical models is essential for drug discovery and target identification in oncology. These models need to recapitulate the properties of three-dimensional heterogeneous tumours and cannot be adequately recapitulated *in vitro*. Similarly, the effects of drugs need to be tested *in vivo* so that the effects of the tumour microenvironment, drug access and target specificity can be assessed.

Nevertheless, we are continually developing *in vitro* cell culture and three-dimensional tissue assays and comparing them to the *in vivo* models in an effort to establish animal replacements.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Use of non-invasive imaging where appropriate to minimise cohort size, so that we do not have to generate large cohorts of animals to be culled at different time points during the course of the experiment.

Ensure that the maximum amount of information is obtained and analysed per experiment to reduce the need for repeats. Power calculations will be used to estimate the number of mice required to identify a statistically significant effect. For example, by assessing the variance of tumour formation from a tumour cell cells xenografted into mice, it is possible to estimate the number of mice required to identify a particular tumour reduction effect of a certain level of statistical significance.

## **Refinement**

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

### **Refinement**

Mice are widely used for preclinical models for cancer research. The generation of genetically altered strains that are immunodeficient makes it possible to model the growth of human tumours as xenografts, which have been shown to be effective predictors of clinical response to drugs. The animals are maintained in ventilated cages using sterile food and bedding and all procedures are carried out in laminar flow cabinets to avoid infections.

Animal suffering will be minimised by keeping tumour burdens within acceptable limits. Therapeutic drugs will have been assessed for toxicity and therefore we expect high tolerability of the regimes.

No protocol is of substantial severity.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 286. Understanding lung injury, inflammation, airway remodelling and pulmonary fibrosis</b>
<b>Key Words</b>	lung injury, inflammation, fibrosis, TGFβeta
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Lung injury, inflammation, airway remodelling and fibrosis are complex processes that occur when wound repair goes wrong. generation of the pro-scarring molecule Transforming Growth Factor  $\beta$  (TGF $\beta$ ) is a key event in their development. This project will investigate how TGF $\beta$  is generation in lung cells, identify the pathways and molecules controlling this process and determine how lack of regulation of TGF $\beta$  in the lung leads to the development of these chronic fibrotic lung 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)?**

These studies will increase our understanding of how lung injury, inflammation, airway remodelling and fibrosis occur. They will increase our knowledge of fundamental wound repair principles in the lung, and will ultimately lead to the development of desperately needed therapies to treat fibrotic lung diseases that are currently incurable and are amongst the most severe conditions a patient can develop.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice and rats will be used and we would estimate that approximately up to 6,600 mice, and 300 rats, will be used over the 5 year period.

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

Each of the animal models requires induced lung injury resulting in development of lung fibrosis and/or airway remodelling. Previous experience suggests that all of

these animals will experience weight loss and increased breathing rates and that they may also have a hunched appearance with their hair standing up on end. In aging studies, genetically modified mice may spontaneously develop lung diseases including emphysema or fibrosis, resulting in the development of symptoms similar to those exhibited by animals following lung injury. The overall level of discomfort for both the lung injury and aging studies is expected to be moderate and progress will be carefully monitored to ensure the well being of all animals during the course of these studies. All animals will be humanely killed at the end of the studies.

## Application of the 3Rs

### Replacement

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

#### Replacement

Where possible we have replaced screening or “blue sky” experiments in animals with experiments performed in test tubes, or using techniques that involve use of tissue samples eg lung slices. However, the processes which control the scarring of the lungs seen in patients with fibrosis are complex and appear to involve interactions between cells of the lung with the cells which circulate in the blood. As it not possible to model all of these interactions in the test tube or tissue samples due to the lack of blood supply, some studies will require live animal experiments.

These experiments will only be performed when there is initial evidence that these studies will lead to meaningful data that may change the way we approach patients who suffer from lung injury and fibrosis.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

To reduce the number of animals used in experiments we use inbred strains of animals to reduce any genetic variability; perform appropriate power calculations to avoid excessive sample sizes; measure endpoints which are reliable and have the lowest variability; use non-invasive measures of injury and fibrosis, such as CT, CT/SPECT or CT/PET, where possible; and measure as many different endpoints as possible from a single animal.

## **Refinement**

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

## **Refinement**

Mice will be used for the majority of these studies as they represent the lowest vertebrate species that develops lung injury, lung inflammation and pulmonary fibrosis in response to various challenges. Rats offer some advantages over mice in terms of size and development of fibrosis in response to certain stimuli and will also be used in some studies. We have refined our laboratory procedures to minimise variability in the measurable outcomes and this has allowed us to utilise the smallest effective dose of injurious agent or therapy. As a result in many of our studies we have been able to shorten the duration of experiments, reduce the severity of the symptoms and minimise the suffering that might be experienced by the animals whilst still obtaining meaningful data. Within the scope of these studies we will further refine our methods including developing further novel non-invasive imaging strategies to measure real-time changes in fibrosis and inflammation in a single animal both during disease progression and in response to therapy.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 287. Contribution of inflammation to tumour initiation</b>
<b>Key Words</b>	inflammation, pre-neoplastic cell, cancer, live imaging
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No

(d) protection of the natural environment in the interests of the health or welfare of man or animals;

No

(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No

(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No

(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The project is aimed to gain knowledge on how the tumour starts to growth in our body, with a focus on understanding how cells with a potential to become tumour interact with the host immune system in their local environment in the whole organism. This is a much under studied area but knowledge gained would help us to develop early detection methods for cancer and cancer prevention therapies. We aim to 1. determine how gene expression changes in immune cells makes them promote cancer development; 2. identify key genes that are important for tumour initiation; 3. manipulating immune cell function to prevent cancer from developing 4. finally we want to identify lead compounds that could be developed into cancer prevention 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)?**

This project would enhance our understanding of the mechanisms that are important for regulating tumour initiation in vivo and with the potential to provide novel targets and/or drugs that could be developed into cancer prevention therapies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use no more than 15700 zebrafish including wild type, transgenic and zebrafish carrying mutations, over the period of five years. 10,000 of which are only for breeding and maintaining purposes. 5000 will be for generating novel transgenic zebrafish that are required for carrying out this project. We will however, try to minimise those actually used. We also request no more than 500 transgenic zebrafish

for adult experiments over 5 years. Another 200 juvenile zebrafish will be used for determine drug dosage.

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

There are no obvious adverse effects expect for breeding and maintaining and generating GM zebrafish unless they carry transgene or mutant that compromise their health, such as genes cause weakened immune system, in which case fish will be monitored frequently to ensure their normal behaviour and health status. These are deemed as mild procedures. All of these fish will be humanely euthanised once they pass their egg production peak or when they no longer required for the project, which is like to be before 18 months of age. Chemical treatment of adult fish is a moderate procedure, as chemical might have unexpected effects on zebrafish physiology. However, all chemicals will be tested in larvae before we will use a dose tolerant protocol to test the effects in adult fish. Therefore we expect very minimum adverse effect, which we will monitor the behaviour change of experimental fish very frequently ( at least twice a day) and all animals show sign of ill health during treatment will be humanely euthanised in order to obtain tissue sample for further analysis. At the end of the treatment protocol, all animal will be euthanised and kept for further analysis.

## Application of the 3Rs

### Replacement

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

### Replacement

The development of cancer is a complex process and host derived factors and cells are known to be important for tumour initiation. Therefore in vivo experiments using the intact animal are crucial for studying tumour initiation. In most of our experiments we opt to use zebrafish larvae under 5 days old before self-feeding, which is not protected. However, we need to keep sufficient numbers of adult fish for generating under 5dpf larvae. Ultimately, we need to use small numbers of adult zebrafish from our tumour model to test efficacy of the treatment that we developed using larval models. We propose that these experiments are of moderate severity, and will utilise non-invasive in vivo imaging to minimise suffering to the animals and maximise physiological relevance.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will utilise statistical methods to determine the smallest adequate sample sizes that are able to detect the effects of genetic manipulation.

We will work with zebrafish facility staff to improve husbandry conditions and egg production efficiency so as to reduce the number of breeding stocks that we need to hold.

### **Refinement**

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

### **Refinement**

We use a less sentient vertebrate, zebrafish, to minimize the potential for suffering. Furthermore, we will use imaging techniques that do not harm the animals. Finally, statistical methods will be employed to ensure maximal information is gleaned from each experiment, and to increase experimental reproducibility.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 288. Somatic evolution and carcinogenesis</b>
<b>Key Words</b>	skin, cancer, mutation, oesophagus
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Evidence from sequencing normal human tissues argues that by middle age, they carry a large number of cells with DNA changes (mutations) linked to cancer. It is from these that cancer emerges in later life. The mutations are selected by Darwinian evolution over decades and an equivalent evolutionary selection is observed in mice. This project will identify interventions such as drugs that could alter mutational evolution to deplete tissues of cancer linked mutations in mouse models, which can then be investigated in human studies.

**What 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 advance our knowledge of the origins of cancer and the knowledge gained may, in the long-term, lead to the discovery of new strategies for cancer prevention. The work should further provide valuable information on how cancer associated genes alter the function of cells in aging tissues. This work will also advance fundamental scientific knowledge of how evolutionary selection operates in tissues. 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. The transgenic animals developed will be valuable to other scientists interested in studies of cancer and aging.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will require no more than 21400 of normal and genetically altered mice over a period of 5 years. We envisage 70% of mice will be used for breeding the required transgenic strains or as controls not receiving active treatments.

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

The project includes protocols to investigate the impact of certain mutations linked to cancer in cell behaviour, tumour development and progression. To do this we will use exposure to ultraviolet or ionizing irradiation or administration of chemicals and drugs via, for example, drinking water or injections to normal or genetically modified animals in order to label cells, modify cell behaviour or cause cancer. These treatments are generally not associated with adverse effects at the time, but if they do and animal exhibit signs of distress such as weight loss, starry coat, hunched posture, they will be humanely killed. Animals will be closely monitored for appearance of tumours that may affect animal welfare. If this occurs and animals exhibit signs of distress such as weight loss, piloerection, hunched posture, they will be humanely killed. Some mouse strains carrying particular mutations may develop diseases other than cancer and experience moderate clinical signs such as scaly skin or eye ulceration. These strains will be closely monitored and if these signs appear and deteriorate animals will be humanely killed.

## Application of the 3Rs

### Replacement

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

#### Replacement

This project studies how cells with altered genes spread through tissues over many months and may cause cancer. The environment of the tissue in a living animal is critical in determining which altered genes emerge during evolutionary competition. Some aspects of this process can and will be simulated in cell cultures whenever possible. However, critical aspects such as the presence of an immune system and the long-term nature of the experiments involved cannot be replicated in culture. The research therefore requires the use of animals, and in particular, mice with genetic alterations are key to research in this area.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The experimental plan uses advanced DNA sequencing software so that analysis of each animal generates a large body of data. This means fewer animals are needed per experiment when compared to older designs of cancer experiments.

By labelling cells using fluorescent markers fewer mice can be used compared to conventional approaches as hundreds of clones may be analysed per animal.

Any new animal models we create will be archived in international repositories and made available to other researchers around the world. This will help reduce the number of animals used to make these models by other scientists.

Similarly, experiments will be conducted to enable publication of results in open access scientific journals and in accordance with the NC3Rs' ARRIVE guidelines. Sequencing or genotyping data will be archived at EMBL-EBI's European Nucleotide Archive (ENA) which is openly accessible to any researcher around the world.

Additional reduction will be achieved by implementing appropriate breeding strategies, adequate experimental design (by using the NC3Rs experimental design Assistant and a power calculator at [www.biomath.info/power](http://www.biomath.info/power)), and the use of ex vivo systems whenever possible.

### **Refinement**

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

### **Refinement**

We are using mice as these are the only species in which the required genetic tools are available.

We will comply with best practice guidelines, e.g., the Home Office Minimum Standards for Aseptic Surgery, the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery and the Guidelines for the Welfare and Use of Animals in Cancer Research (British Journal of Cancer (2010) 102, 1555-77).

All methods proposed in this project use refined techniques that minimise animal stress and pre-serve welfare. For example, oral treatments will replace injections whenever possible. Use of a refined bone marrow transplantation method by injection that does not require whole body irradiation and eliminates the side effects typically associated with this procedure.

The husbandry, care and welfare of the mice are provided by a dedicated team of animal technologists. Animals at risk of disease because they are likely to develop tumours or carry genetic alterations that may cause other diseases will be frequently monitored for signs of distress.

Following injection of gene inducing agents some animals may lose weight, and will receive increased supportive care including a special diet until they recover.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 289. Models of breast cancer heterogeneity and biomarkers</b>
<b>Key Words</b>	Breast Cancer, Metastasis, Diagnosis, Treatment
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Breast cancer is the most commonly diagnosed cancer in the UK; 12,000 women die each year due to this disease. Breast cancers are broadly classified into distinct types by expression of combinations of the oestrogen receptor (ER), growth factor receptors and other biomarkers such as basal cytokeratins. Recent data have suggested that there may in fact be as many as 10 different types of breast cancer that may have different biological “drivers”. As is the case with other cancers, breast cancer is not curable if cancer cells spread to other parts of the body and multiply to form new tumours at these sites (development of metastases). A type of breast cancer with high frequency of metastatic development is triple negative breast cancer (TNBC). TNBC is defined as negative for the receptors ER, progesterone receptor and HER2. As TNBC is defined by the lack, rather than expression of these receptors, there is currently no specific targeted therapy for this type of breast cancer. TNBC is very varied and there are currently thought to be 6 types of TNBC. TNBC is a very aggressive disease, leading to metastatic spread very quickly after diagnosis of a primary breast tumour.

We aim to identify the biological mechanisms that cause and drive these different TNBCs, which is ~15% of all diagnosed breast cancers, leading to the identification of novel drug targets, clinical factors and biomarkers for the treatment and prognosis of TNBC.

We are studying these types of breast cancer using state of the art laboratory techniques (molecular, genetic, pathological) in combination with the proposed experiments in this project.

The aims of the project licence application are to establish and expand clinically relevant models of human breast cancer and to establish these models in the context of high-risk breast cancers. These models will allow us to discover new treatments for both primary breast cancer and metastasis.

**What 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 aims to identify new targets for therapeutic intervention in a very aggressive form of breast cancer that will translate into new treatments or combinations of treatments for human tumours. We also are testing whether our laboratory “organoid” model systems are equivalent of animal tumour models and this may benefit animals by reducing the number used in pre-clinical testing that could be undertaken in the laboratory.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will only use mice in this project. Over the course of the 5-year project we aim to use up to 10,000 female immunodeficient mice that will bear tumours of different types. We will also use up to 1500 female mice from transgenic models that produce spontaneous mammary tumours. This number is subject to variation following results from our pilot studies. However we design each experiment so that we use only the number of animals that mathematically allows us to discriminate the response between e.g. drug and non-drug treated groups of mice.

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

We will establish and study models of breast cancer and assess the effect of various treatments, with the overarching aim to impact patient treatment. Animals are not expected to develop adverse effects in this project. Mice will be housed in cages with sterile bedding, food and water. Trained competent personnel with experience of using mice in cancer research and who are familiar with the effects of anti-cancer drugs on rodents will perform all procedures. Protocols that require animals to bear tumours (Protocol 3-8) are of a moderate severity as tumour implantation is done through either surgical implantation, surgical removal of primary tumour or injection

into specific organs. Throughout procedures, strict aseptic technique and analgesia will be used to ensure minimal adverse effects are found. Tumour growth will be monitored very closely and all animals will be humanely killed by Schedule 1 methods when tumours reach a certain size.

## Application of the 3Rs

### Replacement

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

#### Replacement

At present it is necessary to perform animal studies as an intermediate step in the translation of laboratory findings to the human clinical setting. Laboratory-based models cannot, as yet, replicate the complex interactions between tumour cells and tumour stromal cells, including immune cells and the blood vessel network. In particular when examining the response of tumours to therapy, the utilization of an animal model provides the advantage of generating many tumours of the same or different type that may be analysed simultaneously to examine molecular and genetic profiles correlating with clinical response. Our group is contributing to the development of 3D “organoid” laboratory models made from patient material that may help to replace some animal experiments in the future.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Pilot studies will be conducted prior to every protocol in order to assess the suitability of the scientific procedure and to reduce to the minimum the number of animals likely to suffer distress or pain. It is important to stress that mouse models will not be used for random compound screening and all experiments will be hypothesis-led on the basis of extensive laboratory studies, established clinical practice or currently on-going clinical trials.

Power calculations will be used to determine group size. The number of animals will be kept to the minimum needed for a statistically valid result.

We will use non-invasive imaging where appropriate to minimise cohort size, so that we do not have to generate large cohorts of animals to be culled at different time points during the course of the experiment.

### **Refinement**

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

### **Refinement**

Initially it is our intent to use NSG (NOD.*Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup>/SzJ*) female mice in our studies. The use of these specific species is justified by its ability to accept transplantation of foreign tissue due to its depressed immune system. However, other immunodeficient strains may be used if found to be more appropriate.

Every invasive procedure will be performed under general anaesthesia with analgesia given as appropriate to ensure minimal distress is caused to the animal.

The growth of tumours and development of metastasis will be closely monitored overtime, using imaging methods where possible to keep tumour burden to a sub-clinical endpoint, and it will be made sure the animal only incurs the minimum possible discomfort and pain.

The animals are maintained in ventilated cages using sterile food and bedding and all procedures are carried out in laminar flow cabinets to avoid infections.

Animal suffering will be minimised by keeping tumour burdens within acceptable size limits. Therapeutic drugs will have been previously assessed for toxicity and therefore we expect high tolerability of the treatment regimes.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 290. Mechanisms of Cardiovascular Remodelling</b>
<b>Key Words</b>	Cardiovascular disease, Lesion Formation, Aortic Stenosis, Angiogenesis, Risk Factors
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Diseases involving remodelling of the heart and blood vessels (such as heart attack and stroke) are the biggest single cause of death in developed (and increasingly in developing) countries. The purpose of this project is to improve our understanding of the processes that regulate remodelling of the heart and blood vessels, to identify new therapeutic targets, and test promising new 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)?**

This project will clarify the processes that block arteries, preventing blood reaching sensitive organs (e.g. heart, brain, limbs). It will also investigate control of new blood vessel formation, which is important for supplying oxygen and nutrients, during tissue growth and repair. Identification of key signalling factors, will highlight new targets for medical. These factors will be modified using existing and novel medicines, and new methods of administration, to assess their potential for treatment of clinically-important conditions (heart attack, stroke, heart failure, gangrene, cancers, respiratory failure).

**What types and approximate numbers of animals do you expect to use and over what period of time?**

A 5 year programme will predominantly use mice, as relevant models of the conditions under investigation that can be investigated using genetic modification and the latest imaging techniques. Rats are more appropriate for some investigations provide and, in limited cases, rabbits may provide improved representation of disease/ response to treatment in humans. Specific investigations

will use zebrafish, which provide relevant and accessible models of inflammation and angiogenesis. The predicted numbers are: Mice: 13,750, Rats: 3250.

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

Regulation of cardiovascular remodelling will be assessed in established rodent and fish models of injury and repair; making use of genetic modification to identify mechanisms contributing to remodelling. High fat diets and/ or surgical intervention will induce changes in cardiovascular structure and function. Surgical procedures involve directly damaging an artery, removing/ blocking an artery to reduce oxygen supply to tissues (including the heart), or implantation of devices/ compounds to stimulate growth of new blood vessels. Adverse effects will vary for different protocols. Dietary manipulation is expected to produce few overt effects (eg. weight gain, mild metabolic dysregulation). Surgical manipulations may be associated with temporary pain following surgery, mild weight loss, and transient irritation at the site of skin incisions. There is a small possibility of infection but this is reduced by aseptic technique. Surgical induction of lesions can cause temporary lameness but is otherwise well tolerated and not associated with death or disability. Similar effects (lameness, impaired exercise tolerance) can occur with hindlimb ischaemia (femoral artery removal). Gangrene and autoamputation reported in some strains will be avoided by analysing the response to lower levels of ischemia in pilot studies. Pulmonary arterial hypertension is well tolerated in rodents. Coronary artery ligation and aortic stenosis are associated with peri-operative and post-operative (occasionally sudden) death in up to 20% of animals but this approach is necessary for investigating causes and treatment of heart attack. The impact of these techniques will be managed by good surgical technique and post-operative 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 suffering. Such procedures will only be used for translational proof-of-concept experiments.

## Application of the 3Rs

### Replacement

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

### Replacement

Cardiovascular remodelling in humans is a slow (decades long), symptomless process. This makes it difficult to detect onset of disease, and to predict (often life-threatening) cardiovascular events, and to determine the role and significance of initiating factors.

Animals provide essential models of components of the remodelling process, with changes developing over much shorter timescales. They also provide tissue samples at planned intervals during disease progression. Mice will be the main species used, primarily because this enables investigation of pathways of interest using genetic modification. Transgenic manipulation will be used to refine experiments (e.g. by deleting a gene of interest) or by producing animals which more closely mimic aspects of human disease.

Replacement will be achieved by the use of *ex vivo* and *in vitro* techniques, often using immortalised cells lines. In many cases, these experiments use human cells and tissue (e.g. arterial ring) culture instead of animals. Furthermore, collaboration with colleagues in physics has advanced mathematical modelling of blood flow shear stresses, to improve understanding of the influence of haemodynamics on lesion formation and vascular network development. Generation of complex, scaffold-free bioartificial blood vessels *ex vivo*, using cultured human cells, will help clarify the mechanisms that regulate arterial formation, stabilisation and lesion development.

The translational potential of this work is strengthened by comparing results with data obtained from cellular, molecular and functional analyses of tissues samples (and cultured cells) from patients and healthy controls. In addition, many of the imaging techniques (eg. magnetic resonance, ultrasound) used for *in vivo* analysis are directly comparable to modalities used in patients.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

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 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.

## **Refinement**

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

## Refinement

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. Surgery is 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.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 291. The welfare implications of low atmospheric pressure stunning in pigs</b>
<b>Key Words</b>	Pig, Welfare, Slaughter, Stunning, Decompression
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Approximately 167,000 pigs are slaughtered for food every week in the UK. Meeting this demand requires a humane method that can cope with high throughput. The law requires that animals are stunned (rendered unconscious) before slaughter. Pigs are commonly stunned using carbon dioxide gas as it is reliable, allows pigs to be killed in groups and enables high-throughput. However, this practice gives rise to important welfare concerns because it induces breathlessness and above certain concentrations, pain. Achieving unconsciousness with lack of oxygen (hypoxia) is more humane, but achieving this by exposure to other specific gases has been explored and is technically problematic and too expensive for commercial use. Low Atmospheric Pressure Stunning (LAPS) is a possible alternative, whereby animals lose consciousness and die by being placed in a chamber where gradually reducing air pressure reduces oxygen availability. This is called hypobaric hypoxia and is equivalent to rapidly ascending to high altitude, which is reported as not unpleasant or painful to humans experiencing similar rates of decompression. The aim of this project is to systematically evaluate, for the first time, the potential of hypobaric hypoxia (low atmospheric pressure stunning (LAPS)) as a humane method of commercial stunning for pigs. LAPS has already been validated as a humane method to kill chickens and therefore there is already robust equipment commercially available.

**What 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 an urgent need to find alternative humane methods of slaughter for pigs and LAPS is the most promising option. This project will generate detailed data on the behavioural, physiological and pathological responses of pigs to LAPS, including

negative effects on welfare. We will also conduct a parallel detailed evaluation of CO2 stunning to determine whether LAPS is more humane than current methods. The results of the research will directly inform policy makers in the UK government; if LAPS is found to be a welfare friendly approach to stunning for pigs then an application will be made to the EU commission to add it to the permitted approaches in Regulation (EC) no. 1099/2009 On the Protection of Animals at the Time of Killing. If successful, commercial application of LAPS has the potential to improve the welfare of 8.6 million pigs in the UK each year, and many millions more globally

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use 300 pigs 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 levels of severity? What will happen to the animals at the end?**

The project has moderate severity because (1) we will implant pigs with electrodes to measure their brain activity (essential to accurately assess time to loss of consciousness) and (2) we will expose pigs to carbon dioxide gas which is known to be extremely unpleasant (despite being routinely used commercially). Given that the purpose of the work is to assess the welfare impact of non-recovery stunning methods, the animals will not survive the procedures. To understand the welfare impact of LAPS, pigs will be exposed to LAPS and carbon dioxide and their behavioural and physiological responses compared. We will use measurements of brain activity (EEG) to determine when the pigs become unconscious, so that we can determine what welfare harms occur up to that point. Some pigs will just be placed in the LAPS chamber for the same amount of time as a LAPS cycle then removed, after which they will be culled by one of the humane methods used for research animals. This is to determine the effect of being placed in a novel chamber. We will train pigs to indicate that they want to leave a situation, and then measure these responses during LAPS or exposure to carbon dioxide.

## Application of the 3Rs

### Replacement

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

### Replacement

There are no alternatives to the use of pigs for this work because intact animals are required for the study of the specific welfare effects of each method of killing on this species. We have fully considered alternatives but the nature of animal welfare assessment is that many body systems contribute to the animal's conscious experience which cannot be adequately reproduced by other methods.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We have carefully calculated the minimum meaningful numbers of animals for each experiment, based on previous studies. We will employ a factorial design 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 where possible, the same pigs will be used for behavioural, physiological, pathological and/or meat quality assessments, which will further strengthen the data we gather in terms of commercial relevance.

### **Refinement**

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

### **Refinement**

Our use of pigs is essential as we are investigating a welfare issue specific to this species. Measurement of brain signals requires the surgical placement of very small electrodes through the skull. We will refine this by using anaesthetic techniques that have proven efficacy and suitability in pigs and we will provide post-operative pain relief. After surgery, the pigs must be individually housed to prevent damage to the electrodes, but we will minimise the duration of individual housing and will ensure pigs can see, hear and smell neighbouring pigs which reduces stress. For surgical work, we will use smaller pigs as they have thinner skull bones and this will make the surgery less traumatic. We will train the pigs so that they are accustomed to handling and all testing and stunning equipment before each trial. In trials where we need to train pigs to complete an action when they are in the chambers, pigs will be selected according to their competence in the task (e.g. pushing a button with their snout and receiving a food reward) and this will occur at their home farm. Emergency methods to euthanase pigs will be in place in case of unexpected events. As the work proceeds we may find that LAPS is not a suitable method for stunning pigs because of unexpected and/or unavoidable welfare costs or from technical or application difficulties such as failure to achieve a timely death similar to commercial standards. As such, we have built into our project key decision points, where we will carefully consider our findings to determine if the work should continue.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 292. Mitochondrial Dysfunction and Therapy in Atherosclerosis</b>
<b>Key Words</b>	Atherosclerosis, Vascular disease
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The project's objective is to better understand the implications of how and why blood vessels become blocked with fatty and cell material called atherosclerotic plaque and then find a treatment.

We aim to understand how the cells of the plaque respond to accelerated vessel ageing by removing genes required for DNA repair, essential for plaque cell health.

The project aims to develop the best possible model of atherosclerosis in which only the cells involved in the disease are modified and this is performed in the most elegant way possible through adding a low dose drug to the diet to remove genes predicted to be important in the disease.

If successful we propose a test a treatment in which other useful drugs are delivered directly to the plaque to improve the life span of the plaque cells. The hope is that by stabilising the plaque cap cells we can prevent plaque rupture heart attacks and strokes

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Atherosclerosis is a human disease that accounts for 31% of all deaths, around 17.5 million per annum. The majority of these are caused by heart attacks and strokes caused by arteries blocking with fatty material called plaque. When the fatty plaque ruptures they also cause a blood clot that deprives the heart or brain of oxygen which then dies. Existing strategies rely on life style modification such as diet and exercise which can't always be maintained or relied upon. Surgical intervention to

bypass the blocked arteries or stenting them open with a metal cage in a process called angioplasty, has significant costs and limitations and complications from the procedure itself. The plaque structure ruptures because a thin layer of cells called the “cap” ages more quickly than the rest of the vessel, most likely because it has to continually repair itself and this is finite biological process called cell division. Cell division is inhibited by the accumulated toxic plaque components including dietary fats and chemicals called free radicals that damage the DNA. The DNA is essential not only for replication as it provides the blue print of all cells components, but is required for generating the chemical energy for life. This project proposes to better understand the molecular processes of how the cell copes with damage and then extend the life of the plaque cap cells by switching the way their cells generate their energy for cell division. We have tested drugs in vitro that suggest it may be possible to reprogram the way cells generate energy and then target these drug in ultra low doses to just the faulty plaque cells. This would be a significant refinement in current drug and drug delivery technologies and have global impact to cardiovascular patients with atherosclerosis. This project provides the opportunity to test healthier, less dangerous, less toxic treatments, with fewer side effects that could offer patients longer and healthier lives.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice: 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 levels of severity? What will happen to the animals at the end?**

Specifically, mice will be bred and at 6 weeks of age the mice will be weaned and a tissue biopsy made to identify the wildtype from transgenic animals. This involves the smallest piece of tissue possible (~ 2-4 mm<sup>2</sup>) being removed from the ear under full anaesthesia which heals rapidly and offers mild discomfort. Mice are then weaned onto a high fat “western diet” that causes the arteries to develop fatty plaques also called atherosclerosis. The diet contains a low dose of drug (tamoxifen) that will remove activity of a gene important in generating energy for the cells of the arteries. This step will only affect the arteries and leaves all other body cells unaffected. Pilot data already shows this is well tolerated and a significant improvement over using injections and offers mild discomfort. At the end of the study the mice will be humanely culled and their blood and blood vessels collected. All mice in these groups will develop atherosclerosis and the effect of removing the important gene will be compared between control and experimental groups. Some of the mice will have their blood pressure, blood glucose and blood lipid levels measured on up to 3 occasions, usually just before the Western diet is started for baseline measurements, half way through the study at 7 weeks and again at the study end at 14 weeks (20 weeks of age). Some of the mice may have genetic alterations that affect the way their food is metabolised for energy. Therefore an insulin and/or glucose tolerance

(GTT/ITT) test may be made to determine changes in this important pathway that can affect atherosclerosis. In a similar way to the way the test is performed in humans at risk of diabetes the mice will be given a single dose of glucose or insulin. The smallest possible volume of blood samples (often a single small drop ~15ul) to achieve a test result will be taken at regular intervals and used to check how long the glucose can stay in their bloodstream before being removed from the blood by insulin these procedures are also mild. Drugs already approved for use in humans can often be found to treat other conditions. These medicines used for humans improves the lifespan of heart cells. If these drugs could be targeted to atherosclerotic plaques they may also improve the survival of the cells that cover plaques. Thicker stronger plaque cap cells are less likely to rupture and thereby prevent heart attacks and strokes in humans, responsible for 1 in 3 of all human deaths. We propose to test this idea in which these drugs are encapsulated inside small vesicles. These are delivered in to the blood stream and designed to preferentially bind the plaque and have minimal effects on other body cells. While this procedure is expected to be mild as the carrier is a naturally derived lipid substance and the drug is already used in humans. However, as this approach is new it has been categorised as moderate due to the potential of an unknown effects such as the accumulation of the carrier or drug in the liver or immune cells that may cause a very low chance of anaphylactic like response. To address this a staged and stepped escalation processes will be used to protect the very first recipients. If successful, patients of the future could have a targeted drug therapy that would avoid hospitals and lead to longer healthier lives.

## Application of the 3Rs

### Replacement

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

#### Replacement

Atherosclerosis is specifically a human specific disease that can not be modelled in non mammalian systems. As such, the ApoE transgenic mouse provides the best cost to benefit ratio and most realistic model to obtain the best quality data to date.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We have conducted pilot studies that predict the minimum number of animals required to find a statistical difference.

These data inform on the minimum numbers required for these studies without wasting resources or underpowering the study.

### **Refinement**

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

### **Refinement**

Transgenic mouse models of atherosclerosis are the simplest mammalian system that permits these molecular manipulations and yet retain the degree of complexity required to translate to human models of disease. The protocols have been refined where possible to use diet rather than the previous standard of using intravenous injections to induce the genetic rearrangement in the floxed mice. This is a significant refinement and reduction in undue suffering.

We can now confirm the use of this approach as we have successfully been able to prove removal of our gene from the vessel of treated animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 293. Brain mechanisms underlying cognition and emotion</b>
<b>Key Words</b>	
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The overarching aim of this project is to identify the neural, genetic and neurochemical circuitry in the brain that underlies the cognitive and emotional impairments that are important symptoms of psychiatric disorders such as depression, anxiety and schizophrenia. This includes identifying how genes and physiological stressors impact upon the development and subsequent functioning of this circuitry, how this affects cognitive and emotional processes, and how current therapies (ie antidepressant drugs) interact with this circuitry to treat these symptoms. These are important questions because over 40% of patients suffering from neuropsychiatric disorders are not helped by current therapies for reasons that are unknown, and when the therapies are effective, we don't understand why and thus can't predict which patients will do well on which treatments. This severely limits treatment options and treatment development. It is recognised that this is because we have very little understanding of the different brain mechanisms that can cause these symptoms, and until we understand how the neural, genetic and neurochemical circuitry within the brain contributes to the normal and symptomatic cognitive and emotional processing we will not be able to improve treatment strategies for the sufferers of psychiatric 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)?**

This project will provide a basic understanding of how some frontal brain areas contribute to a variety of cognitive and emotional behavioural impairments (for example, compulsivity, anxiety and loss of sensitivity to rewards) that are common in patients with neuropsychiatric and neurodegenerative disorders. It will provide an understanding of how damage to different brain mechanisms contributes to the different cognitive and emotional processes that cause these impairments, such as problems in switching attention away from negative stimuli or problems in predicting when negative events may occur. By identifying the underlying psychological, neural, genetic and neurochemical causes this will not only help stratify patients but also

improve their chances of getting personalised therapy. For example, if you are anxious because you find it difficult to switch attention away from negative things due to dysfunction in one region of prefrontal cortex, this will require different treatment than if you are anxious because you can't predict when negative things will happen due to dysfunction in a different part of the prefrontal cortex. It is this basic knowledge that is currently lacking. Thus, understanding the different brain circuits that mediate different aspects of such psychiatric symptoms, and combining it with information about how particular therapies interact with such circuits will help us to identify particular symptoms and eventually target existing therapies more effectively.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 340 marmosets 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 levels of severity? What will happen to the animals at the end?**

All animals are housed in either stable male/female pairs or live in their family groups in cages that exceed the UK and EU guidelines and contain an extensive array of environmental enrichment aids. Animals may occasionally be housed without a partner in the event of an argument with their cage mate or if their cage mate is humanely euthanased upon study completion (e.g., in cases where timing of brain assessment is critical). A new partner will be provided at the earliest possible opportunity, depending, e.g., on availability of opposite-sex partners, experimental status, etc. A typical study lasts between 18 months to 2 years. During that time marmosets are likely to receive behavioural testing 5 days a week on a range of cognitive and emotional tests that either last 15 minutes or 40 minutes. The rest of the time they are in their home cage with their partner or family. Over that 18 month to 2 year period they are likely to have between 3-5 general anaesthetics, 2-3 involving a surgical procedure such as brain surgery and implantation of a measuring device, the remaining for restraint purposes only in order to e.g. perform brain scans. Normally, the animals recover well from their surgery or general anaesthesia and are back in their home cage within 2 hours of coming round from the anaesthetic. With all surgical procedures, animals will be fully recovered from one surgical procedure before undergoing another, with a minimum of 2-3 weeks between procedures. At the end of a study the animals are euthanased. In such a study an animal will undergo behavioural testing either in the home cage or in a specialised apparatus. The latter is a purpose built test box including a computer and touchscreen. It allows animals to be presented with positive stimuli (e.g. food rewards and visual and auditory stimuli predictive of food rewards) and mildly negative stimuli (e.g. mildly aversive loud noise (0.3-0.7sec) or darkness and visual and auditory stimuli predictive of these negative stimuli) to study learning, attention and emotion. Animals learn to voluntarily enter a transport box for transfer to the testing apparatus, to which they have been gradually acclimatised to minimise stress. Testing away from

the home cage is limited to 40 min, typically once, but very occasionally twice a day, and is halted if the animal exhibits signs of distress. No adverse effects are associated with behavioural testing, and even when mildly aversive stimuli, such as brief loud noises, are used, animals enter the transport box for testing. Animals undergoing restricted access to water during more intellectually demanding experiments utilising a liquid reward receive 2 hours of unrestricted water 5 days a week in addition to rewards received during testing. Water restriction does not affect the weight of the animals, who often ignore the water when it is returned to their cage, suggesting that they are not very thirsty. To study the brain mechanisms underlying behaviour and cognition, selective surgical procedures may be carried out under anaesthesia. Animals are gently caught from their home cage by an experienced handler and carried to the surgical suite. Premedication with a sedative is achieved via an injection into the muscle which causes only mild, momentary discomfort. A gas anaesthetic is used thereafter to ensure no pain is experienced during the surgical procedure (typically lasting 3-6 hours depending upon the procedure). Through small holes made in the skull, we can infuse substances that permanently or temporarily alter brain function in a discrete region or insert an implant that allows the later injection of substances to the implanted region. The latter is fixed in place using screws attached to the skull and dental adhesives. We may also temporarily implant devices to measure local brain function. Animals are monitored closely throughout the procedure and during recovery, and are usually fully recovered and back in their home cage eating, drinking and behaving normally within 2-3 hours. Long-lasting pain relief is given prior to surgery via an injection under the skin, and for several days after as an oral treatment delivered in marshmallow to minimise the need to catch them. Extra care is taken during the first week after surgery to observe any changes in normal behaviour or appearance. Long term implant sites are cleaned regularly throughout the life of the animal to prevent infection. Surgical procedures (lasting 90-120 mins) are also performed in some animals to implant a small radio transmitter into the abdomen to record physiological measures of emotion (heart rate and blood pressure) in animals that move freely during behavioural testing. Brain imaging (typically lasting 90 mins) may be carried out using anaesthesia to keep the animal still so as to ensure good quality images. Animals receiving certain brain scans may have an intravenous access device implanted under the skin to allow the injection of a radioactive substance without the stress of injecting directly into a vein. Following these surgeries animals typically return to the home cage within two hours.

## Application of the 3Rs

### Replacement

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

## **Replacement**

The aim of the proposed work is to investigate how neural circuits in the brain control cognition and emotion. To do this, functional brain circuits are required. Furthermore to be able to determine the contribution of a particular brain region or circuit to the expression of a certain behaviour it is essential to be able to alter its function. As such interventional experiments cannot be done in humans for ethical reasons, and cell cultures are unable to contribute to a functional, behaving circuit, animal models are indispensable for this work.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

We are very aware of the need to minimise the number of animals that we use in a year while optimising the validity of our scientific results. For this reason, and to keep numbers to approximately 70 per year, we screen all our animals for their suitability for studying particular behaviours and for their genetic background to optimise which animals go into which study. We also use brain scanning to ensure the precise targeting of the location within the brain which are of interest, and plan to investigate the use of imaging as a way of measuring brain structure, connectivity, chemistry, and function, allowing individual animals to act as their own control rather than requiring both control and experimental animals. All new surgical techniques are piloted in rodents first where possible, and any new techniques are tested first in one or two animals to ensure the experiment is optimised. We repair surgical implants, when possible and when there is no risk to the animal, rather than implanting additional experimental animals. We regularly consult with local statisticians to ensure that we are using the optimal group size for the results that we see, to ensure that we use the minimal number of animals while optimising the mathematical power of our analyses.

## **Refinement**

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

## **Refinement**

Primates are used specifically because their brains, in particular, those brain regions most important in regulating our cognitive abilities and emotions, such as the prefrontal cortex, are far more similar in structure and function to that of humans than lower species, e.g. rodents. To illustrate this, the cerebral cortex, that region of the brain with the most sophisticated processing abilities, makes up 80% of the brain

mass in humans and 60-70% in primates, compared to just 26% of the brain mass in rodents. Marmosets are a particularly valuable species to use for the proposed work as their relatively small primate brain makes it possible to target cortical and subcortical structures and to make regionally selective neurochemical interventions with relative ease, with little risk to the animal. Often, the same approaches cannot be used in larger primates, such as the macaque, because the surgical procedure involves too many brain entries, which increases the risk of collateral problems such as damage to major internal blood vessels. Having a breeding colony in the same establishment as the experimental program affords us considerable experimental control over the entire lifetime of the marmoset. This is an important factor, particularly when studying negative emotion and its regulation, since it is known that stress and early life experiences can have an enormous impact on the cognitive and emotional regulatory processes under study. The on site breeding colony means that animals do not have to experience the stress of transport to the laboratory and allows us to separate some of the environmental and genetic influences on behaviour. We constantly review all of our behavioural and surgical techniques to ensure that we refine procedures in order to minimize potential animal suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 294. The role of the immune system in tissue damage</b>
<b>Key Words</b>	
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We want to understand how and why we develop an abnormal immune response to organs like the skin and kidney. Understanding this abnormal response is important because it causes damage. Our experiments aim to identify how to stop the damage in a safe manner.

The immune system comprises a large network of cells and molecules that can detect and eliminate harmful substances e.g. toxins or disease-causing microorganisms. Induction of an immune response in this setting is appropriate and necessary to maintain health. However, if the immune response is uncontrolled or induced inappropriately (i.e. in the absence of any harmful substances) it can cause damage to organs like the skin and kidney through a process termed inflammation. Inappropriate immune responses are seen in many human illnesses including cancer and kidney diseases. The reason for our animal experiments is to work out the fundamental mechanisms driving abnormal immune responses. By doing this we will be able to identify where to develop drugs to prevent unwanted damage and to develop drugs to promote repair. There is a need to do this since although some of our available treatments are very effective in certain conditions, the side-effects are significant. Only by a detailed understanding of pathways that cause injury will we be able to develop more selective treatments that will work more effectively and have little or no side-effects.

In this application we aim to generate and analyse genetically modified mice in which we have removed one or more immune genes. Studying how their immune response to an insult differs from wild-type mice is a powerful way of revealing the function of these genes in the development of organ damage

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Our research will provide valuable insights into the mechanisms leading to the development of human diseases whose underlying cause are abnormalities in the

immune system (e.g. cancer, autoimmunity and renal disease). Our results will advance the knowledge that clinical researchers and biomedical companies use to develop new treatments for these conditions

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We estimate that we will use approximately 33000 adult mice, including breeding, during 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 levels of severity? What will happen to the animals at the end?**

None of the experiments planned under this project involve procedures that are expected to cause the mice severe distress or discomfort. The majority of the animals will only be subjected to mild/moderate procedures. For example, the administration of a substance or bleeding which have only minor adverse effects, such as transient pain after the injection. A small proportion of animals (approximately 20%) will undergo moderate surgical procedure (e.g. skin punch biopsy), which will be performed under general anaesthesia with pain-relief peri- and post-operatively. Some animals (approximately 15%) will be exposed to a tumour challenge. These animals will be monitored daily to check for signs of adverse effects (e.g. signs of lethargy and of deteriorating body condition) and humane end points have been defined to avoid unnecessary suffering. In all cases animals will be killed before they develop signs of ill health. Some animals (less than 15%) may develop renal impairment spontaneously or as a result of the experimental procedures. By measuring the presence of protein and blood in the urine (leaking through a damaged kidney) we can readily identify this and mice will be culled at the onset of clinical signs of renal disease. All procedures have been designed to be terminated as soon as the animals appear to be suffering.

## Application of the 3Rs

### Replacement

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

### Replacement

The immune response is a very complex and dynamic process that involves a number of different cells and molecules. Experiments using intact animals are essential to study the immune response since *in vitro* cell culture systems or *in silico* modelling are at best only able to recapitulate partial interactions. Despite these limitations, every *in vivo* study will be preceded and followed by *in vitro* work with the aim of developing new experimental approaches that can replace animal work. For example we are currently working on 3-dimensional skin culture systems. However,

presently the tissue changes present in skin cancer or kidney damage cannot be studied using isolated cells alone and whole animal studies are still required. For these reasons the use of genetically-modified mice remains the most scientifically robust experimental approach to achieve the objectives in our application

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

To ensure that we always minimise our use of animals we: (i) use strategies that tell us which are the most important experiments to perform to answer our objectives; (ii) ensure that all animal experiments are designed so that we use the minimum number of animals to answer the experimental question; (iii) maximise the information that we obtain from each animal experiment.

To reduce the number of animals in each experiment we perform statistical calculations that tell us the minimum number of mice needed so that we can be scientifically confident of the experimental results. These calculations are termed power calculations and from our previous work typically result in us using 5-6 animals in each experimental group. These calculations are continuously updated as we generate new data and this means that in some cases we can perform future experiments using fewer animals per group.

We maximise information from each experiment by:

- examining multiple organs and tissues simultaneously.
- breeding together mice in which both copies of the genes are changed to reduce the generation of mice without the required alteration to the gene.
- using inbred mouse strains to reduce experimental variability
- applying, whenever possible, longitudinal imaging studies to maximise the data output from live animals.
- utilising strategies to minimize bias such as blinding.

## **Refinement**

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

### **Refinement**

Our animal research uses only mice. Mice develop immune disorders that are functionally comparable to human disease. We can also reliably measure changes in markers of organ damage (e.g. protein leaking into the urine in kidney disease) that are comparable to those measured in clinical practice.

Mice have proved invaluable in experiments studying the immune response because of the availability of genetically-modified strains lacking specific immune genes. The alternative to this would be to administer to healthy mice compounds that specifically inhibit gene function. This is technically challenging, not widely available, and often toxic to the animals.

To minimise animal suffering the experimental protocols have been refined so that they are all mild or moderate. In our program the majority of experiments are typically mild in practice. In all cases these models have been chosen because they accurately mimic features of the human disease without causing systemic distress to the mice. To our knowledge, there is no alternative less severe *in vivo* protocol. However, should such a protocol arise during our research we would adopt this to refine our existing protocols. However, we have not seen any significant or unexpected complications with our current models. In all experiments we design the protocols to keep the experimental duration as short as is necessary to achieve the scientific objectives. Animals will be housed in groups where possible, with appropriate environmental enrichment and fed according to institutional recommendations.

Procedures will be performed under local, general or terminal anaesthesia as appropriate and with pain-relief wherever necessary. In all experiments, animals will be inspected daily to ensure general well-being and any animal showing signs of illness will be humanely killed by schedule one or another approved method at the end of each study.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Preclinical evaluation of cancer therapeutics</b>
<b>Key Words</b>	cancer, chemotherapy, immuno-oncology,
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>No</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>Yes</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

- Identify tolerated dose levels of test drugs and side effects not predicted by cell culture based model systems.
- Study the effects of the drug on the body, and also the effects of the body on the drug.
- Demonstrate that anticancer activity can be shown at specified doses and with dosing schedules that are tolerated.
- Identify the best tumour models to use pre-clinically that correspond with a specific therapeutic target.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The aim of the data generated in these studies is to provide pre-clinical supporting information for clinical trial applications. A drug requiring evaluation will be supplied to Epistem along with summary evidence supporting the rationale for testing the agent. By having a much more thorough investigation into the efficacy and mechanism of action of a drug they will be able to make a more informed decisions on whether to proceed into clinical trials, reducing the risk of later stage failures. This will speed up the clinical trials and make them less expensive. The benefit is therefore a reduced number of unproductive human volunteer studies (and a reduced risk of adverse effects) and most importantly the development of improved and more effective therapeutics. The benefit to patients will be the identification of new anti cancer drugs. These studies will help identify the best potential drugs early in the drug development process.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We would expect to run 150 studies on behalf of sponsors using approximately 7,500 mice and 2,150 rats over the 5 year duration 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 levels of severity? What will happen to the animals at the end?**

Side effects of tumour treatment can include lethargy, anaemia, loss of appetite, diarrhoea, dysuria, bruising, bleeding or peripheral neuropathy. Animals exhibiting these signs will be humanely killed. This is likely to manifest as weight loss. A general dose limiting sign will be a 15% loss in bodyweight, and animals showing this will be considered unwell. Any mouse reaching a 20% bodyweight loss, or any rat reaching a 25% bodyweight loss along with other signs of distress will be humanely killed (schedule 1 method). Subcutaneous tumours may grow to a size that could cause discomfort or interfere with the animals' ability to satisfy thirst or hunger. Also, tumours could ulcerate through the skin dependent on the cancer type, or if intra-tumoural therapy is administered. Animals will be killed if their ulcers do not heal within 48 hours or if their tumour reaches more than 15mm in any direction. Injection at the tumour site may cause temporary bleeding which should stop within a few hours of injection. In the unlikely event that bleeding does not cease, and if the animal shows signs of discomfort, it will be humanely killed. Orthotopic (implanted at the natural tumour site, eg a breast cancer cell line injected into the mammary fat pad) tumours may have site specific adverse effects and elicit metastatic disease. Metastatic disease will be monitored by imaging of the whole body wherever possible. However if such techniques cannot be employed using a specific model, any deviations in physiology or behaviour will be treated as indicative of metastatic disease, and animals will be humanely killed when there is loss of condition consistent with the severity limit as defined by the Home Office regulation. For leukaemias, animals may gradually become weak, lethargic and lose body weight. Infiltration of the spleen or liver can lead to enlargement of these organs which may be palpable. Any animals showing signs of distress or symptoms at the limit of moderate severity will be humanely killed. Immunocompromised mice will be maintained in Individually Ventilated Cages in a barrier environment to avoid unwanted infections. If animals develop unwanted infections or surgical wound complications, they will be given antibiotic treatment after advice is sought or will be humanely killed. Animals will receive analgesia following surgical procedures such as bone marrow aspiration or orthotopic tumour implantation. Prolonged periods of anaesthesia can lead to animals losing body temperature. To counteract this, animals will be warmed throughout the procedure, either by the use of heating mats, warm air blowers or temperature regulated stages. All work will comply with the UKCCCR (United Kingdom Co-ordinating committee on Cancer Research) guidelines for the welfare of animals in experimental procedures.

## **Application of the 3Rs**

### **Replacement**

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

## Replacement

The programme requires that the models used are ones which closely mirror human disease. All compounds to be tested would have previously been screened in relevant *in vitro* models to determine those candidates suitable for *in vivo* testing. Rodents (rats and predominantly mice) are suitable for these studies as the work cannot be conducted in lower vertebrates, invertebrates or cell lines due to the poor resemblance of these options to the clinical setting. Animal models address issues which current *in vitro* tests cannot accurately determine.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Animal models will be restricted to the minimum number of animals needed for a statistically valid result. The number of animals used will be the minimum safely necessary to allow meaningful statistical analysis of the data generated.

The most important aspect of the proposed programme of work that will reduce the number of animals used is careful selection of drugs, on the basis of preclinical data. Only those potential drugs that offer a realistic prospect of therapeutic exploitation will be investigated.

The investment by the team in the purchase of small animal imaging technology also reduces animal numbers in these experiments. The development of disease can be followed in each animal over time, abrogating the need to humanely kill satellite groups to examine disease progress, and thereby reducing total animal numbers.

## Refinement

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

## Refinement

Rodents provide a cost and time effective platform in general for most pre-clinical testing. For the purposes of oncology testing, the use of higher species is not required because there is a wealth of knowledge on different types of cancer in rodents, as well as decades of in-house expertise with such models. Internal expertise, and more recent technological advances, such as the use of whole body imaging, allows for a more refined study design that will minimise the number of animals. These techniques will maximise the output and will provide a more thorough assessment. They will also help in selecting the best models.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 295. Investigating the regulation of energy homeostasis</b>
<b>Key Words</b>	AMPK, cancer, diabetes, metabolism, obesity
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to investigate the role of a protein (called AMP-activated protein kinase) in regulating metabolism in animals. Metabolism is the sum of all of the reactions within a living organism, and is usually divided into catabolism (the process of breaking molecules down to release energy) and anabolism (the process of building molecules which requires energy). Mounting evidence suggests that defects in metabolism underlie many human diseases, including obesity, type 2 diabetes, and cancer. The rationale behind my group's work is that understanding the basis for the regulation of metabolism will provide better strategies for preventing and treating these diseases. In order to survive, living cells need to balance the supply of energy (in the form of ATP) with demand, and this forms the basis for the way in which all organisms balance their metabolic processes. ATP is the molecule which all living cells use to supply energy. We are studying one of the key mechanisms that living cells use to monitor energy levels. This pathway involves a number of enzymes called protein kinases. An enzyme is a special type of protein that speeds up chemical reactions within living cells, and a protein kinase is a specific type of enzyme that carries out a regulatory role in cells. We will generate and analyse genetically modified mice that have one or more of these proteins deleted, or that express forms of the proteins that have altered activity. Studying the development of these mice and how their metabolism differs from wild-type mice, will help to determine the function of the proteins in the control of energy metabolism. An increased understanding of these processes will provide important information for the design of new therapeutic approaches for treating and/or preventing the progression of metabolic diseases, including 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 benefit of this research will be to provide valuable insights into the mechanisms leading to the development of human diseases whose underlying cause is likely due to defects in energy balance. The potential implications of this research are that the

results could lead to novel therapeutic approaches to treat these diseases as well as better strategies for preventing these conditions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice for our studies and we estimate that we will use approximately 25000 mice during 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 levels of severity? What will happen to the animals at the end?**

Most experimental animals will only undertake mild procedures, such as food intake and basic metabolic studies which have only minor adverse effects, such as weight loss from dietary manipulation and transient pain after systemic injection. Some animals will undergo metabolic studies which will be of moderate severity and this will include a number of surgical procedures which will be performed under general anaesthesia with pain-relief peri- and post-operatively. In some cases, animals will be used to look at tumour progression in the liver or prostate. These animals will be monitored regularly to check for signs of adverse effects (e.g. signs of lethargy and failure to respond to gentle stimulation, overt signs of deteriorating body condition). In most cases, animals will be used before 40 weeks of age, and at this age we do not expect serious adverse effects. For all experimental protocols, animals will be killed using a schedule 1 method or another approved method at the end of each study.

## Application of the 3Rs

### Replacement

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

### Replacement

Energy homeostasis is the process by which an organism balances its energy levels over time in response to changes in energy supply and energy demand. It is a complex process involving a number of different tissues and integration of multiple signalling pathways. To analyse the systems regulating this process experiments on intact animals are essential. Characterisation of the metabolic responses themselves requires the use of animals e.g. measurement of glucose and fat metabolism. These parameters cannot be reconstituted using experiments carried out in a test tube (these types of studies are often termed “in vitro”, from the Latin meaning in glass), nor can they be modelled in other systems, necessitating the use of animal models. Whilst experiments using isolated cells in culture, rather than living animals, are useful in providing insights into processes such as cell signalling and enzyme regulation, these studies do not adequately replace whole body physiology. For

these reasons the use of genetically modified mouse models is currently the best way to carry out the physiological analyses in the proposed work.

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

The choice of genes that we will study is based on previous cell based and in vitro biochemical approaches. Only genes encoding proteins that have been shown to be involved in regulating energy metabolism in other experimental systems will be examined. Although these in vitro studies cannot predict the effect of manipulating the gene in living organisms, they provide valuable clues enabling us to prioritise our experiments and keep animal numbers to a minimum. In designing our experiments, we plan to obtain the maximum possible data from a single animal. When breeding genetically modified mice we use strategies to maximise the use of offspring where possible. All studies are designed with careful statistical considerations with respect to sample size, utilise strategies to minimize bias such as blinding and randomisation and involve precise and reproducible assays which together ensure that the information we gain is robust while using the minimum number of animals.

### **Refinement**

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

#### **Refinement**

Our studies will use the mouse, which represents the lowest mammalian species available in terms of displaying physiological and disease states seen in humans. The use of genetically modified mouse models is well established and has proved to be a powerful tool providing novel insights into the roles of many proteins in mammalian physiology. In particular, the mouse has provided an excellent model for studies of metabolism and metabolic diseases. All the procedures are classified as either mild or moderate. Procedures will be performed under local, general or terminal anaesthesia as appropriate and with pain-relief wherever necessary. We will use the most refined technical approaches to minimise welfare costs.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 296. Mechanisms behind T-cell function and regulation</b>
<b>Key Words</b>	T cells, Immune regulation, Immunotherapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

T cells are an important population of white blood cells that protect us from infections and cancer. T cells sense germs or damaged cancer cells through a specialised structure, called the T cell receptor (TCR). Each TCR is unique, enabling T cells to respond to any potential threat to the body. As there exists both a large number of T cells and many unique TCRs in the body, this presents a great challenge to study. Scientific approaches in the past have used techniques to label T cells with green proteins when their TCRs sensed a threat. However, these green proteins can remain in a cell for up to a week, many days after a T cell has responded. We have developed a new Tool, which labels T cells temporarily blue (within hours of TCR sensing a threat), meaning we can follow T cell behaviour with much greater precision. Using this system, we aim to reveal how T cells behave under normal healthy conditions, as well as in diseases such as allergy, infection and autoimmunity. In addition, we will look to understand how drugs may alter the function of T cells in autoimmune 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 project will advance scientists' understanding of how T cells respond during allergy, infection and autoimmunity. In addition, the breeding protocol will create new tools for use by others within the scientific community to take forward their own research. The project has the potential to identify new targets on T cells that we could design drugs to alter how T cells respond to threats in the body. This could help the development of future immunotherapies – which are drugs designed to alter how the immune system works. In addition, a key objective of the proposal is to investigate how a particular type of immunotherapy – called peptide therapy – works through altering the behaviour of T cells. This could better inform strategies for treatment of autoimmune diseases, such as multiple sclerosis, diabetes and arthritis.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The project will use both normal and genetically modified mice (which express the blue colour when T cells are activated). Over a period of five years, it is anticipated that a total of 3000 mice will be bred in order to address the aims and objectives of the project. In addition, of these mice, 2000 are anticipated to undergo direct procedures.

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

To study allergy, a refined model is used which results in a slight reddening and thickening of ear skin 24 hours after applying a substance to the skin. In addition, to test T cell responses during infection, we will use a well-established model that does not result in clinical symptoms for mice. Infection will be performed via infection with droplets in the nose, with the mouse anaesthetised for a short period of time to minimise suffering. Multiple rounds of anaesthesia will be avoided to reduce the cumulative harm to mice. For studying T cell responses during autoimmunity, it is necessary to use a mouse model of multiple sclerosis (mMS). These models require the injection of proteins immersed in oil containing heat killed bacteria under the skin of mice. The majority of these studies will look at the early stages of mMS, before more severe symptoms set in. These experiments will be restricted to no longer than 2 weeks duration to assess T cell responses during the initiation of mMS disease. However, to test drugs to potentially prevent mMS, a small group of mice may experience moderate symptoms (such as paralysis of tail and back legs). This is unfortunately necessary in order to reveal whether drugs are effective at preventing or improving disease. In some experiments, mice will receive substances in the diet, or be given orally, that can alter genes within T cells. All animals will be carefully monitored by trained staff throughout experiments, and all mice will be humanely killed following the end of experiments.

## Application of the 3Rs

### Replacement

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

### Replacement

T cells are part of a very changeable and complex biological system, which involves numerous cells that migrate around the body and interact with each other. Whilst simple test tube models exist for investigating their behaviour in the lab, these do not always predict how T cells may behave in the actual body.

In order to study T cell responses that are relevant to human disease, we must use a species which shares all the major parts of the immune system. Amongst species that share the main components of the immune system with humans, mice are the least sentient option. 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.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will use a very refined model of MS, where each T cell has a TCR that makes them activate when they sense nervous system tissue. This model (called Tg4 MS model) is very reliable, and can induce disease in 100% of mice, which will mean experiments can be performed using only 3 mice as disease controls (compared to 5-8 using other MS mouse models). In addition, T cells from these modified MS model mice can be cultured in a dish in the lab and tested for how they response to nervous system tissues, reducing the number of mice that have to undergo experimentation.

## **Refinement**

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

### **Refinement**

REDACTED, we can capture more information during the responses of T cells to infection, allergy and during autoimmunity. This will require fewer mice to be used. The Tg4 mMS model is very reliable, and protocols have been designed to induce moderate disease levels in the greatest number of mice. In order to make sure mice suffering is minimised, we have a dedicated scoring system for disease, which grades from 1 (mild) to 5 (most severe). Any mouse that develops grade 1 or 2 disease will be checked on by trained lab members twice a day. Mice that develop grade 3 disease will be humanely killed. Protocols have been refined to generate no greater than grade 3 disease. However, a balance is required that minimises disease whilst also maintaining a level and incidence of disease that is sufficient to enable whether a given treatment is effective.

Following potentially painful injections, mice will receive Sudocrem® to reduce irritation and prevent ulceration

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 297. Investigations into Lumpy Skin Disease virus</b>
<b>Key Words</b>	Poxvirus, Lumpy skin disease, Capripoxvirus
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>Yes</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to understanding how lumpy skin disease virus (LSDV) causes disease, and to improve diagnosis, prevention, control and eradication strategies against lumpy skin disease (LSD).

The objectives of the project are to develop an experimental model of LSD and use it to investigate the pathogenesis of the disease, how the virus is transmitted from one animal to another, and to test improved and new vaccines.

**What 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 this project will be better control and prevention of LSD. This will benefit regions currently threatened by LSDV (Europe and Asia) and regions where the disease is endemic (Africa and the Middle East). It will directly benefit farmers, particularly in low and middle income countries where food security can be precarious.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

10 rabbits, 40 mice and 750 cattle

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

A group of 500 cattle on affected farms (should a LSD outbreak occur in the UK) will undergo procedures of mild severity (blood sampling, oral and nasal swabs). 62 cattle will have mild severity 178 cattle will undergo mild to moderate pain, harm and distress 10 cattle will undergo severe pain, harm and distress 10 rabbits and 40 mice will undergo mild pain, harm and distress. The major adverse effect for the cattle is lumpy skin disease, which can result in fever, ocular, oral and nasal discharge, and lymphadenopathy in addition to firm cutaneous nodules up to 5 cm diameter. These

can be found all over the body but particularly sparsely-haired areas such as the head, udder, scrotum and perineum. The nodules may become necrotic and ulcerate. Vesicles and ulcers can occur in the oral and nasal cavities. In severely affected animals necrotic lesions also develop in the respiratory and gastrointestinal tract. The group of 500 cattle will be discharged from the controls of the Act by releasing to a farm. The remaining cattle and mice and rabbits will be euthanased by a schedule 1 method.

## Application of the 3Rs

### Replacement

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

#### Replacement

LSDV is a very host-specific virus and does not cause disease in any species other than cattle. In order to study the disease cattle must be used.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Reduction will be achieved by using the optimal experimental design, minimising variability, by full and prompt publication of results, and by maximising the use of the tissues and samples generated.

### Refinement

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

#### Refinement

Refinement will continue throughout the lifetime of the programme to eliminate or reduce to the minimum any possible pain, suffering, distress or lasting harm. Initial pilot studies will optimise the LSD experimental model and design humane end points which will be used to minimise pain and suffering in future studies. Environmental enrichment will be employed to minimise the contingent suffering.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 298. Effects of testosterone on atherosclerosis</b>
<b>Key Words</b>	Heart disease, Testosterone, Treatment, anti-inflammatory
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Heart disease is the single biggest cause of death and disability in the developed world, yet new treatments are uncommon. Testosterone protects men from heart disease, but how it does this is not known and it is therefore not usually considered as a treatment option for male patients with cardiovascular risk and low levels of the hormone. Additionally, drugs that stop the blood from clotting, known as antithrombotics, may also have some beneficial effects in reducing the progression of heart disease. How they do this specifically within the blood vessel wall where heart disease typically starts is unknown. Using a mouse model of heart disease we will evaluate the effect of different treatments (including Testosterone and Rivaroxaban - an antithrombotic) on the dysfunction that occurs in the main arteries leading to 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)?**

Improving our understanding of what happens in heart disease is of great importance in developing approaches to treat patients with this condition as much is still unknown about how this disease develops. This project will increase our understanding of how the disease develops in the blood vessels as will test how specific treatments take effect to potentially provide the basis for the development of new therapies. Testing existing drugs like rivaroxaban and testosterone, which are already approved for other uses (thromboembolism and hypogonadism respectively), in the new context of heart disease treatments is extremely valuable as it can save years and hundreds of millions of pounds in drug development studies needed for new compounds. As a result, this could also accelerate the time for such therapies to reach the patient.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will use approximately 1500 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 levels of severity? What will happen to the animals at the end?**

The procedures that the animals go through are expected to have only very few adverse effects and moderate severity of discomfort. Pain will be reduced during and after castration surgery with anaesthetics and mice will be monitored closely and provided with the best health care to ease, reduce and eliminate suffering. The test treatments are not expected to cause any adverse effects and administration produces only mild and very short-lived discomfort from an injection which will be reduced through anaesthetic. A high fat diet used to develop heart disease in this study is well tolerated by the animals and causes no adverse effects. This study will use only enough animals to answer the experimental questions. All animals will be humanely killed at the end of the study to allow detailed investigation of the heart and surrounding blood vessels in relation to heart disease; something that cannot be otherwise achieved in live animals.

## Application of the 3Rs

### Replacement

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

### Replacement

The use of animals in the proposed study has been carefully considered and we are not aware of any alternative which does not use animals that would allow the experimental questions to be answered. Mice are mammals and develop heart disease in a similar way to humans providing a good model for studying details of this disease. While we will use isolated cells relevant to the development of heart disease grown in culture to complement the work, such experiments do not allow for the influence of the whole-body and systems within it to be fully considered and therefore cell experiments cannot be used as a replacement of the animal study.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Every attempt has been made to reduce the number of animals used through careful experimental design in consultation with statisticians familiar with animal studies. Throughout the project animals will be regularly monitored and experiments altered

as necessary to ensure only the correct number of tests are performed to answer the specific questions of the study. These ongoing decisions will again be made with input from statisticians.

### **Refinement**

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

### **Refinement**

The use of mice for the study of atherogenesis is advantageous due to the similarities in the development of the disease to human. The specific mouse used in this project develops heart disease at an accelerated rate promoting disease in the arteries similar to that found in humans without the need for long experimental times and prolonged discomfort.

Any negative impact on the animals will be reduced through providing the highest levels of skilled care by trained and competent personnel. Discomfort of the experimental animals will be minimised through the use of appropriate anaesthetics during any procedures where it is considered necessary.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 299. Molecular mechanisms underpinning behavioural stress responses in the rodent</b>
<b>Key Words</b>	Stress, adaptation, genes, coping, rats
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project aims to understand how stress acts on the brain. This is a very important question because many people in our society (1 in 4) are suffering from a stress-related mental disease like depression, anxiety and schizophrenia. In addition to the personal trauma experienced by the patients, their family and friends, it is also an enormous burden for our society costing over £100 billion per year. Presently, we don't exactly know how stress acts on the brain and how it can cause disease, particularly after repeated exposure. As long as this remains unknown we can't develop new and improved medication to help these patients. It should be mentioned that most medicines used at the moment to treat patients were discovered by chance and actually we don't completely understand why they help some (but not all) patients. It is of great importance therefore to understand the impact of stress on the brain.

Stressful events result in the secretion of stress hormones ('glucocorticoid hormones') from the adrenal glands, which are hormone-producing glands located close to the kidneys. These hormones can act on nerve cells in our brains and influence their functioning. Importantly, research so far indicates that glucocorticoid hormones play an important role in the development of stress-related mental diseases. Presently, however, we do not know how these hormones act on nerve cells. For proper functioning of nerve cells (so the whole brain and thus the person can function normally), many substances ('molecules') are interacting in these cells. Many of these molecules are the result of the expression of genes located within the DNA in the nerve cell nucleus and may be affected by stress hormones. Thus, our aims are:

1. To determine which genes are affected by stress-induced glucocorticoid hormones
2. To investigate how these hormones are changing the affected genes after stress and the consequences for nerve cell function and behaviour
3. To study whether, in addition to these hormones, other molecules are involved in these effects
4. To study how repeated exposure to stress disrupts the normal effects of glucocorticoid hormones on genes, nerve cells and 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)?**

This project will deliver the following direct benefits: 1. A vast increase in, publicly available, knowledge about how stress and stress hormones impact on the brain, which includes: - The identity of the genes affected by stress/stress hormones - How this results in changes in nerve cell function and behaviour - Through the comparison of juvenile and adult rats, we will learn why adolescents are more vulnerable to developing a stress-related mental disorder than adults - How repeated stress disrupts the normal action of glucocorticoid hormones in the brain 2. The scientific results will help the development of computer models, which can reduce the need for animal experimentation in the future. In the medium-term, the results of this project will help to identify the genes that are critically affected by stress hormones. These genes should then be further investigated as potential targets for novel drugs for the treatment of stress-related mental diseases. Identification of the genes will also help to screen for stress-sensitive individuals amongst applicants for high-stress jobs, such as air traffic controllers or the armed forces. In the long-term, this project will benefit the development of new drugs for stress-related diseases. The results of this research may also contribute to the so-called personalized medicine in which each patient will receive their personally 'fitted' medication. Importantly, this project will help to develop life style changes and treatments to prevent the development of these diseases.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The work to be conducted under this licence will use up to 3994 rats under three protocols of up to moderate severity during 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 levels of severity? What will happen to the animals at the end?**

To investigate the effects of stress on the brain, rats will be exposed to various stressful experiences such as swimming in a pool of water. None of these challenges cause any pain and the distress they cause is of transient nature. Certain drugs will

be used to inhibit/activate key stress-related molecules before, during or after the stress experience, which will result in no more than quickly passing discomfort and no lasting harm. Whenever possible, administration of agents will be on a voluntary basis, for instance via liquid food. There is a small chance (<10%) that drugs may induce seizures, skin reactions, hair loss and weight loss which will be counteracted by close monitoring, topical treatment and increasing ease of food consumption. Surgeries such as removal of the adrenal glands will be conducted competently and aseptically under general anaesthesia with appropriate pain relief and recovery time. Overall, the expected severity level is moderate. All rats will be killed at the end of each study either whilst under a general anaesthesia or using a Schedule 1 method.

## Application of the 3Rs

### Replacement

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

#### Replacement

This type of research requires the use of intact, freely-behaving animals because coping with (responding to and adapting to) stress including expression of behaviour involves many different organs and cell types communicating together. This condition cannot be mimicked in a cell culture setting since the processes under investigation are extremely complex with many unknown factors and variables playing a role. Moreover, cell cultures do not show any behaviour as an experimental endpoint. Computer simulation programmes are not currently available, although work carried out under this licence may contribute to develop these.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The number of rats needed has been estimated based on over 30 years of prior experience and on the advice of a professional statistician in order to use the minimal number required to ensure strong statistical confidence in the scientific findings. We have optimised our scientific methods such that we can study many genes and molecules using a single animal, in some instances more than 15,000 genes. This advancement has enabled us to greatly reduce animal numbers. Furthermore, the state of our technologies are such that the scientific results generated show relatively little variation, which helps to keep the required number of animals low and yet allow us to draw strong scientific conclusions from the data.

### Refinement

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

### **Refinement**

Our protocol and techniques have been refined over many years to ensure the minimum stress is applied to the animals in order to achieve our scientific aims.

All rats will be handled for a few minutes for a number of days before the start of the experiment so the animals can get used to being picked up and held.

The applied stress protocols in our license do not cause any pain and in most cases only quickly passing discomfort. The stress protocols have been designed and optimised such to just present the level and duration of distress required to answer our research questions. The nature of our stressors is mainly mildly psychological. We have refined our stress procedures in a manner that enables us to study the impact of stress on the brain, at the same time keeping the level of distress for the animals as low as possible.

All surgeries will be conducted under general anaesthesia using aseptic techniques and appropriate levels of pain relief administered as well as interventions to minimise possible side effects (increasing room temperature to help recovery, providing mashed food/food in cage if weight loss is expected, and enhanced monitoring during vulnerable periods).

Drugs and other substances will be administered at the appropriate dose and manner to minimise suffering and side effects whilst giving the best possible pharmacological action.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 300. Examining the control of female meiosis and early development</b>
<b>Key Words</b>	Oocyte, Aneuploidy, Women's Health
<b>Expected duration of the project</b>	1 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We want to understand how a healthy egg is produced. This has a number of facets but the most prevalent to go wrong is having the right number of chromosomes. Eggs that are made with the wrong number of chromosomes are described as being aneuploid. Such aneuploid eggs go on to form aneuploid embryos, and these are mostly non-viable and die on or before the time of implantation. It is estimated that up to 60% of ovulated eggs from women are aneuploid. This leads to infertility, early pregnancy loss and birth defects – because a few aneuploidies can result in live births. The most prominent type of aneuploidy is trisomy 21 (Down Syndrome).

Currently we do not know why eggs should end up being aneuploid at such a high a rate. So we need to investigate this phenomenon if we are to move forward and to either develop ways of reducing aneuploidy or screening for it more effectively. Especially intriguing is how the rate of aneuploidy increases with maternal age. There is an increasing trend to have children later in life, hence the relevance of aneuploidy to human fertility is on the rise.

Mice also show a high rise in aneuploidy as they age and are therefore an appropriate tractable system to study this area- without the ethical issues of using human oocytes. Put simply there are also too few human oocytes available for research to produce much scientific progress. Therefore in order to understand why aneuploidy happens and how a healthy egg is produced this project will investigate the process in mice

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Women and couples: the main benefactors in the long term are going to be women who will have an increasing control on their reproductive health. Knowledge from this project will help underpin future strategies to develop ways of improving egg health during the 40 years of reproductive life women have. Especially relevant is finding ways of reducing the effects of ageing on aneuploidy. However any strategy to do this has to be based on scientific knowledge of how aneuploidy comes about. Such knowledge will then aid in developing appropriate methods to circumvent or reverse the deleterious effects of ageing.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Numbers are reduced during the entirety of the project (3Rs) by using only hormonally treated animals, a process that increases the numbers recovered. I request animal numbers on this 5-year Project licence of 3500. This equates to 14 mice per week (50 week year), 700 pa, 3500 over the 5 year window. This oocyte number is the average we can use in the experiments we perform following oocyte collection (on average just under 3 mice a day). This is based on the average week using (2,3,3,3,3) mice. Some experiments use more (e.g. Western blotting), while some (e.g. pilot experiments) use less. It is based on a historical average for the same sets of experiments that we have performed in the laboratory under previous grants and licences, which use the same mix of experiments.

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

Female mice are used as a source of eggs. The protocol is very mild, and involves either 1 or two intraperitoneal injections of hormones. These hormones mimic endogenous hormones, and help follicle growth and ovulation. This simple and quick procedure ensures that we can reduce animal numbers by obtaining the most useable numbers of oocytes per mouse. The only feasible adverse effect is infection from injection, however this is minimised by only using sterile equipment and solutions.

## Application of the 3Rs

### Replacement

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

### Replacement

Replacement: the long term goal would be to replace the use of mice. However, there are no in silico models or cell cultures that can be used. The only available source of oocytes is from the ovary.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Reduction: we only use mice that have been hormonally treated, so reducing the numbers we need to a minimum. Hormonally treated mice will produce more eggs than non-treated.

## **Refinement**

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

### **Refinement**

Refinement: we are using a priming and superovulation hormonal treatment that has been refined over the past 40 years. One of the great advantages of using mice is that this procedure is so common, it has been refined over decades to be used with the utmost effect.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 301. Development, function and regulation of the immune system</b>
<b>Key Words</b>	Lymphocytes, immunology, cancer, vaccines, signals
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The immune system must be precisely regulated so that it will fight off infections and tumour cells effectively, while not damaging the normal organism. T lymphocytes (“T cells”) are cells found throughout the body and which have key roles both in attacking “foreign” material but also regulating the activity of the immune system as a whole. Our research objective is to systematically map and identify the key signalling pathways and molecules that control lymphocyte function. One focus is how lipid and protein kinase pathways integrate information from antigens, cytokines and nutrients to control metabolism, inflammatory cytokine production and T cell migration/trafficking and hence determine cell fate choices in lymphocytes.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

When T cells fail to function correctly the immune system fails, often resulting in death. The present project will characterise molecules that act as messengers inside T cells to regulate their function. The control of T cells is critical for immune responses. Understanding the signalling pathways that control T cell activation is essential to identify targets relevant for the treatment of autoimmune and inflammatory diseases. The laboratory has an integrated research program to explore signalling pathways inside lymphocytes. These studies will generate new information about mechanisms that control immune responses and identify new targets for therapeutic intervention in the immune system that can be used for vaccination, to fight bacteria and to treat cancer.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This application is to support a group of 8 scientists for a period of 5 years and proposes to use up to 20,000 mice including wild type and genetically modified mice.

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

Based on previous experience, more than 95% of the mice to be used in this programme will exhibit only mild outward signs or none at all. The great majority will be killed humanely for the collection of tissues and cells to be studied in detail in the laboratory. Some animals (up to 5%) will be challenged with microorganisms or tumour cells. Infections are likely to cause deviation from normal welfare in some of these mice, but all will be killed humanely at as early a scientific endpoint as possible. Similarly, tumour studies will be conducted (in up to 5% of animals) in a way that will ensure that only minimal changes in welfare are caused. Some animals (2-3%) will be irradiated, to permit the introduction of immune cells derived from other mice (akin to bone marrow transplants as carried out in humans). We will use a standard procedure for this and mice will receive welfare support to ensure that the effects of the irradiation are minimised and resolve completely.

## Application of the 3Rs

### Replacement

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

### Replacement

Lymphocytes isolated from peripheral blood from normal human donors can be used to study some aspects of lymphocyte behaviour but immune responses are very complicated and require that lymphocytes traffic between the blood lymphoid organs and peripheral tissue. To identify important modifiers of immune responses, it is necessary to carry out immune function tests in live animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We work in partnership with skilled animal experimentalists and use well validated mouse models. We ensure through constant discussions and consultation with statisticians that the number of mice we use for experiments is appropriate. We constantly seek to reduce our animal usage through improvement of in vitro models. The minimum number of mice required to show scientifically and statistically significant data are used.

## **Refinement**

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

## **Refinement**

This project will use mice because mice are an appropriate, well established model for studies of the mammalian immune system and are a good model for the human immune system. We will adopt early scientific endpoints for all experimental interventions.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 302. Repairing the damaged brain after hydrocephalus</b>
<b>Key Words</b>	hydrocephalus, axon regeneration, neuroprotection, scarring
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The objectives of this project are to determine changes that occur after induction of hydrocephalus, a condition where fluid builds up on the brain and causes damage to brain tissue by a build-up of pressure. We are particularly interested in learning how neurons deal with the injury that makes them vulnerable to death, the scar tissue that forms after injury and the pathways involved in pressure regulation.

This will allow for a better understanding of the mechanisms of hydrocephalus and will help us to identify therapeutic drugs that will be used to protect nerve cells from death, dissolve scar tissue and reduce raised pressure.

**What 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 provide important data that will improve our understanding of the changes that occur after hydrocephalus and provide an insight into what is required to promote nerve cell survival, removal of scar tissue and promote nerve regeneration. This will underpin the discovery of novel therapeutic drugs that will be used to promote nerve cell survival, scar tissue removal and reduce raised pressure in the brain.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats: 2,050 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 levels of severity? What will happen to the animals at the end?**

Potential harm results from hydrocephalus, which will be created under general anaesthesia. In the vast majority of cases, there is no adverse response to induction of hydrocephalus. However, hydrocephalic animals do show retarded weight gain, a dome-shaped head and gait instability. There are clear guidelines in place in our facility to ensure that suffering in animals is minimised by either administration of pain-killers or termination of experiments. Soft mash will be provided on the floor of cages as well as injections of fluids and extensive care within the first three days after induction of hydrocephalus. We will remain vigilant for any adverse effects and will promptly provide pain relief or treatment if appropriate, or humanely kill the animal. Animals will be killed by Schedule 1 methods or perfused with 4% paraformaldehyde under terminal anaesthesia for histological analyses.

## Application of the 3Rs

### Replacement

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

#### Replacement

There is no adequate substitute for using the *in vivo* models described in this application. Establishment of potential clinical relevance of regulatory molecules interacting in a dynamically changing hydrocephalic site can only be achieved in an animal model. A less sentient animal such as fish cannot be used since they spontaneously regenerate their damaged axons after injury and achieve complete recovery of function. Therefore, rats and mice are our prototypic laboratory animals and have been rigorously characterised by ourselves for the hydrocephalus paradigm and shown to be representative of the human condition by us and others. The tools for the project have all been prepared in relation to the models described herein and continuity of the study in these species will be essential for significant progress to be made in a timely and efficient manner.

However before embarking on each experiment, we will consider key issues, which include: is the research necessary?; what has already been done in this area?; what models have been used?; what are the best methods/procedures?; alternative consideration for potential pain and distress?. We will then search online databases such as Pubmed and Web of Science for alternatives to animal experiments and systematically review the number of hits using defined search terms that will seek out alternatives to animals. All of these strategies will be used to address possible alternatives to animals, prior to embarking on experiments in live rats.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Some of the end-point measurements (e.g. nerve regrowth, scar formation etc) may be essentially qualitative and for these we use 3-6 animals per treatment group. In most experiments with quantitative end-points, 6 animals are randomly assigned to each treatment group, a number calculated as the minimum required to provide statistically significant results. This has been determined on the basis of our previous experience with these procedures, the methods of analysis and after consultation with statisticians to calculate power.

All experiments will be designed and appropriately powered using the NC3Rs experimental design tool to ensure compliance with ARRIVE guidelines.

## Refinement

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

## Refinement

The models selected closely resemble the features seen in humans after hydrocephalus.

All therapeutic agents are evaluated and optimised *in vitro* prior to *in vivo* application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are conducted on all harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. These refinement steps significantly reduce animal usage and severity.

Before conducting each experiment, it is discussed with the NACWO and NVS routinely to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to acquire the scientific endpoints. We will also 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 the next experiment.

The surgical technique for inducing hydrocephalus has been refined to minimise injury to the animal and aid with recovery. In addition analgesics are given post-operatively (following day) to minimise pain from the operation.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 303. Nutritional programming of metabolic disease</b>
<b>Key Words</b>	Suboptimal nutrition, pregnancy, early life, programming, adult health
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our overall purpose is to identify the mechanisms leading to adult disease, secondary to suboptimal maternal nutrition during pregnancy and/or lactation, and to investigate the efficacy of interventions that are potentially translatable to humans.

The key questions are:

1. **How does suboptimal gestational and/or lactational nutrition affect maternal and offspring health and what are the underlying mechanisms?**
  - What are the key maternal factors (e.g. hyperinsulinemia, hypertension) in an obese mother that program insulin sensitivity, cardiac dysfunction, energy balance in offspring?
  - As proof of principle, can we mimic these programming effects (e.g. insulin) by exogenous introduction of these maternal factors directly into the fetuses?
  - Does altered maternal diet lead to altered vascular function and/or blood flow between the mother, placenta and the fetus?
  - Does altered maternal diet lead to defects in cellular proliferation, cellular apoptosis, hypoxia or oxidative stress in maternal, placental, fetal or adult offspring tissues?
  
1. **What are the effects of pharmacological and/or exercise intervention to the mother, and the metabolic health of her offspring?**
  - Does perinatal maternal exercise in an obese mother improve her insulin sensitivity and cardiovascular tone and that of her offspring?
  - Does perinatal pharmacological treatment improve her insulin sensitivity cardiovascular tone and that of her offspring?

- Do the interventions affect cellular proliferation, cellular apoptosis, hypoxia or oxidative stress in maternal, placental, fetal or adult tissues?
1. **What are the effects of dietary/pharmacological intervention in the offspring on the programmed effects on metabolic health?**
    - Does dietary/pharmacological treatment to the programmed offspring improve insulin sensitivity and cardiovascular tone?
    - Do the interventions affect cellular proliferation, cellular apoptosis, hypoxia or oxidative stress in neonatal/adult offspring

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Lifestyle (increased physical exercise) intervention to an obese mother around the time of pregnancy is likely to improve her metabolic fitness and the long-term health of her offspring. This is an important translational message.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over the 5-year period of this project, we expect to use no more than 6,900 adult and 7,500 neonate Rats; 11,600 adult and 10,400 neonate Mice.

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

The large proportion of animals to be used in this licence (81.9% of the adult rats and 87.5% of the adult mice) will experience no adverse effects. Female animals fed differing diets, with or without exercise or pharmacological intervention, will then be paired with a male. Both mothers and offspring health will be monitored throughout adult life using longitudinal assessments of a) non-invasive cardiovascular imaging techniques, sometimes with a general anaesthetic to negate stress b) metabolic testing involving All animals will be killed humanely at the end of the experiment. 15.2% of the adult rats and 10.8% of the adult mice will undergo recovery surgery under appropriate general anaesthesia. Following the surgery, they will experience minor discomfort with itching around the wound stitches. This will be managed with appropriate analgesia and antibiotics. Animals will be killed humanely at the end of the experiment. In addition to this, 2.9% of the adult rats and 1.7% of the adult mice will undergo surgery (without recovery) under general anaesthetic throughout the experiment and be killed by an anaesthetic overdose at the end of the experiment.

## Application of the 3Rs

### Replacement

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

## Replacement

Although experiments in cultured cells can provide a lot of useful information on specific workings at a cellular level, we need to study how different cell populations behave and interact as part of a complex environment in a living animal. Each tissue type e.g. brain, fat tissue, heart, working muscle or liver, are themselves made up of different cell populations (including stem cells which go on to divide and mature into fully functioning adult cells). The different tissue types send out and respond differently to the signals present in the peripheral blood system such as occurs in the whole living animal. This level of complexity cannot be attained in cell culture based experiments.

Our previous work has used the strategy of identifying specific mechanisms due to suboptimal maternal environment in animals which lead to cardio-metabolic disease in the offspring, and then using this information to guide parallel human studies (e.g. in Danish low birth-weight men). This successful strategy clearly underlines the value and justification for our work using animals, which has significant parallels with the human pathophysiology. We will where possible, use a similar approach to translate our observations in animal models into humans by working on human serum and biopsy material and we will continue to use a forward and back translation approach.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

It is important to note that as the environmental stimulus is made to the mother, it is the mother which becomes the statistical unit for all our studies, and this is therefore reflected in our animal numbers as we are constrained to using only 1 offspring in each litter for any given outcome. We will collect all tissues and organs at post mortem-even ones that are not required at the time for any particular study. These include brain, heart, aorta, lungs, liver, pancreas, fore-gut, hind-gut, kidneys, intraperitoneal fat, retroperitoneal fat, gonadal fat, vastus lateralis & biceps femoris muscle, brown fat, bones and testes or ovaries. This extensive bank of tissues allows us to follow up several lines of disease pathology, so that we and other groups through internal and international collaboration, are able to facilitate later studies without the need for additional numbers of animals, thus significantly reducing the need to use more animals.

Where possible we will follow up observational studies in the animal with non-animal cell systems to gain mechanistic insight into specific cell signaling pathways. These studies will help define the specific pathways involved and thus inform specific intervention, resulting in a reduction in the number of animals and a high degree of refinement to the proposed intervention models. For example, we observed that maternal obesity “programmed” a loss of IRS1 (an insulin signalling molecule) in the

fat tissue of the offspring of obese mouse dams. In the same tissues, we also observed a gain in the expression of a small RNA molecule (microRNA) known to negatively regulate IRS1 protein levels. In order to investigate if these programmed changes could be replicated in a cell system, we obtained precursor cells from the fat tissue of these mice and grew them into mature adipocytes in vitro. This experiment showed us that despite being grown and outside the animal, these precursor cells carried the information encoding the programmed phenotype observed in the mature fat cells of the animal. This strategy will allow us to **reduce** animal numbers further by adopting a cell system widely used in studies of regulation of fat metabolism for our more complex studies. We will also couple the use of this cell system with a global approach to identify other proteins regulated by the overexpression of any microRNA in a non-biased manner and without *a priori* bioinformatics prediction.

We will use non-invasive echocardiography with recovery anaesthesia to monitor cardiac function longitudinally, in the same animal that **reduces** the number of animals required. Isoflourane is very well tolerated in every animal as we maintain anaesthesia for no longer than 20 minutes and recovery is quick (under 1 minute). This provides substantial gain in power and data quality and robustness thus reducing animal numbers.

We are introducing some new techniques: 1) Labelling with a marker for impaired oxygenation of fetal tissues and placenta and 2) Ultrasound imaging of the pregnant dam at critical stages of fetal development. These 2 techniques will inform on the effects of maternal condition to her offspring in early life and during organ development, even before the pups are born. In the first approach, a chemical which binds to tissues affected by reduced oxygenation, will be injected into the pregnant animal shortly before killing. The label will identify both placenta regions/specific cells and developing fetal structures affected by maternal condition/treatment using histology with antibodies. In the second approach, we will monitor maternal heart function and blood flow in major vessels connecting the mother to the fetus as well as fetal heart function using ultrasound. Additionally, by administering various drugs that modulate contraction or dilation of the heart and blood vessels during the ultrasound, we can gain more specific information on the types of loss of function and the mechanisms involved. Similar information will be gained by using this ultrasound technique in the adult offspring.

We currently use a technique for labelling fetal tissues that are actively growing during gestation by injecting a dye into the mother which is trackable. We now wish to extend this approach to measure the expansion of cell populations during early postnatal life.

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

Females from our breeding colony will be randomised (by animal technicians blinded to the study) to receive experimental diets and or interventions. At sexual maturity, they will be mated with male studs that are refreshed every 6 months to optimise breeding and minimise ageing paternal effects on the offspring. Offspring are again randomised (by technicians blinded to the study) to be weaned onto control or obesogenic diets with or without pharmacological intervention. Where possible, investigators carrying out cardiometabolic measurements will be blinded to the experimental groups.

In our exercise intervention studies, we apply the knowledge that rodents are nocturnal and therefore they respond better to exercise training at the beginning of their wake cycle (which is after 6pm in the evening when the lights are out). Our researchers therefore go in to train the animals in the dark with a red headlamp to minimise disturbances to their circadian rhythm.

We use non-invasive TDNMR that does not require anaesthesia to measure body composition of mothers and offspring. As this allows longitudinal body composition to be measured in the same animal, it also reduces the number of animals required. We combine this data with other repeated longitudinal measures such as non-invasive tail cuff blood pressure and Echocardiography. Finally, at post-mortem, we take blood to measure metabolites and tissues for molecular studies. All the data can be correlated and then also compared to aged controls to identify if there is an advanced aging phenotype. From this, we can identify markers at the cellular level indicative of ageing, which enables us to assess the effects of early nutrition on life span without the need for maintaining mice and rats for their full lifespan. During the next 5 years, we hope to acquire access to more advanced imaging equipment that would allow in-utero measurements and measurement of ECG in conscious animals.

Offspring of obese or undernourished mothers may be affected by a lack of oxygenation during development in the womb. Introducing a chemical that directly labels tissues and cells so affected is a refinement to various indirect techniques currently in use (e.g. manual techniques using whole blood, which are prone to errors as once a fetus is removed from the amniotic sac, it is exposed to ambient oxygen levels). Ultrasound imaging of both the mothers' and offspring heart and vessels whilst still in the womb greatly advances our understanding of blood flow and therefore the availability of nutrients to the developing embryo.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 304. Targeted therapies to modulate inflammation in alcohol-induced injury</b>
<b>Key Words</b>	Liver, alcohol, hepatitis, therapy, inflammation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

**Clinical Need :** In common with much of the developed world, the UK is currently experiencing a rapid and dramatic increase in mortality from liver disease. Mortality from liver disease in the under 65's has risen 500% since the 1970's, with 80% of these cases presenting as an emergency, either because of alcohol-related liver damage or decompensated cirrhosis. This means that the cost to the NHS linked to alcohol-related liver disease is estimated at £3.5 billion per annum.

The most severe form of alcohol induced liver disease is alcoholic hepatitis (AH), characterised by a rapid onset of jaundice and/or ascites following alcohol consumption. This is particularly challenging to treat and up to 65% of patients will die within 1 month. Importantly, the current therapeutic gold standards, namely administration of corticosteroids and pentoxifylline have recently been shown to give NO improvement in three-month or one year mortality in a large multi-centre trial. For many, the only option is transplantation, which is ethically sensitive in actively drinking individuals. Thus we are a population with rising alcohol consumption and very little in the way of non-transplant therapy to treat those who succumb to liver damage. This is important because there is currently no proven effective therapy for treating AH

**Our solution :** We wish to apply our knowledge of the molecular pathways that cause liver inflammation in response to alcohol consumption to gain a wider understanding of alcoholic liver disease and design new therapies for patients.

The overall purpose of our project is **to understand whether it is possible to target the processes of inflammation in order to treat alcohol-induced liver disease.**

Thus we wish to use a mouse model of alcohol-induced liver injury to address the following specific aims

- i) To understand the contribution of platelets to development of and recovery from alcoholic liver injury
- ii) To understand the contribution of white blood cell populations to development of and recovery from alcoholic liver injury
- iii) To test whether inflammation and liver damage following administration of LDC/ethanol can be modified by administration of therapeutic agents

These studies will be informed by our prior identification of candidate molecules in both human and murine mouse models.

We also regularly review the scientific literature to ensure that we are using the most refined animal models and so that we can respond to new developments in model design, particularly where newly emerging *in vitro* techniques could replace animal 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)?**

This work will significantly enrich the knowledge base in our field of expertise as it is directly intended for testing novel molecular interactions with the potential to translate to clinical treatments using novel compounds or new targets for existing drugs. Our mechanistic knowledge will be important for the scientific, medical and pharmaceutical communities. We also hope to identify new treatments that we can use in patients with alcohol-related disease. This is important for patients because not all will respond to current treatment options. We are primed to move rapidly into early phase clinical trials through the NIHR Biomedical Research Unit with the partnership of the pharmaceutical industry. Our pioneering studies have already illustrated common mechanistic regulators of disease in several organs and extension of these studies has the potential to not only identify new therapeutic targets but also to extend the licensed use of pre-existing therapeutics. Thus our data is thus likely to be used by basic scientists and clinical scientists to inform the design and outputs of their own experiments. As required by our funding partners, data originating from these studies will be published in high impact scientific journals confirming with the ARRIVE guidelines provided by NC3Rs, and presented at national and international symposia and conferences. Thus benefits from our work include transfer of knowledge, training opportunities for future clinicians and academic scientists as well as improvements in treatment for UK patients and the healthcare industry.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice for our experiments and expect to use up to 800 over the five year term 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 levels of severity? What will happen to the animals at the end?**

This model is classed as severe because it is necessary to induce liver injury and inflammation in our mice. We would expect all of the mice in the untreated injury groups to exhibit some degree of weightloss (<15%) and deterioration in condition (ruffled coat and reduced mobility) for a transitory period after alcohol administration. All animals are humanely killed at the end of the experiment.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

The complex disease pathways we are interested in involve the interaction of several cell types and regulatory signals that are hard to recreate *in vitro*. We also do not have access to samples from humans in all stages of alcohol-induced injury. Mice share the main components of their immune systems with humans, and established alcohol injury models recreate the patterns of disease seen in humans. A wide range of genetically manipulated strains and therapeutic reagents are available for mice and thus they are the best model for us.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We have prior experience using the model, which will inform the design of experiments in this study. Importantly we have noted inter-individual variation in response and so our experiments are powered to take this into account and in conjunction with our local facility we have devised a flexible dosing approach based on clinical scoring to maximise our outputs and minimise animal loss. We have built in checks in our workflow to ensure that experiments do not progress if statistically significant results are not evident upon an intervention. Similarly experiments run serially with outcomes from initial animal groups informing the design of subsequent experiments. For all experiments the scientific team meet regularly to discuss data and seek advice from local statisticians and clinical staff. Importantly our experimental design strategy is informed by use of the NC3R's experimental design assistant (EDA : <http://www.nc3rs.org.uk/experimental-design-assistant-eda>)and in

conjunction with adherence to the ARRIVE guidelines, to ensure the minimal numbers of animals are utilised in order to gain valid experimental outputs.

Many of the molecular pathways we investigate operate in more than one organ. Therefore to maximise the useful information we can collect from each animal, we will collect blood, liver and other solid organs. These samples can later be used to investigate the wider significance of our pathway or therapeutic intervention. We work closely with collaborators at other institutes and have a policy to ensure-tissue is shared with our colleagues so that maximal use is gained of each individual animal and that new knowledge generation is facilitated.

### **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

Although many alcohol injury models are used worldwide, few both recreate the histological picture seen in humans AND meet the strict welfare conditions we adhere to in the UK. We have chosen a model that is quick to perform, recreates human alcoholic hepatitis, and has been refined by our past experiments. This means we individually tailor our monitoring and alcohol exposure to ensure weightloss and loss of condition are minimised.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 305. Models advancing knowledge and treatment of paediatric brain cancer</b>
<b>Key Words</b>	Paediatric, brain, Cancer, Treatment, Biology
<b>Expected duration of the project</b>	2 year(s) 9 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our main objective is to understand the development of and find new treatments for four types of paediatric brain tumours.

Ependymoma (EPY), Medulloblastoma (MB), High-Grade Glioma (HGG), and Choroid Plexus Carcinoma (CPC) are the most common solid cancers to affect children. Each presents a unique set of clinical challenges, and all require new treatments. With few exceptions, childhood brain tumours remain one of the biggest killers from disease in children and require aggressive surgery, radiation and chemotherapy that have changed little in several decades. Radiation is especially damaging to the developing brain and results in devastating long-term cognitive side effects for survivors. Fewer than 70% of all patients are cured following initial therapy and once these tumours recur they have a dismal prognosis. Importantly, Ependymoma and CPC are relatively insensitive to chemotherapy and there is therefore a great need for effective new treatment strategies. By investigating the development of these detrimental diseases, we will advance the understanding of the underlying biology of all four tumour types. By exploring new and innovative treatment strategies we will hopefully be able to translate new treatments into the clinic.

HGG is a particularly lethal form of childhood brain tumour, the origins of which are only recently beginning to be understood. In order to direct the design of new treatments further study is needed to understand how the tumours initiate and how they progress. The recent development of mouse models for this disease type is a critical step in that process. The research proposed in this project will use these models to fill gaps in knowledge about the disease and be crucial in understanding the mechanisms of acquired resistance to 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)?**

Our project holds great promise to make fundamental and much needed progress in advancing understanding of the origins, biology and treatment of paediatric brain tumours. The benefits of this project are numerous and include but are not restricted to: (i) advancing the knowledge of four paediatric brain tumours (ii) Provide brand new and repurposed drugs for clinical testing.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will be working with mice (including genetically engineered mouse models). We expect to use around 20,665 mice over the licence 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 levels of severity? What will happen to the animals at the end?**

A number of the animals required in this project will be used to breed subjects for further study. Animals used purely for breeding and that do not undergo any procedures except for ear notching will be humanely killed when they are no longer required. Some of the animals will develop adverse effects, including cancer as a result of their genetic makeup or because tumour cells have been implanted and allowed to grow. This may require administration of an inducing agent or a virus that will switch on/off particular genes. This administration will only cause momentary discomfort, but all animals will be monitored closely for clinical signs related to tumour growth, including loss of 15% body weight, limited normal behaviour, loss of movement on one side. Animals in distress will be humanely killed. Tumour development may be monitored using imaging techniques, such as MRI scans. These methods may require anaesthesia and/or administration of imaging agents, which will not result in any harm to the animals. Some of the animals that develop tumours will be treated with surgical resection and/or irradiation, in order to mimic the clinical standard. Many of these will go on to receive treatments with anti-cancer drugs, including potential new therapies. All animals on treatment studies will be closely monitored and may be blood sampled to follow uptake of the drugs which should only cause only mild momentary discomfort. Any animal that displays clinical symptoms such as those listed earlier, will be humanely killed. At the end of the study all animals will be humanely killed, and tissues collected post-mortem to gather as much information from the study as possible.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

## Replacement

Because our approach requires the use of specific cancer-susceptible cell types at specific points in development, this is currently only possible by using live animals that fully recapitulate the complexities and cell populations present in development.

Regulatory and research bodies require preclinical assessment of potential therapies in animal models prior to their translation to the clinic. Therefore, our translation of optimal new therapies to the children's cancer clinic requires the animal studies proposed here. Nonetheless we will continue to use *in vitro* drug sensitivity studies, including radiation/chemotherapy combination studies *in vitro* to optimise the selection of agents and thereby minimise the use of animal models in exploratory studies.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

## Reduction

Our use of *in vitro* methods limits the number of animals required for the *in vivo* investigation stage. Furthermore, each of our *in vivo* mouse model experiments has a careful statistical design that is aimed at minimising the use of animals while ensuring robust and meaningful statistical end points. These animal numbers are selected in collaboration with our statistical colleagues and our extensive experience with brain tumour mouse models. In addition, we have optimised the use of material from each mouse, often harvesting fresh cells for culture, frozen tumour for RNA and DNA studies and fixed material for histology from the same animal.

## Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

## Refinement

Mice are the only species employed in our protocols. They are the least sentient species that best fit our criteria for the following reasons: Their lifespan (approx. 2 years) allows for "development to humane endpoint" studies; the scientific community has a range of techniques to manipulate the mouse genome, allowing us access to many transgenic/knock-in/knock-out mice with which to answer specific key questions; mouse gestation is less than three weeks, and embryogenesis in this species is extremely well documented in the literature, allowing us to look at the effect of genes on normal brain development. Non-animal models cannot recapitulate the complex context existing in developing tissues in which cancers actually form and are treated. The advancement of knowledge and development of concepts to improve human and animal health and well-being requires the use of

living animals. Exhaustive literature searches in brain tumorigenesis show that our tumour systems are the most accurate models for the study of these diseases.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 306. Developing an ocular melanoma model for drug discovery</b>
<b>Key Words</b>	ocular melanoma, uveal melanoma, Patient derived xenograft, MEK
<b>Expected duration of the project</b>	2 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Uveal melanoma is a rare form of cancer that arises from the eye. Unfortunately it often spreads to the liver and treatments are mostly ineffective. In this project we hope to find out how and why this cancer is resistant to a type of drug called selumetinib. We anticipate that the results of the study will help us design new combinations of drugs (including selumetinib or related drugs) that will overcome this resistance.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Uveal (also known as ocular) melanoma is a rare cancer arising in the eye. Unfortunately it spreads to the liver in about half of all cases and is invariably fatal. At present there are no established therapies for uveal melanoma, and resistance to drug therapy is very common. We intend to decipher the main mechanisms by which this occurs thus developing new combinations, which may help control the disease and prolong survival.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use approximately 300 mice 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 levels of severity? What will happen to the animals at the end?**

The severity limit of the experiments is moderate, and most animals will have limited adverse events. All animals will have tumour cells implanted under the skin and these will be allowed to grow up to about the size of a pea. Some animals will have

drugs given to them which may cause some adverse effects, however in all cases the side effects will have been established previously and a dose used to minimise these. At the end of the experiment the animals will be killed humanely and tissue extracted for experiments.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

Wherever possible we will perform experiments in cell lines or directly on human samples. However, these experiments cannot model many of the effects of growing tumours inside humans such as the presence of other cells, growth of blood vessels into the tumour and varying concentrations of oxygen and nutrients in differing parts of the tumour.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will only use enough animals to establish our model systems and for the purposes of our assays. Where we perform experiments to contrast different treatments, we will perform calculations to identify the minimum number of mice needed to show a meaningful result.

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

#### Refinement

While simpler organisms may be used to perform experiments on the basic biology of cells, the experiments we will be performing need us to be able to grow tumours derived from humans. Mice are the simplest model system, which will allow us to do so and then treat with drugs. We will monitor the condition of mice daily and weigh at least weekly. Where animals appear sick we will observe more closely, and if they are not recovering, the animals will be euthanized. Tumours will not be allowed to grow beyond a specific limit which has been established in previous experiments. Where drugs are given, these will be used at a dose that has been shown to be tolerated well by animals without significant adverse effects.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 307. Neuroimmune mechanisms of CNS degeneration and regeneration</b>
<b>Key Words</b>	Neurodegeneration, Brain repair, Immune system, Infection, Microglia
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The immune system is essential for protecting us from infection but there is also growing evidence that it can have both harmful and helpful functions in the brain that can influence the risk of brain disease and the response to injury. Brain tissue is particularly sensitive to some forms of inflammation but conversely, some types of inflammatory activity are needed to help the brain heal after injury. The molecular and cellular mechanisms which influence the balance between harmful and helpful actions of the immune system in brain disease and injury are not well understood. It will be vital to better understand these so that the damaging effects of the immune system can be targeted to treat brain disorders without affecting the helpful functions, such as protection from life-threatening infection.

The aims of this project are to:

1. Identify key cells and molecules that control and cause the harmful and helpful effects of the immune system on brain injury and disease
2. Test if manipulating these cells and molecules can minimise the damage and complications caused by brain injury and disease and enhance brain repair and recovery

**What 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 the project will lead to a major step forward in knowledge about how the immune system positively and negatively influences brain injury and disease. This new knowledge may include the identification of new therapeutic targets, that with further development beyond this project, could have the potential to reduce death and disability caused by brain injury and disease. For example, the information

produced in this project could inform and lead to future testing of therapeutic agents in human trials. The new knowledge we create will be important for the progression of our own studies but will also help other scientists and medical professionals to develop their own work thus ensuring that progress towards treatments of brain injury and disease can be made as quickly as possible. We will share our data where appropriate so that this is possible. It is likely that the work we do in the project will develop and refine existing methods and techniques and potentially produce new approaches. These could benefit the scientific community by providing better ways to gain insight to how the brain is damaged. It is also possible these developments will help to further reduce the risk of adverse effects on animals used in research.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use around 4000 mice overall and around 50 larger animals (e.g. pigs) 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 levels of severity? What will happen to the animals at the end?**

Adult animals will be used and will be housed in spacious and well-maintained cages within a dedicated animal facility and provided with free access to food and water at all times. Trained animal care staff will ensure that best practices of animal husbandry are applied. Our project is aiming to understand processes that influence brain injury and disease therefore animals will undergo procedures that are designed to replicate aspects of the human conditions e.g. stroke, Alzheimer's disease. Some experiments will involve the surgical blocking/narrowing of blood vessels supplying the brain or the precise injection into the brain of substances that cause cell death or inflammation. Experimental infection may be achieved by injection of bacteria into the nasal cavity. Mice may be irradiated to deplete bone marrow cells and then receive bone marrow transplants from other mice. Brain scans will be performed on mice and pigs. The above procedures will be performed under general anaesthesia. Other procedures will include blood sampling from superficial vessels, administration of drugs or fluids by injection (usually into the abdominal cavity or under the skin) or into the food or drinking water. We will also assess the memory and other thinking skills of mice by placing them in experimental mazes. For many procedures, there is likely to be only a transient impact on the animal and a rapid return to normal behaviour without any intervention e.g. blood sampling, drug injections, brain scanning. For surgical procedures and those causing brain injury/disease there is the potential for the following: • Change in eating and drinking habits • Weight loss • Signs of neurological injury e.g. limb weakness, memory problems • Impaired movement The duration and frequency of these will depend on the individual experiment but it is expected that weight loss and eating/drinking habits will be commonly observed and recover within a few days. Signs of neurological damage may persist permanently (as they often do in humans). Death is not an expected

outcome and animals that are not able to perform normal functions will be humanely killed.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

Many experiments will not require animals because we can use cells cultured in the laboratory, computer simulations and analyse samples taken harmlessly from patients. However, we need to use live animals for some experiments because it is not possible with current knowledge to recreate the complex interactions between the brain and immune system in isolated cells or computer models.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Each animal will be used for a procedure or set of related procedures only once.

We will design experiments in a way that ensures the minimum number are used – this will include using statistical methods that can accurately predict the number of animals necessary to meet experimental objectives based on previous data collected. Experiments will be conducted and data collected in ways that minimise the introduction of confounding variation

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

#### Refinement

Almost all studies will use adult mice. Although not identical, mice have many things in common with humans in the way that their brain and immune cells function that means information from mice can be used to predict how the same processes work in humans. Experimental reagents and methods for data collection are also most advanced for mice.

We will use a small number of larger animal species (pigs) for selected experiments because they provide a bridge between the anatomy/physiology of rodents and humans thus further improving predictions from animals to humans. The larger brain

can also enable study of certain aspects of neuroimmune function not possible in rodents. We predict that using a small number of pigs may enable many fewer mice to be used.

Brain injuries and diseases in humans can be devastating conditions therefore to accurately mimic aspects of these conditions it is necessary to use animal models that cause brain damage and changes to brain and body function resulting from this. However, we will do this in the most refined way possible with the minimum severity and follow well established care protocols to minimise the frequency, duration and severity of adverse effects. This will include the use of anaesthesia, regular monitoring and recording of animal health by trained staff, routine administration of fluids to maintain hydration, administration of drugs to provide pain relief in consultation with vets, enrichment of cage environments to encourage eating and drinking, and the use of defined humane limits that will not be exceeded. Throughout the duration of the project, we will be responsive to further developments within the scientific and animal husbandry communities that could be applied to further refine experiments.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 308. Production of polyclonal and monoclonal antibodies</b>
<b>Key Words</b>	Monoclonal, polyclonal, antibody
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Development of novel antibodies for research

**What 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 tissues and antibodies produced from the animals used in this licence will enable a wide range of in vitro or ex vivo studies to be undertaken. These include development of potential clinical applications relating to immunotherapy and cancer treatment. Other benefits are related to the development of new diagnostic reagents for understanding of diseases in medical research.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

600 mice and 100 rats

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

Animals will be injected with reagents to produce an immune response. This should not result in any adverse effects for the animal. Animals that remain conscious during blood sampling or are immunised to produce antibodies and other immunologically related cells and tissues will experience the skilled insertion of a hypodermic needle or the minor puncture of a superficial blood vessel. Transient inflammation or irritation may be experienced around the injection site. However significant adverse effects are not expected to occur and the level of severity is classed as Mild. At the end of the protocols the animals will be either humanely killed for the collection of tissue and cells or undergo deep surgical anaesthesia in a non-

recovery process to obtain maximum amounts of blood to containing the valuable antibodies resulting from the immunisation schedule

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

Antibodies are produced by a living immune system involving activation of specific cells in response to antigens e.g. infective micro-organisms or in a laboratory situation specific molecules e.g. proteins.

This means that laboratory animals of excellent health status and known genetic background are required to produce the highest quality of antibodies for research.

We are aware that this is an area where there is a great deal of research into developing non animal alternatives for antibody production such as phage display. At the moment these have not shown sufficient sensitivity for us to use in all the areas of research we undertake. Often we require a whole molecule so that we can work on the effector functions of the Mab.eg complement lysis of bacteria or killing/non proliferation.

However over the course of this licence further new technology may well emerge so we will review the literature for non-animal alternatives before undertaking any new work and will only use an animal model when an alternative is not suitable

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

For monoclonal antibody production, extensive experience has informed research groups that , to ensure a good humoral response is obtained in at least one mouse, a minimum of 3 mice per group are required. Smaller groups may lead to waste of valuable antigen, delay in obtaining valuable antibodies if no response is obtained, and requiring repetition with the use of more animals.

For polyclonal antibody production, where experience indicated a particularly good immune response can be obtained from the antigen then 3 animals per group may be used. In mice the less specific response in means that typically 3 mice will be immunised for monoclonal antibody production when only small quantities of antibodies are required or only small quantities of antigen are available to stimulate the immune response

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

The animal species to be chosen for antibody production is determined by the type of antibody required, the volume of serum required, the nature of the antigen and the likelihood of an immune response by the species. Mice and rats are typically chosen for immunisation to provide the required specific antibody producing spleen cells for monoclonal antibody production and can be also used for polyclonal antibody production where only small volumes of serum are required.

Animals will typically be group housed and monitored at least once per day by a trained and competent animal technician. Bedding and environmental enrichment will be provided for all animals to enable them to live normal, good quality lives. Experimental procedures may involve a limited number of injections and/or small blood samples (the latter using local anaesthesia) over a period of several weeks. These will be conducted according to best practice guidelines by trained and competent staff. Procedures will be classed of being of Mild severity and have only a transient impact on the animal. Any concerns regarding the health or welfare of an animal will be discussed with the Named Veterinary Surgeon or the humane killing of the animal. At the end of the procedures animals will be killed using a recognised humane method detailed in Schedule 1.

After every experiment we critically appraise what we do to seek out any ways to improve our models to reduce harm to animals. This strategy has been highly successful and our models continue to show improvement in this area.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 309. Strategies for Brain Repair</b>
<b>Key Words</b>	Brain repair, transplantation, neuroscience
<b>Expected duration of the project</b>	0 year(s) 6 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

This project seeks to develop novel strategies for treatment of brain damage, whether caused by injury or disease, with a particular focus on the development of novel cell and gene therapies for Parkinson's disease (PD), Huntington's disease (HD) and stroke

**What 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 underpins clinical trials of fetal tissue transplant-ation in HD and PD taking place now, and provides the biological foundations for the next generation of major new applications using more efficient sources of cells, including pluripotent stem cells.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Rats and mice. The project will use approx. 300 rats and 500 mice over 6 months.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

The project involves surgical, anatomical, physiological and behavioural procedures of mild, or at most, moderate severity, including breeding genetically modified animals, that express modest impairments of motor and cognitive disability, that are the targets for structural repair and functional amelioration. The experimental procedures are reliable, and serious adverse effects are rare and not expected, but procedures are in place for rapid alleviation of distress in the case of unexpected adverse events being detected. All animals are killed at the end of each experiment by the most humane methods appropriate to the species.

**Application of the 3Rs**

## **Replacement**

State why you need to use animals and why you cannot use non-protected animal alternatives

### **Replacement**

Motor and cognitive behaviours are complex features of the living sentient animal, dependent upon the intact functioning of a complex living nervous system, and impaired in human neurodegenerative diseases. The survival, growth and connectivity of cells in this complex environment cannot be adequately modelled in vitro or in simulation. Thus, in order to develop effective new cell-based therapies for devastating human conditions, the experimental use of live animals is the only way to model the disease processes, to determine the survival integration growth and connectivity of cell repair processes, to test the effectiveness of alternative cell therapy procedures, to develop the transplantation technology and to test protocols for safety and efficacy prior to human application.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

All protocols are designed for maximum sensitivity, and experiments are designed to maximise power to detect significant results with the smallest numbers of animals achievable. Non-animal alternatives e.g., tissue culture are used to optimise all cell preparation protocols prior to assessment in animals, but ultimately the in vivo situation cannot be avoided if the goals for human health are to be achieved.

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

The organisation of motor and cognitive functions and of the brain systems that underpin them are relatively consistent among mammalian species but differ progressively from non mammalian brains. Rats and mice are used as the least sentient mammals to model the relevant systems and functions disturbed in human neurodegenerative disease. These species tolerate well living in the laboratory environment, and provide the most extensively validated models for addressing the physiological, anatomical and behavioural functions under investigation. All animals are housed in licenced facilities and cared for by professionally trained staff following procedures designed to optimise health and welfare, operating under a rigid

inspection system to ensure compliance with full and continuous attention to welfare regulation and best practice.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 310. Hypoxia and Cancer: Molecular Mechanisms and Therapeutic Strategies</b>
<b>Key Words</b>	Hypoxia, cancer, metastasis, immunotherapy
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The understanding of the relationship of oxygen to cancer is key to a better understanding of cancer progression. Work by many scientists has shown that cancers are typically lacking in sufficient oxygen (a condition known as hypoxia), and that this lack appears to drive cancer dissemination to distant sites in the body, or secondary cancer (metastasis). Metastatic disease is the leading cause of death for cancer patients. We have shown that the mechanisms and molecular players activated in response to hypoxia during cancer growth and dissemination play an essential role in allowing or preventing the cancer progression, and in fact activate different cell types in different ways. During the tenure of this license, we aim to manipulate the hypoxia pathway in a way that elucidates what is necessary for tumour growth and colonization of distant organs. We will also determine how hypoxia affects the ability to treat those cancers and prevent secondary disease, namely by exploring the role of both the immune cells, which can be activated to remove and kill cancer cells, and the blood vessels, which allow the transport of tumour cells from the site of origin to other organs. By increasing our understanding of how the response to low oxygen increases cancer progression, and which cells are responsible for than phenomenon, we expect to find tools circumvent or avoid those responses to both treat cancer and prevent metastasis.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

Most current therapies are very toxic, and cause a great deal of damage to non-cancerous tissues and organs, often also resulting in resistance and refractory disease. Also, there are no therapies to specifically target secondary cancer. Understanding these pathways will help us predict cancer progression as well as

specifically target the treatment type, the treatment duration and time frame, to specific patients and specific cancer types, potentially avoiding or reducing the use of cytotoxic drugs in some cases. Targeted therapies that are specifically focused on individuals and their cancer type would greatly minimize the often devastating side-effects of treatment and increase efficacy; Our new findings in immune cell activation have great potential to transform this therapeutic avenue by making it applicable to cancer types that so far have been considered unresponsive to this approach.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice will be used exclusively. We will use approximately 20,000 mice over the five year period of this protocol.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

Animals will develop tumours, but in most models these will not cause any pain or discomfort within the time frame of the experiment. We will also use early humane endpoints as well as pain relief in order to prevent any unnecessary animal suffering. All mice will be humanely killed at the end of these experiments and tissues taken for further analysis.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

Cancer is a very complex disease, and progression of this disease cannot be fully modelled in any other system than an animal. Unfortunately, growth of cells in dishes cannot recapitulate the complex interactions necessary for the development and maintenance of cancer. We do use cell cultures to test simple hypotheses, and when feasible, to better understand how molecules that affect cancer growth interact with each other.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Pilot experiments are always performed using a small number of animals to refine the experimental procedure and design, so as to minimise overall animal numbers used.

Any questions that can be answered using isolated cells, or combinations of cell types, will be preferentially used so as to avoid unnecessary use of animals.

We will only produce mice in response to very specific and required experimental demands.

### **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

Mice are ideal models for the studies we propose within this license for several reasons, including their high physiological and metabolic resemblance to humans, which makes cancer origin and progression in these animals similar to that seen in humans, and the research results likely applicable for further applications in human disease.

We use the earliest endpoint possible to stop experiments, that still allows scientific value. We use routine monitoring of mice that may develop tumours to ensure that animals only develop cancers to pre-determined stopping points. We randomise and blindly assess results so as to avoid biases and confounding factors, and determine the endpoint of experimental animals using their welfare as the primary criteria.

Animals will always receive pain relief and anaesthetics if and as needed.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 311. Bioelectronic Medicines</b>
<b>Key Words</b>	Electrophysiology, Implantable Devices
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The purpose of this project is to understand how implantable devices and electrical signals can be used to regulate the nervous system to treat disease and organ dysfunction.

To do this we must first gain a better understanding of the anatomy (**Protocol 1**) and function (**Protocol 2**) of the nervous system, and how it exerts control of organ function. Secondly we must ascertain whether electrical regulation of the nervous system can be accomplished safely and effectively over time (**Protocol 3**). Thirdly we wish to apply this regulation treatment in animal models of disease, such as diabetes (**Protocol 4**), infertility (**Protocol 5**), and arthritis (Protocol 6), to understand the magnitude of treatment effectiveness.

**What 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 molecular medicine treats a wide range of ailments in billions of people. However, there are a multitude of side effects and treatment resistant populations. Additionally continual reliance on molecular medicine is expensive, socially limiting, and in most cases only a treatment and not a cure. The potential for Bioelectronic medicine is huge. Through implantation of devices that regulate the nervous system, and in turn the target organs, one can reverse organ dysfunction and disease states completely. We have chosen to focus initially on 3 disparate diseases that we have clear reason to believe that bioelectronic medicine could be a success, therefore ultimately improving people's quality of life. Type 2 Diabetes is wide spread, and will continue to devastate people's lives in the developed and developing world. Reliance on molecular medicine is debilitating. Polycystic Ovarian Syndrome affects millions of women and currently has no clinical treatment options. Rheumatoid Arthritis is a chronic progressive inflammatory condition affecting millions.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mouse (550 over 5 years) and Rat (2750 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 levels of severity? What will happen to the animals at the end?**

Work will initially involve non-recovery models for assessment of anatomy and physiology, and by definition no side effects will be encountered. Recovery studies will only be initiated once correct surgical approach and treatment refinement has occurred in non-recovery models. The expected adverse effects are associated with the 3 disease models, and they will manifest as hyperglycemia and weight increase (T2D), loss of ovulation (PCOS), and joint swelling in the limbs. This will not lead to any behavioural effects in these animals. Implantable devices will be thoroughly refined and miniaturised for use in rodents. The only expected side effects are related to post-surgical complications, such as infections, broken sutures, local inflammation and swelling. Levels of severity will not exceed moderate. All animals will be killed by a schedule 1 or perfusion-fixation methods.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

A limited amount of testing has been done without using animals to give confidence nerve stimulation may treat disease. The science cannot be advanced further without using animals. Only a whole body system biology approach will give conclusive evidence and understanding that manipulation of the nervous system can be an effective treatment of disease.

A computer model does not yet exist to test nerve stimulation as a treatment of disease.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Pilot studies in small numbers of animals (e.g. 1 to 3) will be used to develop optimal methods, assess feasibility and outcome measures, and to estimate required group sizes for larger studies. Statistical advice will be sought on adequate animal numbers for each recovery study. Imaging techniques will be used to monitor evolutions within the same animal over time.

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

Rodents will be used for all experiments because they are the lowest order species with a nervous system that is anatomically and functionally similar to that of humans.

Aseptic surgical techniques, anaesthetics and pain preventing medicines will be employed to minimise potential of post operative infection and pain. Veterinary care will be provided throughout.

We will work with manufacturers, to ensure a continued refinement approach is adopted for all implantable devices, electrodes and leads. We will work toward fully implantable devices as advancement to percutaneous leads or head caps. We will always use the least minimally invasive option for each study.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 312. Pre-clinical Pharmacology of Inflammatory Disease</b>
<b>Key Words</b>	New drugs, Inflammatory disease, Translational
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	<b>Project 313. (iii) improvement of the welfare of animals or of the production</b>

## conditions for animals reared for agricultural purposes.

**Yes**

(c) 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 paragraph (b);

**No**

(d) protection of the natural environment in the interests of the health or welfare of man or animals;

**No**

(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

**No**

(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

**No**

(g) forensic inquiries.

### **Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

With the increasing incidence of inflammatory respiratory diseases and gastrointestinal inflammatory diseases there is an ever increasing problem both in terms of global economic impact caused by these diseases, but also on an individuals quality of life, which is impaired through underlying pain and social impact of the disease. Therefore there is clear need for research to develop improved and novel treatment option. Therefore the aim of this project will be to test novel agents/drugs to treat such inflammatroy diseases as part of the pre-clinical drug development process. From this work, efficacy of novel agents/drugs will be established and used to asssit in identifying agents/drugs for further evaluation in early clinical studies.

### **What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The ultimate benefit from this project would be the identification of drugs that can potentially treat respiratory and gastrointestinal inflammatory disease, which then successfully progress through human clinical trials. Other benefits are refinement of the disease models so that they are more effective predictors of drug efficacy in humans.

### **What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 12000 mice, 2000 rats, 1200 guinea pigs and 100 rabbits 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 levels of severity? What will happen to the animals at the end?**

Inflammation of the lung or gastrointestinal tissue will be induced with inflammation inducing substances administered either locally or systemically. Following the dosing with these substances it is expected that the animals may exhibit changes in appearance and behaviour, e.g. become more subdued, un-groomed, suffer from diarrhoea (gastrointestinal inflammatory studies) and lose weight, as well as experience transient respiratory depression. We are not expecting these adverse effects to go beyond moderate severity and expect that they will be transient in nature if observed. If these are seen the animal will be closely monitored and humanely killed if no improvement is seen or the condition deteriorates. The animals may also be dosed with established or test substances, for which information on any adversity will be sought prior to commencing studies. Therefore no adverse effects are expected but, as for all studies under this licence, some test substances may result in unexpected adverse effects. Again, if these are seen the animal will be closely monitored and humanely killed if no improvement is seen or the condition deteriorates. At the end of the protocol the animals will be humanely killed and their lung or gastrointestinal tissue analysed to ascertain if the medication has reduced the inflammation.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

Prior to animal studies, tests on cells can be carried out to get an idea of the toxicity and the efficacy of a drug on the target cell type, but animal models are still needed in order to identify the effects on the whole body. It is possible that the influence of, and processing by, a multi-organ system will alter the behaviour of the drug.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Experimental study designs will use the minimum number of animals to achieve meaningful results based on valid statistical calculations such as power

analysis. Design of such studies will also look to minimise data variation by using randomisation and blinding techniques where applicable.

Where possible all scientific readouts will be harvested from one animal in order to reduce further animal numbers.

### **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

Inflammatory mechanisms in guinea pigs, rats and mice are well characterised and resemble what is seen in humans. Similarly the models and methods are well characterised in the literature and have been extensively used over the last 20 years by the company to provide an adequate inflammatory response in the target organs for pharmacological manipulation without causing undue pain and suffering to the animal.

Anaesthesia will be used where appropriate during procedures to reduce suffering and analgesia will be given following any surgical intervention.

The animals will be closely monitored following procedures, and checked regularly throughout the course of a study with clearly defined end points and limiting clinical signs so that any adversity is spotted

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 314. Determining dynamic changes in tumour infiltrating lymphocytes to enhance checkpoint blockade therapies</b>
<b>Key Words</b>	Tumours, checkpoint inhibition, T cell, migration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

The aim of this project is to provide fundamental new information on the movement of immune cells into and out of tumours and how the cells change over time. This basic information of how cells change over time is crucial in improving therapies, but is difficult to generate without new approaches. Using a mouse model that we have used in the lab to assess immune cell migration, we will apply this knowledge to understanding migration and changes in the cellular response to tumours. There has been a huge advance in therapeutic treatment of cancer, an approach called 'checkpoint inhibition'. This therapy can have fantastic effects, but only some patients with certain tumours currently respond. This really suggests that we do not understand enough about what is happening in some tumours and if we knew more we could better direct the therapy. Understanding cellular movement is important as it can tell us whether certain cells preferentially move into and out of tissues or are specifically retained in a certain location. This understanding can then help to determine how different immune cells can exert their effects. Through understanding when certain cells have entered a tumour, we can assess changes over time which can reveal important information of how the immune cells are responding to or interacting with the tumour. Overall, these data will help to optimise existing therapies and potential develop new approaches to enhance anti-tumour 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)?**

The key benefit from this work is new data concerning the movement of immune cells into and out of the tumour and the immune tissue in which the response to the tumour is made. This information can only be generated using new mouse models which enable labelling of cells at specific sites. We will use our expertise to provide

new data to scientists and pharmaceutical companies on the movement of cells into and out of tumours and how the cells change over time. Our data will certainly reveal how molecules that may become therapeutic targets change over time. We will also demonstrate which cells are resident and which cells are migratory. Other scientists and pharmaceutical companies can use this information to refine their therapies or design alternative approaches. A lot of the work done in this Licence will be in collaboration with industry to ensure that the knowledge gained can be rapidly translated to therapeutic strategies. Armed with this knowledge, we can develop more refined efforts to enhance the immune response to tumours, ultimately to widespread benefit in patients through better therapeutic approaches.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Approximately 3500 mice will be required to perform the planned experiments over the 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 levels of severity? What will happen to the animals at the end?**

Animals used in these experimental procedures will be given tumour cells under the skin resulting in the formation of a local tumour at this site. These tumours do not spread so discomfort to the animal is minimal. This will cause local discomfort at the site of the tumour and inflammation at this location. Some mice will receive carcinogens on or under the skin to induce local tumour formation. This approach will irritate the local skin and cause discomfort. The mice will then experience a local tumour at this site with the discomfort associated with this. In some experiments mice will undergo minor surgery including exposure of the kidney to graft tissue under the kidney capsule. Mice will only undergo one form of surgery. Mice will be handled frequently (ranging initially from approximately three times per week, increasing to daily towards the end of each study). During these times animals will be injected and / or monitored for tumour burden. Through good handling techniques, distress caused to the animal from being restrained will be minimised in terms of time and discomfort (a single animal will typically be restrained for less than 30 seconds). Mice will be monitored for tumour burden frequently and tumours will be scored for size, position and ulceration. Should tumours limit mobility, appear ulcerated or reach a maximum permissible size, animals will be humanely killed by a schedule 1 method. Mice will be humanely killed by a schedule 1 method when the tumour reaches a certain size (1.2 cm mean diameter).

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

## **Replacement**

This project requires animals as we seek to understand the movement of cells into and out of tumours to inform treatment of cancer in humans. Such interactions cannot be modelled in vitro due to the many complex parameters and multiple three-dimensional environments involved. Thus we require an in vivo approach to recapitulate the complex situation present in patients. We can use existing mouse models to accurately assess cellular migration in several different tumour models. These have been selected as they are currently used to assess therapeutic treatments that have been demonstrated to work very well in some patients.

We regularly review the literature to keep informed of any new developments in experimental approaches that might enable the replacement of animal experiments with in vitro work.

The use of the animal models described means that our data can rapidly inform current treatments, since the models used are currently those that inform clinical work. The insight our work can provide into the action of checkpoint inhibitors is of a very broad applicability to cancer treatment and is fundamental information that can improve our understanding of how these therapies work and how they can be improved.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

Small scale pilot experiments will be used to establish models building on expert advice from collaborators using these approaches. Experiments will be designed following NC3Rs EDA guidelines, using power calculations and previous advice from in-house statisticians. We reassess group sizes as our experience with models develops and we will continuously look to use the minimal number of mice that provide robust experimental data. I have been publishing the results of my in vivo analyses of immune responses for over 12 years in high impact journals reflecting the expertise we have in the appropriate design of this type of experimental work. Publication of this work requires peer-review and this process ensures robust assessment of our experimental work and dictates that our experiments are well performed. We will continue to do this. Should further assistance be required we will reach out to local statisticians and/or the local NC3Rs advisor.

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

## Refinement

### **Choice of Species:**

The mouse provides an excellent model in which to study the relationship between the immune system and tumour growth, since mice are well characterised immunologically, and their immune systems closely resemble those of humans. In addition, several genetically modified mice lacking various immune molecules/cells have already been generated and provide an ideal opportunity to perform detailed analyses of immunological function. Fundamental to this study is the use of specialised mice in which violet light can be used to label cells at a specific site to allow direct analysis of cellular movement. This in turn enables the dynamic changes in the cells to be assessed and factors affecting this movement to be precisely tested.

### **Choice of Models:**

Tumour models that are proven to work in the mice have been selected based on the suitability for use in our mouse models and the use of these tumour models by many labs to inform treatments of human patients.

### **Minimising Animal Suffering:**

In all procedures animal suffering will be minimised through good animal handling techniques and strict adherence to monitoring procedures outlined in detail in Section E. These monitoring procedures ensure that any potential adverse effect of tumour growth is spotted before pain or distress is caused to an individual animal. In the case of the tumour models described herein, particular attention is paid to ulceration of tumours and to effects on mobility as well as to the general well-being of individual animals.

### **Review:**

We critically review experimental approaches at the end of each experiment and look constantly look to refine our work as it progresses.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 315. Pig models of poisoning &amp; drug toxicity</b>
<b>Key Words</b>	poisoning, antidotes, treatment, mechanisms
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Poisoning is a major global health problem causing hundreds of thousands of deaths each year. Self-poisoning with medicines ('attempted suicide') is responsible for 10% of all medical presentations to hospital in the UK. Medicines such as diltiazem and paracetamol are responsible for several hundred deaths each year in the UK.

Self-poisoning is an even greater problem in rural Asia. Here pesticide self-poisoning is a major public health problem and one of the three most important means of suicide worldwide, killing more than 150,000 people each year. Many of these suicides occur from organophosphorus (OP) insecticide poisoning, but other types such as paraquat and aluminium phosphide can be devastating.

The study of poisoning in humans (clinical toxicology) is a neglected area of medicine, with little active research. Few animal models exist with which to study what happens after poisons enter the body - information that is essential to find novel treatments. Few effective and affordable antidotes exist for severe poisoning.

This project will use pigs to identify effective antidotes for poisoning and to better understand what poisoning does to the body. This will be done by giving poisons to anaesthetised animals and studying the effect of poison and treatment. Lessons learnt from these animal models will be rapidly considered for studies and trials 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)?**

This project will increase our understanding of how poisons affect the body, in particular how OP insecticides cause our muscles and nerves to stop working and

the lungs to become damaged. It may also find new treatments (or antidotes) for cyanide poisoning that are better at saving lives than our current treatment options

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use up to 150 pigs over 5 years. Previous work shows that detailed studies in a small number of pigs are able to provide scientifically powerful data that will guide human treatment

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

All animals will be anaesthetised at the beginning of the study so that they are unaware of any study procedures. They will then have minor surgical procedures to place monitoring and blood sampling tubes into an artery and veins so that blood samples can be taken for tests and the condition of the heart carefully watched. The wounds will be stitched up after insertion of the tubes. Poisons and/or treatments will also be administered via these tubes or by a tube placed into the stomach. All animals will be cared for by veterinarians who will closely monitor for adverse effects. They will be watched for the effects of the poison and how this is controlled (or not) by the antidote. At the end of the study, the animals will be killed by a humane method and tissues taken for analysis after death. There are no severe protocols on the license and no animal suffering except that associated with routine administration of sedative or other drugs before anaesthesia.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

It is not possible to set up models of poisoning in humans or to test new antidotes that have not previously been tested in animals. Studies done in test tubes or on computers are unable to determine the efficacy of antidotes or therapeutic interventions for poisoning in living humans because they cannot reproduce the complex multiorgan effects of the poisons against which the antidotes must work. Animal studies are therefore required.

Human patients presenting to hospital with self-poisoning are very variable. They have ingested differing amounts of different poisons, at different times, and have received different treatments before coming to hospital. Furthermore, the dose ingested is rarely known and the actual compound ingested may well not be known

for several days, if at all. This marked variation between human patients makes clinical research difficult.

Large controlled studies in hospitals allow the variation to be balanced out but such trials are expensive, difficult, and only to be attempted when there is good evidence from both animal studies and early human studies that there is a reasonable likelihood of effectiveness.

Animal studies can be more controlled, with a specific dose of a particular poison administered at a specific time point, thus allowing much smaller numbers of participants.

We have shown that pig models of poisoning provide a large amount of relevant information on what poisons do in the body and whether treatments work - all information that can be rapidly translated into human studies

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

#### **Reduction**

We work with experienced statisticians to ensure that the minimum number of animals are used for each study, while maintaining scientific quality

### **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

#### **Refinement**

We have chosen pigs (either the Gottingen minipig which has been bred to be small or outbred farm pigs) for our studies because pigs are much closer to humans than rodents. Due to similarities with humans in how pigs handle and break down medicines, they have become an increasingly important model species for understanding the benefits and harms of new drugs.

The large size of the species has several further advantages including: a longer, and more clinically relevant, time course of study for most diseases; ability to repeatedly sample blood and tissues; and the use of readily available hospital equipment for humans to record changes and to image the animals.

Unfortunately, previous animal models of poisoning using rodents have not been closely related to the human situation and their data could not be extrapolated to clinical practice. For example, most studies of OP insecticide poisoning and its

antidotes have involved measuring how many animals survive to 24hrs with or without certain treatments. However, these studies do not mirror what happens in people. The OP pesticide has been given in the wrong form and by the wrong route. The treatment has been started: at the wrong time; with treatment doses that differ from doses used for humans; without the typical intensive care support available to humans; and without the intention of giving the animal comprehensive treatment. Our pig models address all these limitations.

All studies on this license will involve anaesthesia before poisoning. There are no severe protocols on the license and no animal suffering except that associated with administration of sedative or other drugs before anaesthesia.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 316. Understanding the regulation of brain monoamine neurotransmission in health and disease</b>
<b>Key Words</b>	Neurotransmission, Dopamine, Basal ganglia, Parkinson's disease, Acetylcholine
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

**Overall objective:**

Brain cells, or neurons, that use the transmitter dopamine, carry out key functions in our everyday motivated actions as well as our learned habits. We think these neurons tell us about things in our environment that have some motivational value that help us to detect them, and then respond optimally to benefit from them. These neurons die in the neurodegenerative disease Parkinson's disease. There is therefore a need to understand the workings of these cells better so that we can not only advance biological knowledge, but also improve our understanding and treatment of Parkinson's and related diseases.

The work we propose will promote our understanding of how dopamine regulates our everyday behaviours, and it will also allow us to explore at a subcellular level how these neurons communicate from synapses.

We will work towards these goals through a program of work that will identify how neurotransmission by dopamine (and related transmitters) is regulated by neural circuits with other neurotransmitters, neurotransmitter receptors, cellular signalling pathways, regulatory genes, and related mechanisms. We will also examine how dopamine release governs behaviour.

Brain cells, or neurons, that use the transmitter dopamine, carry out key functions in our everyday motivated actions as well as our learned habits. We think these neurons tell us about things in our environment that have some motivational value that help us to detect them, and then respond optimally to benefit from them. These neurons die in the neurodegenerative disease Parkinson's disease. There is

therefore a need to understand the workings of these cells better so that we can not only advance biological knowledge, but also improve our understanding and treatment of Parkinson's and related diseases.

The work we propose will promote our understanding of how dopamine regulates our everyday behaviours, and it will also allow us to explore at a subcellular level how these neurons communicate from synapses.

We will work towards these goals through a program of work that will identify how neurotransmission by dopamine (and related transmitters) is regulated by neural circuits with other neurotransmitters, neurotransmitter receptors, cellular signalling pathways, regulatory genes, and related mechanisms. We will also examine how dopamine release governs 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)?**

This work should therefore advance basic biological knowledge and understanding of many brain functions relevant to our everyday motivations and actions. It shed also light on mechanisms relevant to key brain disorders. In turn, we hope to gain insight into potential future therapies for these disorders for which there are currently still very few effective treatments.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Over 5 years, we estimate that we may use up to 2,600 mice in procedures other than simply breeding and maintenance. We may breed and/or maintain up to 12,000 mice, some of which will be the same ones used in the additional procedures. Mice will be the species used because they are the lowest vertebrates in the phylogenetic tree for which brain dopamine systems are suitably well characterised and comparable to that of humans, as well as there being models for neurodegenerative disease. The mouse is also currently the most tractable mammal for use in genetic studies.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

We will raise genetically altered mice that allow us to explore the functions of key molecules in these mechanisms. In some animals we will insert genes into the brain during general anaesthesia which allow animals to express proteins that can be targeted with flashes of light or designer drug tools to activate the neural circuit we want to explore. Some animals might instead receive a toxin or will be genetically altered to make the animals begin to develop a Parkinsonian condition so that we can understand the disease better, and explore some options that might treat it. Some animals might be given drugs regularly when awake over a few weeks to enable us to understand better the processes which become disturbed, or how we

might treat them. And a small number group of animals will have small microelectrodes implanted in their brains and then be allowed to roam freely so that we can understand how neural circuits are important to behaviour. The adverse effects that some animals might experience might include the effects of brain surgery under general anaesthesia which might include transient pain and bleeding, some disturbances to normal movement particularly some slowness or other slight difficulties in initiating movement, which might compromise their ability or a failure to thrive. Animals will be well supported during these times. At the end of the experiments, the animals will be humanely culled.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

This work is an exploration of fundamental mechanisms of operation of the brain and also studies the adaptive mechanisms and/or the impact of drugs in neurodegenerative disease. Use of live animals and real brains is therefore needed to provide tissue with synaptic circuitry that resembles the in vivo scenario. We are not aware of any alternative which does not use animals that would allow progress to be made towards the objectives. No cell or culture alternative can adequately provide this. We will use virtual neuron computer models in the limited experiments for which this is appropriate.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We will keep animal numbers to a minimum by using power calculations and pilot studies where appropriate. We will also use experimental designs and powerful techniques that are high yield, by allowing multiple refined measurements per sample, or per brain or per animal.

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

#### Refinement

We have chosen mice because their dopamine systems are very similar to those of humans and because they are highly suited to using genetic manipulations that will help us understand how the dopamine system works in health and disease. The genetic tools we can use allow experiments and manipulations to be highly targeted to the cells we are studying, and therefore very refined.

We will select for each experiment the most refined mouse brain preparation. Sometimes brain slices are ideal because they have a good balance between containing substantial normal circuitry, unlike isolated cell preparations, whilst also allowing good access to the neurons we want to visualize and study. Sometimes, we need to use whole animals, when they are the only means to understand brain cell function in relation to behaviour.

The mouse models of disease we will use are the best available, and each one has been chosen because it closely mimics key aspects of the disease and with minimal suffering, and so is very refined.

We will use the lowest severity models applicable to each of our aims. Our general measures to minimise suffering in interventional experiments include appropriate use of anaesthesia, aseptic surgeries, close post-operative care, analgesia, and support. In all cases where an intervention is applied *in vivo*, monitoring systems and humane endpoints will be in place.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 317. Mechanisms of Acute and Chronic Inflammation</b>
<b>Key Words</b>	Inflammation, Lung Diseases
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Inflammation is a normal biological process that the body uses to protect itself against diseases and for restoring damaged tissues back to normal function. However, when inflammatory processes are poorly controlled or directed against normal bodily functions they are harmful to the affected individual and result in diseases such as Asthma and Chronic obstructive pulmonary disease. The inflammatory response involves directing specific white blood cells to sites of disease where they interact with other cells to secrete substances which allow the cells to divide and fight the disease. However, how the cells are directed to and function at sites of disease and damage are not well understood. The overall objective of our investigations is to identify molecules that start and propagate the inflammatory response.

**What 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 involved in the inflammatory response is fundamental to analysing the processes of infectious disease control on the one hand and inflammatory diseases on the other. This understanding is critical for the design of new drugs for common inflammatory diseases for which there are currently few effective treatments.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use only mice (approximately 30000) over 5 years, amongst 20 researchers.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

The following procedures are expected to cause some moderated discomfort, that animals will fully recover from; bone marrow ablation and reconstitution (tiredness

and reduced appetite), splenectomy (pain) and pathogen exposure (weight loss). Other procedures, such as administration of substances, assessment of lung function and taking of blood samples cause mild transient discomfort and no lasting harm. We do not anticipate any severe adverse events. However, we will monitor animals for recognised physical and behavioural changes that indicate ill health. Mice displaying two or more of these will be humanely killed. All experimental mice will be humanely killed at the end of the experimental procedure.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

Due to the multi-cellular interactions involved in the inflammatory response and in inflammatory diseases such as asthma the responses cannot be adequately or fully mimicked by in vitro studies alone. It is critical to perform these studies in mammals since there are significant differences between the biological systems of frogs and fish to that of humans. Where possible we will complement the in vivo work with experiments using in vitro culture systems taking advantage of isolated human cells and cell lines to investigate selected pathways identified in the in vivo models. Throughout the project, where possible, non-animal experiments will be employed. This will include developing in vitro systems such as 'lung on a chip' and exploiting in silico technologies and databases where appropriate.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We have developed our experiments so that we can measure multiple parameters in each animal, thus maximising the information gained from each experimental group and minimising the number of animals used. Combining tests in the same mice allows the data gained to be correlated directly, rather than inferred. Based on previous experience we have calculated the minimum number of animals needed to see desired effects using robust statistical analysis. Also, all experiments will be conducted in accordance with the NC3Rs' ARRIVE guidelines. All of which help to minimise variation and avoid unnecessary repeats.

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

We have chosen the smallest animal possible to represent human disease. While the models chosen closely represent the features of the human disease in the treated animals, they are the least severe and do not promote undue distress to the mice. We constantly monitor animals for signs of ill health and work closely with animal care staff and veterinary surgeons to ensure the best possible husbandry and welfare for mice under procedure. Analgesia is routinely provided to all animals when required.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 318. Neuronal cell development and survival</b>
<b>Key Words</b>	Motor neurone disease, Axonal transport, RNA metabolism, Cell biology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Our objectives are: 1) To understand how defects in the components of the intraneuronal transport and signalling systems lead to the death of motor neurons in motor neuron diseases. 2) To investigate the underlying mechanisms of the roles of proteins implicated in motor neuron disease in response to DNA damage and to elucidate how defects in these proteins could affect the expression of other genes.

Our study will contribute towards our understanding of the mechanisms of motor neuron death caused by defective intraneuronal transport or response to DNA damage. Therefore, our findings will benefit the scientific community with a broad range of interests in neurological conditions. Moreover, working from the mouse models of motor neuron disease to mouse primary cells and neurones derived from reprogrammed mouse skin cells, will aid the understanding of the mechanisms of disease onset and progression. Using this knowledge in human derived fibroblasts and neurones and applying this information back to human conditions and for cross species comparisons at the cellular and neuronal tissue levels will set a paradigm for the effective use of both the mouse and human-derived cells as valuable model systems.

**What 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 benefit patients and their families, who have been affected by motor neurone disease, hereditary motor neuropathies, and some cases of intellectual disability; and health professionals, who work with the above mentioned patient groups. The benefit from the outcomes of this study could be immediate, as our findings could inform the beneficiaries about the causes and basic mechanisms

of the disease. In the longer term, this project will contribute to: 1) our understanding of the relationships between defective axonal transport or DNA repair response with abnormal neuronal cell function and development; 2) and hence, discovering novel drugs and more effective treatment of the above mentioned diseases and perhaps other related disorders; 3) validating the promising drug targets in preliminary pre-clinical settings; 4) informing patients and ensuring best possible care planning.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice, 6000 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 levels of severity? What will happen to the animals at the end?**

A proportion of the animals used in this study are transgenic mice that start showing signs of a progressive muscle weakness in their limbs. The level of severity of the phenotype in these mice is substantial, as this is a progressive condition which leads to paralysis and it is crucial for this research to obtain tissues from all stages of the disease in order to pinpoint the correct pathway that is impacted, or the efficacy of the drug. To minimise the animal suffering we monitor these mice twice a week between from the pre-symptomatic stage. Mice with signs of paralysis will be given dry mash and gel blocks and their food and water intake will be monitored daily. Mice will be weighed once and checked twice every day till end point (righting reflex within 30s is not observed; or 15% loss of body weight) is established. End-stage mice will be monitored 9am-5pm. If the mouse shows severe symptoms, then it will not be kept and will be culled humanely as specified by the Home Office. No mice with severe symptoms will be kept overnight. Another group of mice showing adverse effects in this study exhibit an abnormal gait but have normal feeding behaviour and life span.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

Motor neuron disease targets neurons in the brain and spinal cord and thus it is impossible to have access to these tissues during the development of the disease before the post-mortem stage. This would provide us with data about the very late stages of the disease. Although we will be using skin fibroblasts isolated from patients and reprogrammed cells, we will still need mouse models to have access to tissues at all stages of life and for culturing primary neurons.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will maintain and breed just enough animals for providing us with required tissues and cells for generation of data which are statistically sound.

### **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

Sequencing of the mouse genome has revealed that ~99% of mouse genes have a homologue in the human genome and that for ~80% of mouse genes an analogous (orthologues) gene exists in the human genome. In addition, human and mouse have common biochemical pathways.

Because of the above properties several large international mutagenesis programmes have been generating mutant mice that could serve as model systems for late onset human disorders such as motor neuron disease.

The mouse clearly does not have the same physiology as humans, but does, largely, share the same biochemical pathways as well as genes. Thus we can work with mutant mouse models of human motor neuron degeneration to highlight and interrogate the proteins and pathways that are involved in motor neuron disease.

To minimise the animal suffering we monitor the animals which show signs of muscle weakness or paralysis twice a week between 100 – 120 days of age. Mice with signs of paralysis will be given dry mash and gel blocks and their food and water intake will be monitored daily. Mice will be weighed once and checked twice every day till end point (righting reflex within 30s is not observed; or 15% loss of body weight) is established. End-stage mice will be monitored 9am-5pm. If the mouse shows severe symptoms then it will not be kept and will be culled humanely as specified by the Home Office. No mice with severe symptoms will be kept overnight.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 319.Mechanisms of metabolic regulation in health and disease</b>
<b>Key Words</b>	cancer, metabolism, obesity, diet, imaging
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Little is known about the ways various organs in the animal body communicate with each other using nutrients. However, there is increasing evidence suggesting that metabolic communication between cells and tissues is important for healthy tissue functions and is perturbed in disease. The aim of this project is to elucidate the mechanisms that allow cells exchange nutrients in order to support each other's functions and thereby tissue homeostasis. The project will also investigate how these operations fail or contribute to diseases such as metabolic syndrome and 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 benefits of this project will be fourfold. Firstly, it will elucidate fundamental mechanisms of non-cell autonomous metabolic communication; secondly, it will reveal metabolic pathways that can be targeted for therapeutic intervention in human disease; thirdly, it will validate the use of specific compounds as therapeutic or diagnostic modalities in both non-human and human disease; and fourthly, it will generate and validate new mouse models for human disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to approximately 4500 mice per year will be bred and used under the auspices 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 levels of severity? What will happen to the animals at the end?**

Experimental procedures proposed for this project have either been established or will be refined to minimise the possibility of adverse effects. Possible adverse effects expected may include weight loss, appetite loss, hunching, or temporary shivering. None of the procedures, on their own or in combination are expected to breach the moderate severity threshold. In case of unexpected adverse effects an animal care and welfare officer and a veterinary surgeon will be consulted. Any animal showing more than a moderate level of harm will be killed by an approved method.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

In order to generate data that is relevant to the way in which cells and organs interact with one another inside the body, it is necessary to utilise animals. For example the complex ways in which tumour cells interact with a multitude of different types of healthy host cells *in vivo* is key to understanding cancer progression but this can only be studied in a living animal. However, the knowledge acquired through this project will be used to inform suitable *in vitro* experiments that will aid replacement in the future.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We will carefully plan our experiments so that to attain the best statistical power with the minimum number of animals. We will also aim to maximise the amount of information that can be acquired per animal within the confines of this licence. We will also develop or validate new, non-terminal methods that will allow longitudinal monitoring of biological parameters (e.g. liver function) in a non-invasive manner, such as *in vivo* imaging.

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

## **Refinement**

Mice have been selected because of their advanced genetics, readily available disease models and well established laboratory procedures. In all cases, animal suffering will be minimised by following strict guidelines in accordance with the Home Office and by regularly monitoring animals in consultation with an animal care and welfare officer and a veterinary surgeon. Any animal showing unexpected adverse effects of any procedure will be killed immediately by an approved method. Animal use will be minimized wherever possible by employing the lowest numbers necessary to achieve statistically significant results.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 320.Evaluation of anticancer agents and combinations in orthotopic oncology models</b>
<b>Key Words</b>	Cancer, Pre-Clinical, efficacy, models, imaging
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

As the understanding of the mechanisms behind cancer progression continues to increase, so does the requirement to develop and validate relevant models in parallel to test new strategies. The aims of this project are to provide the scientific community with accessible expertise in terms of available clinically relevant cancer models, knowledge and technical capability to improve decision making on which agents should progress to the clinic and which patients will benefit from the treatment. This project focuses specifically on solid tumours arising in organs of the prostate, pancreas and bladder, all of which are very different in terms of their origin, growth rate, progression and response to treatment.

The objectives of this project are:

- 1) To develop, validate and optimise patient relevant organ specific pre-clinical models of prostate, pancreatic and bladder cancer to enable the testing of anti-cancer agents. .
- 2) To evaluate anti-cancer agents and combination therapies using models developed in objective 1.

**What 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 order to benefit the clients/collaborators who develop the anticancer agents and ultimately patients who are treated with these agents, the development of pre-clinical cancer models that exhibit greater patient relevance by implanting them in relevant organs will allow these novel agents to be tested in more relevant conditions where environmental factors such blood supply, spatial arrangement, interaction with supporting cells and structures will be better represented. These models require

expertise in surgery as well as generating the cells that emit light and then applying the imaging technology to capture the right data and analysis, which is not readily available in most institutions and companies. These models will enable decision on moving programmes forward into clinical trials or in some cases this may result in a specific anticancer programme being cancelled which may seem a negative benefit, but identifying anticancer agents that are either ineffective or unsuitable for further development can be considered a positive benefit in the longer term as it prevents the unnecessary progression of ineffective therapies to early phase clinical trials and allows the redirection of resources and patients to other projects. Once validated, all models are added to the proprietary databases; access to which is free to all users, as well as abstract submission to national and international scientific conferences.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice will be used for this project as they are the lowest species of animal that allow the modelling of human cancer, and offer the opportunity for genetic manipulation to generate specific models relevant to human cancer. Substantial numbers of cancer relevant models are already validated in-house (100+) including prostate, pancreatic and bladder cancer making this species most suitable for this project. Over the course of this project we'd expect to use 7,200 animals to model these cancer types, the different stages of cancer and novel anti-cancer agents and combinations.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

The majority of mice will undergo surgery to implant tumours in the site of origin in the prostate, pancreas and bladder under anaesthesia which are then measured once/twice weekly (or up to three times weekly dependent on growth) throughout the study by imaging under anaesthesia to track internal size before it becomes too large. Imaging is non-invasive and not expected to affect the wellbeing of mice. Dosing of candidate anticancer agents by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response (as assessed by imaging) or humane endpoints as guided by imaging before the onset of any adverse effects. Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects is uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs will result in humane killing regardless of body weight measures. The highest level of severity will be moderate. All mice will be killed at the end of the studies with tumour, blood and tissue collected which will allow further characterisation of treatment effect providing additional information such as how the cancer has spread or whether the drug has reached its target.

## Application of the 3Rs

## Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

Research conducted in a test tube or artificial environment (In vitro) has replaced animal use in early development phases, particularly in the development of screening assays to refine compound selection, target identification, off-target toxicity or toxicity versus normal tissue cell lines, which increase our understanding of the target and candidate agent and therefore guide and refine the steps prior to moving into in vivo, and minimise subsequent use. However, there is still a requirement to use animals for this project as in vitro assays still do not optimally mimic all interactions between cells and tissues, such as blood vessel formation, specific organ environment, spread to other organs and thereby relevant drug access or the many homeostatic mechanisms in play in an in vivo environment that allows relevant tumour biology drug evaluation.

## Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

The use of in vitro studies can be used to identify lead compounds, evaluate dose ranges confirming target modulation/expression and relative off-target toxicity which can be used to inform on relevant doses for use in the equivalent animal models to evaluate drug distribution, target modulation and toxic effects. The use of complex 3-dimensional in vitro assays can be applied to pre-screen studies and compound selection prior to advancement into animal testing (thus reducing animal use). The model development stage of this project will be used to determine statistically powering so the minimum number of mice are used in a study design but still achieve scientific endpoints. The use of imaging technologies can also reduce the number of animals required to generate study outcomes as model variation can be improved by eliminating mice which do not develop the disease appropriately or refining the model so this is minimised.

## Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### Refinement

Mice are the lowest species in which the knock out of the immune system allows growth of human tumours. Mice with a fully functioning immune system also provide

the opportunity to investigate the immune system interplay with a mouse tumour. The mice will have tumours implanted into the prostate, pancreas or bladder i.e. at the site of origin which are more relevant to patients but are more complex and require imaging to track the growth inside the mouse by using prostate/bladder/pancreatic cancer cells that are altered to emit light which is then captured by an imaging system specifically designed for small animals. Organ-specific models are known to better model cancer in patients as tumour grows in the correct environment which facilitates spread to other organs as seen in the clinic and show a reduced response to chemotherapy therefore providing more relevant information on the drug. The use of imaging is also a refinement as data from the internal tumours can be captured in real time, provided additional data that wouldn't normally be visible, only using animals that show tumour, and minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled killing of the mice, thus reducing the duration of model and regulated procedures.

The development of relevant pre-clinical models of oncology in objective 1 is a key stage for the evaluation candidate anti-cancer agents to ensure the right models are being used to answer the questions being asked in objective 2. The following will be undertaken to minimise animal suffering.

- Pilot studies for the establishment of new tumour lines and refinements to surgical techniques will be carried out on an ongoing basis under the advice of the vet and/or the named animal care and welfare officer will be sought in this respect.

All surgical procedures will be conducted in line with established welfare guidelines on aseptic surgery using suitable anaesthesia along with peri and post-operative analgesia.

- Any in-life sampling will be in line with established welfare guidelines and micro-sampling regimens will be utilised where study design supports this.
- The frequency of dosing will be such that animals fully recover between injections and will not suffer more than transient pain and distress and no lasting harm and there will be no cumulative effect from repeated injections.
- Use of pilot tolerability studies to ensure there are no unexpected adverse effects associated with new models or unexpected toxicity because of tumour: drug interactions and to ensure the drug levels used are not associated with any cumulative effects.
- Using cells that emit light to allow imaging to be used to recruit only those mice that have been identified to have the right tumour location and to reduce model duration

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 321.Mechanisms controlling calcium dyshomeostasis in malignant hyperthermia susceptible mice</b>
<b>Key Words</b>	Malignant Hyperthermia (MH), Heat stroke, Volatile anaesthetic, muscle, Calcium dyshomeostasis
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

<b>Purpose</b>	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

To determine the mechanisms causing human MH and exercise and heat stress to provide new targets for treatment and prevention of human 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)?**

Understanding the basic mechanisms of disease are critical to be able to find targets for treatment and prevention. In this case we are fortunate to have genetically altered mouse models that exactly mimic human disease, which combined with drug interventions and other models that allow molecular dissection or the addition of key proteins by gene targeting that lead to disease.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We anticipate using 7000 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 levels of severity? What will happen to the animals at the end?**

Some of the animals will only suffer the effect of having general anaesthesia and no further conscious stress. This includes animals that will receive non-depolarizing neuromuscular blockers. Other animals will be exposed to increased ambient temperature or exercised and monitored for increased body core temperature, the

stress for this is moderate. If a sharp increase is noticed, they will be given a general anaesthetic and the stress will be immediately blunted.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

There are no non animal or non-protected animal models of this human disease. Primary cell lines (which are animal derived) and isolated muscle fibers (also animal derived) will be used for some experiments, but it is impossible to study whole animal physiology in vitro

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

We have done power analyses based on previous studies to limit animal numbers and will constantly be doing ongoing power analyses to determine if the number of animals for any given protocol can be reduced while still allowing reasonable statistical analysis

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

#### Refinement

These animal models have been previously shown to exactly mimic human disease OR to prevent disease OR to enhance survival. All animals used will be housed under standard conditions and every measure taken to assure that the harms to these animals will be minimised. They will be monitored daily while housed and continuously monitored during experimentation.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 322. Physiological biomarkers of poultry welfare.</b>
<b>Key Words</b>	Neuroscience, acute affective state, electrophysiology, chicken brains
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Increasing welfare of poultry (and other farm animals) is high on the public agenda. However, understanding which conditions or management processes affect the animals more negatively is difficult to ascertain. We should not assume that, just because humans might (not) like certain conditions, that chickens would respond accordingly.

We therefore have to ask the chickens. This can sometimes be done with behavioural tests, but there are a number of situations in which it is impossible to use behaviour, because the animal is unable to behave normally (e.g. when being picked up and put into crates for transport).

This project aims to use physiological (neurobiological) indicators as potential measures of the animals' welfare state. We aim to develop short-term, immediate measures, which give us an idea of an animal's immediate emotional state. For this, we are looking at brain activity in brain areas that are known to process emotions.

**What 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 validate these markers, we can use them to assess the welfare impact of different management practices on the animals. If we can assess this, then we can make practical recommendations as to which methods are higher welfare than which others. Because we are doing this research in collaboration with a company that

designs processing systems for broiler chickens, our findings will be implemented quickly.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use broiler chickens of up to 3.5 kg in body mass. Over the 5-year length of the project, we anticipate using 100 animals.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

Because we need to validate that our measures can identify negative experiences, we need to induce these experiences in the animals. However, these are unlikely to be any worse than those they might encounter had they been kept in commercial establishments. For a few animals, some aspects of their environment may be worse than they would normally have experience. However, the potential benefit to the millions of chickens being housed and then killed every year in the UK alone will outweigh the slightly increased negative experiences of a small number of birds. Birds will undergo surgery under general anaesthetic to implant electrodes into the brain. They will be allowed to recover from anaesthesia and heal from surgery before being recorded. Post-surgical pain will be treated with routine analgesics. The recording will be conducted by attaching a wireless recording device to the implant. This will be designed to be as light as possible and to impair the animals' movements as little as possible, so that the impact on the animal is minimal, and we can focus on the impact of the different stimuli we present to it. These stimuli may include negative stimuli, such as brief restraint, brief periods of pain or bad-tasting food; or they can be positive stimuli, such as re-uniting them with flock mates, providing dust baths or providing them with preferred food types. If necessary, the animal will be given time to habituate to the equipment before we start recording. At the end of each experiment, the animals will be humanely killed.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

Because we are interested in the physiological responses of live animals to different environments and conditions, we have to study this in live animals. No in-vitro or computer model can mimic the response of real animals. However, early optimization of the implant surgery will be done first with cadavers, and then with non-recovery anaesthesia before moving to recovery surgeries.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will use the most powerful statistics available to make sure we can use the fewest possible animals for the most possible outcome. Whenever possible, we will conduct power analyses to estimate the minimum effective sample size needed. The experiments will be done within-bird, allowing us to control for a lot of inter-individual variability, and therefore to reduce the sample size needed to obtain meaningful results.

### **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

Because our question is about chickens, we have to answer it using chickens.

Except for the experimentally required negative experiences, we will minimize the animals' negative experiences by closely monitoring them for distress and disease, and by administering antibiotics and/or analgesics when necessary to the animals recovering from surgery. We will use wireless recording methods for the electrophysiology, as this reduces the stress on the animal of being physically connected with a wire.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 323.Pre-clinical Pharmacology of Idiopathic Pulmonary Fibrosis</b>
<b>Key Words</b>	Incurable, Respiratory Disease, New drugs
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Idiopathic pulmonary fibrosis (IPF) is a progressive disease of the lungs caused by the build-up of scar tissue following insult or injury. There is currently no cure for this condition and mean survival is 2-3 years following diagnosis.

Two new drugs have recently been licenced to treat this disease (Pirfenidone and Nintedanib), but they only delay the progression. Much more research is needed to develop drugs which can halt the advancement of this condition.

The purpose of this project is to mimic the human disease in mice, rats or guinea pigs in order to test the efficacy of potential new medicines for IPF

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The ultimate benefit from this project would be the identification of drugs that can potentially treat IPF, which then successfully progress through human clinical trials. Other benefits are refinement of the disease model so that it is a more effective predictor of drug efficacy in humans.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to use approximately 6500 mice, 2000 rats and 1000 guinea 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 levels of severity? What will happen to the animals at the end?**

Scarring of the lung tissue will be induced with a cancer medication called Bleomycin which is dosed directly into the airways. This will be done acutely with a single dose. Following the dosing it is expected that the animals may exhibit changes in appearance and behaviour, e.g. become more subdued, ungroomed and lose weight, as well as experience transient respiratory depression. We are not expecting many of these effects to go beyond moderate severity, but from discussions with fellow researchers who are experienced with this protocol, and having read the literature, weight loss has the potential to be severe. To try and prevent this we will be supplementing the animals' diet with additional nutrition from the outset. Weight loss should only be temporary and is likely to naturally resolve half-way through the course of the study. Animals will be dosed with a test drug, probably daily, once the fibrosis is established; adverse effects resulting from this are expected to be seen in less than 1% of animals due to prior screening of the drugs. At the end of the protocol the animals will be humanely killed and their lung tissue analysed to ascertain if the medication has reduced the lung scarring.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

Prior to animal studies, tests on cells can be carried out to get an idea of the toxicity and the efficacy of a drug on the target cell type, but animal models are still needed in order to identify the effects on the whole body. It is possible that the influence of, and processing by, a multi-organ system will alter the behaviour of the drug.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Smaller validation studies will be carried out initially to ensure the most appropriate dose of bleomycin is used, and to identify the optimal time points for drug dosing and tissue sampling. This will help ensure good quality, reproducible data, so that the fewest number of animals are required to produce statistically significant outcomes. Where possible all samples required for scientific readouts, will be harvested from one animal.

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

The method of inducing lung fibrosis using bleomycin is very well documented and characterised in the scientific literature. It is the most popular model of IPF and the mouse, rat and guinea pig are the most commonly used species as they are proven to display many aspects of the human disease. They also have very well defined immune systems so we can investigate the disease processes at multiple levels to get a very detailed picture of the disease process.

All procedures will be carried out by fully trained and experienced researchers, and for particular techniques, animals will be under anaesthesia to reduce discomfort.

The animals will be closely monitored following procedures, and checked regularly throughout the course of a study. Any animals considered to be approaching severe pain or discomfort will be removed from the study and humanely killed.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 324.Nucleic Acid Sensing by Innate Immune Receptors</b>
<b>Key Words</b>	immune response, vaccination, cancer
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Viruses are infectious agents that cause a variety of diseases, ranging from a common cold to AIDS. The immune system can eliminate viruses, and we are trying to understand how the immune response is kick-started upon infection.

The first step is that the cells in our body recognise the presence of a virus. We know that cells have specialized proteins called receptors that detect viruses. However, how these antennas sense viruses is largely unknown. By investigating the mechanisms of detection we hope to understand how the immune response is initiated during virus infection.

One of the hallmarks of this anti-viral immune response is the production of a group of molecules called interferons. The name stems from the property of interferons to interfere and block the replication of viruses. Interferons achieve this by instructing cells to switch on their antiviral defences. Interestingly, interferons are not only essential as central players in antiviral immune responses. They are also produced during vaccination and are necessary for the development of protective immunity. Moreover, interferons are involved in cancer and may help our immune system to fight malignant cells. These new areas of research hold great promise for the development of new vaccines and novel cancer treatments. We want to obtain a better understanding of the underlying biology, which will be required for the development of new medicines.

Despite all these beneficial functions of the immune system, it is a double-edged sword and can cause problems, too. Patients suffering from autoimmunity are not infected with viruses or other pathogens, but their cells activate a long-lasting immune response that damages the body. Our hypothesis is that the immune

antennas are not tuned to the right signal. We hope to reveal why the immune system is tricked into this false alarm.

**What 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 of this work relates to new knowledge in the area of immunology. Our findings may allow us to develop new ways of boosting immune responses to more effectively eliminate dangerous viruses. Being able to better activate immune responses may also advance vaccination strategies and instruct ways to develop new treatments for cancer. In addition, we envisage inhibition of immune sensors as a treatment in autoimmunity, and our work may provide insights towards such approaches.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice as an animal model, including genetically modified mice that lack specific immune receptors or related molecules (up to ~24,000 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 levels of severity? What will happen to the animals at the end?**

We will breed genetically modified mice. In most cases, the genetic modification will not cause any adverse effects. In some instances, genetic modification may lead to the development of autoimmunity. Manifestations of this include behavioural changes and weight loss. These will be carefully monitored, with clearly defined thresholds such as 15% weight loss, and animals will be killed immediately if these thresholds are reached. The majority of animals will be humanely killed without undergoing procedures and tissue will be used for experimentation. In addition, some animals will be used in models of virus infection, autoimmunity, vaccination and cancer. These models involve administration of viruses, substances or cancer cells. We will use injection, inhalation and the drinking water to administer these agents. Most animals will not suffer at all or will experience only mild and transient adverse effects such as tenderness around the injection site, which typically self-resolves within 24 hours. In the infection, autoimmunity and vaccination models, a small number of mice (less than 10%) may suffer adverse effects that last longer, and this will include weight loss and behavioural changes. In our cancer model, tumour development will occur in all animals. We will regularly monitor animals undergoing procedures and will record and measure adverse effects. Weight loss will not exceed 15% and tumour diameter will not exceed 1.2cm. Animals will be humanely killed immediately if these thresholds are reached, or before if scientifically possible.

## Application of the 3Rs

## **Replacement**

State why you need to use animals and why you cannot use non-protected animal alternatives

### **Replacement**

To answer our scientific questions, our project integrates multiple scientific approaches. This includes biochemistry and molecular biology in the test tube wherever possible to dissect individual aspects of immune recognition. For example, this involves using cells isolated from animals humanely killed by Schedule 1 methods and using existing cell lines. However, we also need to use an animal model because the immune response is a complex process involving many different types of cells and molecular mediators. There is no feasible alternative that would entirely replace the use of a living animal. Where work not involving protected animals is insufficient to achieve our research goals, we will use mice as an animal model, including genetically modified mice that lack specific immune receptors or related molecules.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will use statistical models to determine the minimum number of required animals. We will also design experiments in such a way that many data points can be collected from the same animal. We will use male and female animals, which reduces the number of surplus animals. We will use a breeding strategy - managed by staff trained specifically in maintenance and breeding of mouse colonies - that keeps the number of mice to a minimum. Unwanted genetic changes will be prevented by regular crosses to a reference mouse strain. Finally, experiments will be blinded as much as possible to avoid bias. Taken together, these measures will allow us to obtain robust and reproducible data from a minimum number of mice.

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

We chose mice given that their immune system is sufficiently similar to the one in humans to draw conclusions that are likely to equally apply to man. Once the scientific objective of any procedure has been attained the animal will be disposed of humanely. Specific humane endpoints will be applied. We have chosen those models of virus infection, vaccination, cancer and autoimmunity that are most refined

and cause the least possible harm. We will fully monitor all animals involved in the study and continuously seek to identify new methods for refinement.

Specifically, our virus infection models interrogate the early stages of infection. At these time points, the innate immune system becomes activated and we will study this process. However, at these time points, virus replication has not yet resulted in tissue damage that causes profound disease. Animals will be culled before they reach this later stage. Similarly, in our autoimmunity studies, we are using slowly developing disease models instead of acute onset, severe models. This allows us again to focus on early stage of innate immune activation and to stop procedures before animals become more strongly affected.

Other examples of refinement applied in our work are: (a) Freund's adjuvant will not be used. This is a component of vaccine formulations that has been used in the past and caused adverse effects such ulcerations. We will use other adjuvants that do not cause adverse effects. (b) We will use tumour models that are easy to monitor and do not form secondary tumours (metastasis). (c) Footpad injections will not be used and will be replaced with a refined model (hock injection) that is much less painful but achieves similar scientific aims.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 325. Neuronal communication in the brain of mice</b>
<b>Key Words</b>	Brain, cortex, synapse, neuron, tau
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

A central goal of neuroscience research is to understand how the brain processes external and internal stimuli to coordinate cognitive and behavioural processes. Ultimately, this understanding must relate behaviour to the activity patterns of neurons and their synaptic connections within key circuits of the brain. Elucidating how the activity of these circuits becomes abnormal is also crucial to understanding pathological situations such as neurodegeneration. This project deals with these questions in an unusually direct way - by observing the activity of neurons and synapses in the brains of live mice as they process sensory stimuli (1-4). Optical methods such as multiphoton microscopy now provide the resolution required to image neuronal and synaptic activity in awake animals and we will use these methods to make a circuit-level analysis of sensory processing and neuropathologies such as Alzheimer's Disease.

Our aim is to understand how nerve cells and their synaptic connections convey information (e.g visual or spatial) during health and disease.

Our key questions are:

1. What is the nature of the neural signals by which information is processed and transmitted in the visual system and hippocampus in awake behaving mice?
2. How is the transmission of neural signals at the synapse altered by changes in behavioral state of the animal?
3. How is the processing and transmission of neural signals altered in neurodegenerative disease states

**What 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 have benefits in three broad areas: 1) By elucidating how different types of neuron respond to different types of visual stimuli, it will yield a greater understanding of how the brain executes vision. 2) By concentrating on imaging the activity of synaptic connections, it will reveal how these key neuronal compartments alter visual signals and how they alter the transfer of neuronal signals when the brain enters different states, such as switching from "sleep" to "alert". 3) By studying how the operation of neurons and synapses is altered by the accumulation of proteins that are known to cause neurodegeneration, we hope to suggest novel therapeutic targets and strategies.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

This project will use mice. Approximately 3000 will be used over the 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 levels of severity? What will happen to the animals at the end?**

The large majority of the genetically-altered mice used in this project usually show no adverse effects, particularly at the ages used in our experiments. However, about 5% of mice will be mutants that develop protein depositions in the brain leading to neurodegenerative changes of moderate severity. Some animals undergo surgery to allow us to implant a window through which we can image their brains. They may require post-operative pain-killers but are usually fully-recovered and alert a few minutes after the procedure. Occasionally, this surgery may also include the implantation of an optical fibre or cannula. After recovery from surgery, animals are gradually habituated to the experimental equipment, on which they have their head fixed in place but can run on a ball. They will be rewarded, for example with sucrose, and stress will be minimised by accustoming them gradually to the apparatus, but nevertheless, this can sometimes be somewhat stressful for the mice. At the end of all experiments animals will be humanely killed and where applicable tissues collected and analysed. If animals are suffering for any reason before the end of the experiment and do not respond to treatment, they will also be humanely killed.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

The research proposed here requires the use of animals and animal tissue. The mechanisms underlying the transmission of signals in the brain are complex and involve interactions between different cell types. Studies in cell culture are uninformative as to the physiological properties of these processes, as the properties of the various neurons and their interactions are altered by the tissue culture process. Nevertheless, we will use a number of different experimental preparations, minimising the use of living animals where possible. Where experimentally relevant, studies will use *ex vivo* brain slice preparations to study how signals are transmitted across synapses.

But to investigate how synapses in the brain contribute to the processing of information (e.g. visual information in the visual cortex or spatial information in the hippocampus), we will need to work *in vivo*. This is the essence of our approach: to use the actual, unperturbed, neural circuit as far as possible. Cultures of neurons cannot see or navigate in space or carry out behavioural tasks that reflect the normal functions of the brain, and are therefore not an adequate substitute to understand how the retina or brain works.

As the function of neural circuits is profoundly altered by anaesthesia, many experiments will study unanaesthetised animals. This is also required to study processes such as motivation states. We will always seek to minimise animal use, however, and to maximise the information gleaned from every animal used

### **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

We will collect multiple data from each animal, thus minimising the number of animals required. For example, *in vivo* imaging data of neuronal and synaptic activity will be collected from many hundreds of neurons in the one animal, from several regions of the cortex or hippocampus. The numbers of animals to be tested will be the minimum number required to obtain statistically reliable results, based on previous experience in the laboratory, and from published findings.

To preserve important mouse genetic lines without having to hold stocks of live animals for extended periods we will instead freeze embryos that can later be implanted into a female mouse. The surgical procedures for implantation are demanding and require practise so on occasion it may be necessary to train with re-implantation of un-manipulated oocytes, embryos or blastocysts.

We are also using carefully designed studies that are statistically sound to minimise the number of animals used.

### **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

Mice have similar brain structures and functions with humans and can be bred to express proteins that allow us to see different cell types and the amount of brain activity. They are also small enough to be able to image their brains under a microscope.

The experimental and disease models have been designed to provide robust data while minimising animal suffering. Firstly, they use surgeries from which the animals rapidly recover. Secondly, they study the onset of disease processes. This allows the key triggers to be identified before multiple other processes go wrong, and also means that at these early stages, animal well-being is barely affected.

Animal welfare is monitored throughout the experiments, and animals are humanely killed where necessary.

Importantly for this project, a number of transgenic mouse lines are available, which will allow visualisation of specific types of neuron in the brain and the measurement of fluorescent signals when neurons are activated. Mice have been well-studied to investigate brain function and the sense of vision, as well as diseases such as Alzheimer's Disease, so that these results will be readily integratable within the field and should prove more translatable to humans than studies using lower order vertebrates. Finally, the small size of mice means that more of the brain can be observed using current microscopes than is possible in larger species.

Using a chronic cranial window for imaging involves surgery from which animals quickly recover, and then are stable for several months with usually no decrease in life expectancy or quality of life due to the surgery. Animals will be gradually accustomed to the imaging apparatus and will be rewarded (e.g. with sucrose solution) while under the microscope to decrease any aversion and stress associated with the apparatus. The transgenic mouse models to be used either have no adverse phenotype or, as in mouse models of neurodegeneration, they present some of the symptoms seen in human conditions such as Alzheimer's Disease. The use of such mouse models of human diseases is making possible the scientific studies from which cures will be found.

Where surgeries are carried out, peri- and post-operative analgesia will be used to minimise pain. Animals will be housed with appropriate environmental enrichment, and post-operatively this will be adapted to ensure that there is no chance of catching the cranial implants, while maintaining an interesting environment.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 326. Information processing in the mammalian brain</b>
<b>Key Words</b>	Neuron, Brain, Behaviour, Imaging, Electrophysiology
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Neurons are the basic cellular units of the brain, and are connected via synapses to form neuronal networks. The properties of single neurons and the synapses that functionally connect them to each other are thought to provide the basis for processing and storing information about an animal's experiences and needs. One of the central questions in neuroscience is how particular tasks, or "computations", are implemented by neural networks to generate animal behaviour, and how patterns of neuronal activity are stored during learning.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The results of this project will extend our basic knowledge of the fundamental mechanisms underlying information processing and memory storage in mammalian central neurons. This is essential if we are to understand how neurons communicate with each other and how information is transformed and stored by networks of neurons in the intact brain. In the long term the results of these experiments, and the techniques we will have developed, will provide new approaches of potential value for understanding and treating disorders of brain function such as occur in stroke, hereditary movement disorders, epilepsy and dementia.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Up to 19,000 mice and 850 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 levels of severity? What will happen to the animals at the end?**

Most animals will undergo only mild procedures with a maximum expected severity of moderate. In these animals, adverse effects may include some post-operative pain, controlled by analgesia and some initial stress on head-fixation which will be limited by gradual introduction of head restraint and provision of ample water, sugar-water or food rewards. Animals that are head-restrained in order to image brain activity accurately but supported on a treadmill are free to run or rest voluntarily and do not show signs of stress from the head restraint. Animals may lose weight initially but will be supplied with supplementary gels to aid recovery and supportive food and treats throughout. Any postoperative pain or complications that are not improved or resolved within a timeframe approved by the veterinary surgeon will be killed by an approved method. At the end of the experiments, an approved method of killing will be used and animal brains will be removed for further study.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

Animals are needed in order to study intact brain circuits and their involvement in encoding sensory responses and driving behaviour. There is no lower mammalian species that could be used for addressing the scientific plan.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

Breeding will be carefully controlled so that only animals required for experiments are generated. We will use advanced statistical tests (e.g. Kolmogorov-Smirnov, Wilcoxon matched-pair tests) in order to use the minimal number of data points to provide statistically significant results. Comparisons across multiple experimental groups will be made using the appropriate tests (e.g. ANOVA).

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

#### Refinement

Rodents are probably the lowest species for which direct comparison can be made with the structure and functioning of the human brain. Mice are currently the species

of choice in most areas of biomedical research, as they allow the use of powerful techniques such as the generation of transgenic animals. By using transgenic animals, we can express non-harmful molecules, such as fluorescent proteins, in neuron types of interest, that allow us to look, in a very targeted way, at the activity of cell types most relevant to our research questions, rather than just sampling all cells randomly. This greatly enhances the scientific value of the work as well as reducing the number of animals needed for experiments. As more sophisticated genetic targeting methods are introduced, we will use them to further refine our scientific approach in order to gather data even more efficiently.

We have continued to refine head restraint systems, reducing the weight of the head plate and shaping them so as to minimise any physical impediment on the mouse in its home cage. The introduction of sound proof boxes for behavioural training, providing a quiet environment, and the continued use of treadmills to allow animals to run or rest voluntarily, has substantially reduced stress.

We will continue optimising and adding to these refinements minimise the welfare costs to the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 327.Role of innate lymphoid cells in cancer.</b>
<b>Key Words</b>	Immunology, cancer development, metastasis, inflammation
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Despite recent advances in cancer immunotherapy, there are still many unknowns that limit our ability to harness the power of the immune system in the fight against cancer. The tumour environment can use different mechanisms to promote their growth and evade anti-cancer immune cells. We need to understand these complex interactions in more detail to design better 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)?**

We will explore the function of immune-regulating immune cells on the development and spread of cancer. Our research will specifically investigate the function of immune-regulatory cells, which are critical for controlling a type of inflammation that promotes cancer. More specifically, the proposed research will investigate the role of specific immune cells in different stages of cancer, with the ultimate aim of developing new therapies to combat or control this deadly disease. We will use several physiological models of cancer induction, including surgical administration of cancer cells or cancer-inducing reagents. We will also investigate the effect of radiotherapy, commonly used in humans, on how local radiation-induced inflammation influences the immune response in cancer. Furthermore, we will perform intra-vital live-imaging studies to visualize these actual interactions. Importantly, we will aim to translate these results to human disease. Already there are safe treatments in the clinic that target these immune cells for different diseases, and our work may lead to the “repurposing” of these available therapies for cancer treatment.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mouse. Maximum 5,240 per year.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

Adverse effects relating to tumour establishment, development and the assessment of tumours and the administration of substances and sampling procedures are mild. Our severity limit is 'Moderate'. The majority of mice will experience mild to moderate symptoms. All tumour-bearing animals will be closely monitored and will be killed should clinical indications develop, such as loss of condition, a greater than 20-25% loss in normal body weight, significant abdominal distension, dyspnoea, digestive disturbances or neurological/behavioural abnormalities. Animals will also be killed if the tumour ulcerates or if tumour burden impedes any vital function (such as locomotion, vision, eating or excretion). In all cases, knowledge of the models will be used to guide health observations and to inform decisions on killing of animals before they become severely ill. Animals will also be observed to best ensure the detection of tumour development at unexpected sites. At the end of experiment, all animals will be killed

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

My research dictates the use of animals, as the process of cancer development and spread is currently most accurately and efficiently modeled in mice. Specifically, the role of the immune system for carcinogenesis is best studied in mice for several reasons: 1) We can answer detailed questions about cancer immunology by genetically modulating immune cells in mice. This is still impossible to achieve in humans, or in a petri dish. 2) The complex interactions in immunology and cancer are impossible to model accurately outside of the body. 3) Mice still represent the best model system for studying cancer.

Nevertheless, I have previously developed techniques to model very specific aspects of the immune system in a petri dish. I will employ this philosophy to my future studies, with the aims of substituting animal experiments and/or reducing the number of animals in experiments whenever appropriate.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

Power calculations will be used to determine how many mice are required for studies to show statistical and biological significance.

As mentioned above, we plan to employ (and develop) techniques that reduce the number of mice. These techniques include 'organ in a dish' cultures.

Furthermore, I will collaborate with imaging experts to accurately monitor tumour development over time. This allows for the longitudinal analysis of single animals, leading to more robust control parameters and statistics that will ultimately reduce the number of animals required.

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

## **Refinement**

Mice are the best animal species for my research. As specified before, the many parallels between mice and humans are exploited in normal and transgenic animals. Moreover, as mentioned before, I will employ some of the best established and characterised murine cancer models with state-of-the-art immunology reagents to address questions with important implications in human disease. We will continuously refine these models to more accurately address relevant questions in human cancer research. For example, we amended our PPL to allow targeted radiotherapy treatment, which is known to involve immune responses in the cancer. Also, to accurately study the development of cancer, we will surgically inject tumour cells or cancer inducing reagents locally, which is critical for mimicking how humans develop disease.

We have optimised the procedures to minimise potential pain, suffering or distress, and enhance animal welfare. For example, new types of soft bedding material will be used for recovery from some procedures where the animal will experience pain. Also, we have developed new more refined genetic mouse models, which avoid the previous need for more harmful procedures such as cell-transfusions and irradiation. We continue to strive to develop new refinements that help us address important scientific questions with more refined (and therefore fewer and more humane) animal experiments.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 328. Assessing biomaterial and cell transplant strategies for bone formation</b>
<b>Key Words</b>	Biomaterials, Scaffold degradation, Bone formation, Stem cells
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>Yes</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Tissue engineering is a newly developed scientific field which aims to develop new therapies for the treatment of diseases which cannot currently be adequately treated with existing therapies, for example, osteoporosis or fracture non-union. Tissue engineering techniques use patients' own cells, which are grown within a scaffold (template) made from biomaterials to enable the cells to grow and develop into functional tissues. These biomaterials for scaffolds play an important role in guiding the cells' growth and also initially provide mechanical, structural support. However, the scaffolds have to be degradable. In other words, as the new tissues produce, the scaffolds should break down naturally. It is therefore essential that the breakdown rate of the biomaterials utilised within a tissue engineering therapy, are well defined. The objective of this project is to assess our newly developed biomaterials which enable real-time, non-invasive, non-destructive monitoring of the scaffold degradation rate as well as assessment of bone formation rates. This project will also address whether the incorporation of stem cells to enhance bone formation and therefore promotes better fracture healing.

**What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?**

The ultimate goal of this project is to develop tissue engineering based therapies for patients with bone disease. The successful prediction of the effect of scaffold degradation rate and stem cells' incorporation on bone formation will help to speed up the new therapy development. Our project could accelerate the development of new biomaterials and establish a new technique to test the implants' stability and degradation in real time, non-invasively and non-destructively.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We will use mice and rats for the experiments. We estimate use up to total 2,000 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 levels of severity? What will happen to the animals at the end?**

Both mice and rats will undergo procedures that involve incision in skin or creation of a defect in the non load bearing, non-articular surfaces of the skull. Scaffolds will be placed into small pockets created in the flank of the animals to assess biocompatibility. Small holes will be made in the skull of rats and repaired with newly designed and developed scaffolds, and the healing rates will be tested and characterised. These animals may experience moderate discomfort due to the surgery and the introduction of foreign materials, but anaesthesia, and pre and post operative analgesia will be provided to minimise this. In addition, the animal may experience local inflammation at the site of implantation. This is rare but could cause signs of distress to the animals. At the end of studies animals will be humanely killed so that further analysis can be performed to assess the performance of the grafts and the level of graft integration into the host, and ultimately the potential for the grafts to facilitate and promote bone healing.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

Biomaterials degradation can be initiated by multiple mechanisms. The body fluid, enzymes and mechanic force can all cause the degradation. It is also known that the formation of bone by cells within the biomaterials (scaffolds) depends on the degradation rate of the scaffolds. This complex process cannot be mimicked *in vitro* hence it is necessary to assess the degradation rate and its effects on bone formation in animal models. Although *in vitro* models can give valuable information, they are unable to completely replace *in vivo* models.

We have created ex-vivo models to pre-assess these effects and thus the animal number to be used has been reduced.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

## **Reduction**

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.

We will run pilot experiments with a relatively small numbers of animals where necessary, to establish initial biocompatibility, fluorescence tagging intensity and cell-seeding densities, from which the bony healing rate, scaffold degradation rate and imaging quality can be acquired appropriately. This strategy will minimize the chance of an experiment having to be repeated because it was incorrectly designed.

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

## **Refinement**

To minimise animal suffering, procedures in this application have been designed and selected to be the least invasive and traumatic and as surgically simple as possible. The subcutaneous model will be used to pre-screen scaffolds prior to testing in the cranial model. Only scaffolds showing favourable outcome in the subcutaneous model will progress to the cranial model. The cranial model was chosen as the bone is not jointed or load bearing and has relatively sparse nervous supply, compared to the long bones or the face, for example. This makes it one of the least painful models of bony injury. For most subcutaneous experiments, mice will be used as these are less sentient than rats, but will provide reliable data for assessing the biocompatibility of the scaffolds. For the cranial model, the mouse skull is too small and thin to be used. Such defects are likely to cause significant harm to the animal, and the thin surface is not sufficient for union with the scaffold matrix. For the cranial model, rats have been selected as the most suitable model. Where possible two scaffolds will be used per animal to allow a within animal control/comparison. Non-invasive imaging and analysis of scaffold degradation products in urine will be used for a longitudinal study, rather than sacrificing multiple animals at various time points. These measures will minimise the welfare costs to the animals.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>PROJECT TITLE</b>	<b>Project 329.Preclinical development of interventions against emerging pathogens</b>
<b>Key Words</b>	Preclinical, Emerging, Viruses, Interventions, Pathogens
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

We intend to assess new vaccines and therapies which are required to prevent and treat disease caused by viral haemorrhagic fever viruses (such as Crimean Congo Haemorrhagic Fever - CCHF), Q Fever, and influenza listed in this licence application. We need to know if these have biological activity before we try to use them in people. We can't do this in humans as the diseases are so serious.

**What 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 licence application will enable us to select good candidate vaccines and therapeutics from a range of candidates. By filtering these candidates through our models of infection, we will reduce the number of candidates required to be tested in humans and advance translational research which would otherwise only be able to be conducted in the middle of an outbreak.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We expect to mainly use mice in our studies, although for some infectious agents, rats, hamsters and guinea pigs may have to be used instead. We will use the minimum number of animals required for each process guided by using statistical power calculations. Although it is very difficult to predict how many treatments we will test during the life of this project, our past performance indicates that we will use at least 1000 animals annually for the next five years. The level of usage may, however, increase in any year due to the possibility that a candidate vaccine or therapy may suddenly need a lot more testing as it enters clinical or other critical studies.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

Overall, most of the animals will survive the procedures proposed. Animals which have been infected may develop clinical signs which indicate that they will not survive. In this circumstance, we will try to humanely euthanise them before natural death occurs. All animals which survive procedures will be humanely euthanised before safe and secure destruction within the containment facility. This will help to almost eliminate the possibility of an accidental release of a dangerous pathogen into the environment. Key harms from the infectious diseases: 1. Q Fever - the animals will generally suffer from a weight loss of approximately 20% over a period of three days followed by recovery to their pre-disease weight in a further three or four days. In some species, such as guinea pig, this weight loss is usually associated with a fever of similar duration. Animals may display some clinical signs that they are diseased such as ruffled fur, dehydration, and arching of the back during this period too. 2. Viral Haemorrhagic Fever (such as CCHF) and Influenza - in addition to those signs seen above weight loss may be more severe, approximately 30%, and evidence of hemorrhagic processes, neurological signs, or non-response to handling may be observed - in these instances animals will be humanely euthanised. Other possible harms: 1. Occasionally, antimicrobials & therapeutics suitable for use in man may have adverse effects in laboratory animals such as clearance of the natural gut flora which leads to a rapid decline in weight. Animals displaying this rapid decline will be euthanised humanely according to the criteria laid out in this licence. 2. Occasionally immunogens & therapies might cause enhancement of a disease process which leads to a rapid decline in weight and/or more severe clinical signs. Animals displaying this rapid decline will be euthanised humanely according to the criteria laid out in this licence.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

The infectious agents being researched in this application are difficult to culture in a laboratory. Although this is possible, assessing a vaccine requires analysis of the interaction of a host immune response with the infectious agent. Most tissue culture systems are unable to take into account the complexity of the interaction of an infectious agent with the immune system of a mammalian host. Although human studies of immunogenicity may be possible, animal models of infection need to be used to assess therapies and vaccines against the agents in this licence because deliberate infection of humans with these agents is unethical due to the possible adverse outcome of a failed vaccine or therapy.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

The minimum number of animals per group will be used to satisfy the power requirements of the study. The power of the study is affected by the variability of the measured parameters. Statistical advice is available to carry out power calculations and this advice will be used to minimise animal usage.

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

To minimise severity this licence includes measures to reduce severity as much as is possible. For example, a defined set of endpoints based on weight loss and clinical signs where animals can be euthanised humanely as soon as a terminal decline has been recognised in an individual but there remains a small possibility that animals may die in between monitoring periods.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 330.Repair and regeneration of the injured heart</b>
<b>Key Words</b>	heart, mouse, fish, myocardial infarction, regeneration
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

<b>Yes</b>	(c) 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 paragraph (b);
<b>No</b>	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
<b>No</b>	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
<b>No</b>	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
<b>No</b>	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Heart transplantation remains the only viable cure for adult cardiovascular disease (CVD), as such there is an urgent need for alternative therapies to replace and restore damaged heart tissue either following birth defects or heart attack. Cell transplantation has been rapidly progressed to clinical trials over the last decade, but the outcome has been disappointing to-date. We are adopting an alternative strategy for treatment, based on stimulating resident cells within the heart towards repair. To this end we seek to determine how neonatal mice and adult zebrafish can regenerate their hearts so we can stimulate similar processes to repair hearts in adult mice and ultimately human patients (objective 1) and how to control the level of inflammation and scarring in the heart after injury to enable tissue restoration to occur (objective 2). By combining insights from these two main areas of work we hope to ultimately develop therapeutic approaches to stimulate heart muscle and vascular repair and regeneration and to dampen inflammation and fibrosis (objective 3), thus preventing adverse remodelling and heart failure.

**What 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, as outlined, will help identify changes that occur in the heart in the first few minutes following the loss of oxygen and nutrients after a heart attack, thought to contribute to the early death of muscle cells in the heart, and will also provide insight into mechanisms underlying progression to abnormal heart function and heart failure. This knowledge will help us manage patients who have suffered a heart attack in the first instance and, secondly, may lead to the development of new treatments drugs to stimulate the regeneration of lost cardiovascular tissue and to modulate

inflammation; thus reducing the risk of further heart attack and progression to heart failure.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

The expected approximate usage of animals per annum is as follows: Mice- 2400 for generation of genetically altered lines and breeding and maintenance to supply the project and, usage of 1540 940 adult mice and 600 neonatal mice in surgery and therapeutic agent testing. Zebrafish- 2200 for the generation of genetically altered lines and breeding and maintenance (includes embryos and adults). 2300 adult zebrafish for surgery (heart and tail fin/flank) and cell and compound testing. Medaka – 660 for generation of genetically altered lines and breeding and maintenance (includes embryos and adults). 1200 adult medaka fish for surgery (heart and tail fin/flank) and cell and compound testing. These apply over the 5-year lifetime of the project.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

We are breeding genetically altered mice and fish, the majority of which will be used for breeding and some of which will be used in experiments as adults. In the adult experiments we will need to injure heart muscle in living animals; in these cases, either a blood vessel in the heart will be tied-off to block the blood flow, or a piece of heart muscle will be removed or injured by freezing under general anaesthesia. With heart surgery there is a risk of death, but this is minimized, in our hands, to less than 10%. We will test whether administering cells and/or drugs can induce optimal repair of the heart via new tissue growth and/or reduced inflammation and scarring. In the case of mice, animals will be allowed to recover and given pain-killers; for zebrafish, we will test whether pain-killers are effective without altering outcome. The function of the heart will be monitored in the ensuing days (or weeks) by ultrasound imaging in conscious animals, or by studying the function of the heart in anaesthetised animals. At the end of the study, animals will be humanely killed and tissues removed for further analyses.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

The heart is a complex organ containing many cell types of which arguably the most important are the muscle cells, responsible for the pumping function of the heart and the endothelial and smooth muscle cells, which make up the blood vessels of the

heart. Many of the experiments we propose will be carried out on isolated pieces of cardiac tissue or cell cultures of heart muscle, blood vessel and epicardial cells studied in the laboratory. However cells in a test tube or in a tissue culture dish cannot be used to study the complex changes occurring in the complete heart, nor how it functions in a living animal. Equally, isolated cell populations in tissue culture transform to adopt different functional characteristics, compared to the equivalent cells as they reside in the heart proper, which confounds any experiments to determine the effect of externally-added factors on heart injury and repair.

## **Reduction**

Explain how you will ensure the use of minimum numbers of animals

### **Reduction**

Power calculations (80% power at 5% significance to show a 25% point difference in any one parameter) provide a minimum number of animals. Use of between 8-11 per treatment group ensures statistical significance, given the inherent variation between animals in response to heart injury. An important reduction in number will be by restricting control (sham-operated) animals to the first set of experiments to determine the baseline response of the heart to the surgery itself, in the absence of the final injury insult; once this is standardised sham animals will not be required.

## **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

The choice of species is based on the need to study regenerative animal models, such as the adult zebrafish and neonatal mouse as compared to a non-regenerative model such as the adult mouse and medaka fish which recapitulates the response to oxygen-deprivation and cardiac injury in humans. Moreover, the genetic tools (transgenic and knockout lines/strain in both fish and rodents), the ease of manipulation of individual cell populations and proteins both in circulation and resident within the heart make these models relevant for translating findings into humans. For all surgical procedures in mice pain killers will be administered routinely for the control of post-operative pain and aseptic techniques will be used to minimise the risk of post-operative infection. For teleost fish including zebrafish and medaka fish, pain sensitivity is unclear and no recommended pain killers exist, so we will test those used for routinely elsewhere for effects on fish and on the outcome of our experiments. We have also introduced ECHO as an imaging modality for assessing cardiac function in fish (in addition to MRI), that does not require injection of contrast agents (as for MRI) and moreover is conducted over a much shorter

timeframe thus reducing the length of time of exposure of fish to anaesthetic and risk of over-anaesthesia. Animals will be routinely monitored after surgery for signs of discomfort in recovery and any infection treated with veterinary advice. General anaesthesia will be used for mouse models requiring heart surgery. For the neonatal mouse model this has recently been refined across early stages to ensure that ice-induced anaesthesia is combined with a suitable inhalation agent to ease potential discomfort upon recovery and body warming; working closely with an in-house anaesthetic expert.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 331. Immunology of respiratory infection and inflammation</b>
<b>Key Words</b>	Lungs, infection, vaccination, age.
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Respiratory infections 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. Clinically, these can cause colds, which are a significant economic burden in terms of time lost from work, but can lead to severe disease and mortality in susceptible groups. These groups include the very young, where respiratory infections are the leading cause hospitalisation, the frail elderly, and those with underlying long term health conditions such as asthma. For many respiratory infections treatment is only supportive, there are no preventative drugs, or their cost is prohibitive, and vaccines are not available or effective.

Our work aims to understand the immune response to respiratory infections. Our objectives are to understand how the immune response can protect against infection and how the immune response sometimes leads to too much inflammation in the lungs and disease. In addition we want to determine what is different about the immune response in the very young, elderly and in people with chronic lung disease such as asthma.

We are also interested in understanding the effects of respiratory infection on the rest of the body, such as how infection can lead to muscle wasting.

**What 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. In doing so we will contribute to the field of immunology and respiratory diseases. In the longer term this basic understanding should lead to new therapies which promote protective immune responses, or prevent unwanted and

potentially damaging inflammation. This may include the development of new vaccines for respiratory infections.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

We estimate that we will use approximately 16 000 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 levels of severity? What will happen to the animals at the end?**

In a typical experiment, we would aim to understand the role of a particular component of the immune system in protecting against infection or causing pathology in the lung. This will typically involve altering the immune response using different means, such as using genetically modified mice or by vaccinating the animals, before infection with a respiratory virus. Lung infections can lead to illness in mice and we expect some symptoms of infection including some 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 deficient 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

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

Immune responses to infection are complex and involve an interplay between the bacteria or virus causing the infection, the infected organ and the immune system in ways that cannot be reproduced in culture systems. We need to use a mammalian species due to the similarities in the immune and respiratory systems between these animals and humans.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

We always endeavour to obtain maximal information from each animal we use and take many different tissues from each infected animal in order to gain many different readouts of the immune response to infection. Group numbers are kept to a minimum, but are sufficient to gain meaningful data.

Numbers of genetically modified animals bred will be kept to the minimum numbers required for experiments.

### **Refinement**

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

### **Refinement**

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. Appropriate doses of pathogen are used 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. Any animals showing signs of severe disease are euthanized. Whenever procedures could cause pain or severe discomfort, analgesia is used or animals are anaesthetised. Good, sympathetic, animal handling, injection and blood sampling techniques will minimize discomfort. When pups are used, 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, nesting material, with individually ventilated cages.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 332. The evolution of food hoarding</b>
<b>Key Words</b>	
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>No</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
<b>No</b>	(c) 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 paragraph (b);

No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Evolutionary selection on behaviour has changed brains. However, we know very little about which changes in brains can lead to changes in behaviour. Here we study which changes in brain structure and/or function have led to the evolution of food-hoarding behaviour from ancestral animals that did not hoard food.

**What 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 better understanding of brain evolution, and what kind of changes in the brain lead to evolutionary changes in behaviour. This has a larger relevance for understanding ourselves and our own evolution.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Coal tits (*Parus ater*), blue tits (*Cyanistes caeruleus*), and great tits (*Parus major*); 100 in the first protocol, although this has been increased to 160 since more funding has been obtained. Maximum 300 birds 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 levels of severity? What will happen to the animals at the end?**

We will house coal tits (hoarding birds) and blue and/or great tits (non-hoarding close relatives) in conditions which we expect to increase hoarding motivation (half the birds) or conditions which should minimize motivation to hoard (the other half of the birds). These conditions mainly consist of an unpredictable food supply (high motivation) vs. predictable ad libitum food (low motivation). We will also look at the effect of social rank (being dominant or subordinate) on the motivation to hoard. We will then verify the success of our environmental manipulation by measuring both consumption and hoarding behaviour in the animals. Because we believe that the mechanisms that control hoarding motivation work through the stress hormone corticosterone, we will also monitor corticosterone levels in the blood stream of the

animals. The adverse effects are minimal: - Stress of captivity: mild; minimized by habituating them to captivity in a large aviary with many hiding places and by housing them in pairs in relatively large cages. - Effects of temporary food restriction: mild; minimized by never food restricting for more than 90 minutes at a time, and providing enough food through the day; body mass monitoring - Effects of blood sampling: mild: small possibility of too much blood loss. Minimized by taking very small samples and stopping the bleeding with cotton wool. We always check bleeding has stopped before the birds are returned to their cages. At the end of the study, the birds will be humanely killed to collect brain and other tissues for further examination and comparison between the two species.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

#### Replacement

We are interested in the physiological basis of animal behaviour. Only animals can behave and we therefore need to use live animals.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

#### Reduction

The experimental design is well balanced and multifactorial in order to increase statistical power. We are using the minimum number of animals required to pick up expected effect sizes.

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

#### Refinement

Coal tits are the most common food-hoarding birds in the REDACTED. The only alternative that is relatively abundant as well are rooks and magpies, and these would be much harder to work with, and would probably be affected more by the studies. Great and blue tits are the most common close relatives of the coal tits and therefore provide the best non-hoarding comparison species for the physiological responses of the coal tits to the environmental manipulations we will perform.

The refinement measures are how we habituate the birds to captivity (large aviary, places to hide); how we house them (in pairs); and how we avoid handling the birds as much as possible. For example, to shuttle birds back and forth to the behaviour testing aviary, we let them fly from the home cage to the room, and train them to fly back by turning off the light in the aviary and on in the home cage.

## NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

<b>Project Title</b>	<b>Project 333. Ischaemia-Reperfusion of Thoracic Organs</b>
<b>Key Words</b>	myocardial infarction, ischaemia-reperfusion
<b>Expected duration of the project</b>	5 year(s) 0 months

### Purpose of the project (as in ASPA section 5C(3))

Purpose	
<b>Yes</b>	(a) basic research;
	(b) translational or applied research with one of the following aims:
<b>Yes</b>	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
<b>No</b>	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
<b>No</b>	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) 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 paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

**Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):**

Ischaemic heart disease, characterised by a reduced blood supply to the heart, is the most common cause of death in Western countries. Considerable research effort has so far failed to adequately determine the essential cellular mechanisms responsible for myocardial cell death when deprived of an adequate blood supply (ischaemia) or, paradoxically, when blood is returned (reperfusion). The cellular processes involved in ischaemia/reperfusion injury form the overall objective of the studies outlined in this application, and the information will be used to determine ways in which this injury process can be ameliorated. The proposed studies will address some potentially fundamental and interesting differences between these processes regarding how heart cells respond and adapt to injury. From this information, it is anticipated that new therapeutic targets for the amelioration of cardiovascular disease may be generated.

**What 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 investigate the mechanisms of tissue damage following ischaemia and subsequent tissue reperfusion. It is feasible that these studies may result in the development of a novel methods for the detection of myocardial infarction (cell death due to ischaemia) and for the treatment of acute myocardial infarction and ischaemic heart disease (i.e. patients who survive the initial event), both of which are major causes of premature mortality in the UK.

**What types and approximate numbers of animals do you expect to use and over what period of time?**

Mice – 7,500 over 5 years Rat – 5,500 over 5 years Guinea pig – 1100 over 5 years Rabbit – 600 over 5 years Note that these numbers represent the expected use between 20+ active researchers working on this project.

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?**

This project will investigate the mechanisms of injury and death following ischaemia and subsequent tissue reperfusion. Studies will be conducted primarily in tissues isolated under terminal anaesthesia and with little or no suffering. In a limited number of studies, animals will undergo pre-treatment with pharmacological agents, or by modifying their diet, with few or no adverse effects expected. Animals will be given appropriate care, including pain relief, to limit any pain and suffering. Animals will be continuously monitored for signs of distress and, if necessary, humanely euthanized. Few adverse events are expected with this approach.

## Application of the 3Rs

### Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

### Replacement

Cardiac ischaemia, and reperfusion injury, are complex and incompletely understood phenomenon, involving the interaction of multiple factors, and, as such, cannot currently be studied without animal models. Our understanding of the processes involved, and their relative importance, limits our ability to use computer modelling, though this is a goal we are working towards.

### Reduction

Explain how you will ensure the use of minimum numbers of animals

### Reduction

Experimental data will be continuously analysed and assessed to achieve the aims of the project with the minimum number of animals. All protocols will be refined and conducted by trained individuals, to reduce errors and experiment numbers. Studies will conform to the NC3Rs ARRIVE guidelines.

### Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

## Refinement

Mice and rats are the main species used in this project, and are well-established models for the study of ischaemia and reperfusion. The basic mechanisms of cell death and injury following a reduction in blood supply are similar between small laboratory species and man. In some experiments (particularly those examining arrhythmias mechanisms) larger species such as the guinea pig and rabbit are required mainly because these are the smallest species that share the basic electrophysiological features of the human action potential.

Our experimental protocols have been developed to limit harm to the animals, being as short as reasonably possible and mainly conducted under general anaesthesia. We will continue to make efforts to refine protocols and further reduce the welfare costs.