Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2017

Volume 2 (granted between 1st July to 31st December 2017)

Contents

PROJECT 1: CONTROL OF NEURAL STEM CELL PROLIFERATION AND DIFFERENTIATION

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

Project Title	Control of Neural Stem Cell proliferation and differentiation
Key Words	Neural development, protein arginine methylation
Expected duration of the project	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):

During development a founding population of precursor cells, called neural stem cells, generate all the neurons (cells that relay information) and the supporting glial cells (which provide nutrients to the neurons and insulate them, much like the wrapping of electrical cables) of the central nervous system in a precise spatiotemporal manner. Central to this process is the transition of the neural stem cells from their precursor, functionally unspecialised state to a differentiated state, whereby they become neurons or glial cells. Aberrations in such a transition are linked to neurological diseases, e.g., autism, schizophrenia, mental retardation. The molecular mechanisms controlling neural stem cells' transition from "stemness" to differentiation (generation of specialised cells) remain elusive. REDACTED The group of proteins which are preferentially modified by the novel complex identified by us is involved in the control of expression of genes which are necessary for proper functioning of different neural cells. They are called the RNA binding proteins because they bind and process the RNA which is made when the genes are transcribed from the DNA - a process which ensures that the correct genes are made and processed in such a way as to ensure generation of proteins necessary for proper neural function. Several of the RNA binding proteins which are modified by the complex identified by us are implicated in a variety of neurological disorders, e.g., Fragile X syndrome, amyotrophic lateral sclerosis/fronto-temporal dementia (ALS/FTD).

The aim of our investigations is to elucidate the biological significance of the modifications of the RNA binding proteins, which are mediated by the novel complex we found, in order to understand how these biochemical modifications control their function in normal neural physiology and in the diseased state. Given the great

challenge in treating almost all neurological diseases, any new information about the contribution of different cellular machinery to the progression or initiation of such diseases, will provide valuable and much needed potential targets of therapeutic intervention. Moreover, we may be able to identify new and desperately needed biomarkers, which may be useful in diagnosing such diseases at earlier time points when intervention is more likely to lead to successful treatment. While there is a clear awareness of the involvement of the RNA binding proteins which are modified by the complex identified by us in both normal neural physiology and in neurological disease, very little is known about how the activity of these proteins is controlled. We will address this gap in our knowledge in the proposed work and hope to contribute significantly to the field of both normal neural development and to our understanding of how aberrations in the modifications of these proteins may lead to neurological 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 control of RNA processing proteins' functions is emerging as one of the essential goals in both normal neural development and in a variety of neurological diseases, in which many of these proteins are implicated. Our project is likely to contribute in a major way to defining novel mechanistic insights into neural stem cell biology and the aberrations associated with various neural abnormalities by focusing on the elucidation of a novel control mechanism of the RNA-binding proteins' activity. Therefore, in the longer term, we believe, that the proposed work will add considerable knowledge and expand the repertoire of possible therapeutic targets in treating debilitating neurological diseases.

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

Our work relies on the use of transgenic mice as these are the species which are best suited for genetic manipulations and which provide close models for mimicking human disease. We will minimise the use of animals by using littermates for our investigations. We anticipate that we will be using ca. 1000 mice over the course of this 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?

All the procedures listed on the project licence application are of mild severity. The mice may feel very mild and very transient pain when we are genetically typing them by ear punch or when administering substances intraperitoneally with a syringe. Great care and precautions will be taken to minimise any discomfort and ensure that the animals are allowed to recover comfortably post-administration of substances. The animals will be humanely killed at the end of the experiments. Animals may be injected up to six times with substances that are known to cause little or no pain,

distress, suffering or lasting harm. Where animals are exposed to potentially more painful procedures they will be undertaken under anaesthetic from which the animal will not wake up.

Application of the 3Rs

Replacement

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

Replacement

All neurons and glial cells that make up the central nervous system (CNS) develop from "neural stem cells" in the embryonic neural tube - the structure which eventually develops into the brain and spinal cord. Neural stem cells receive local instructions from different parts of the neural tube, which direct them along different developmental pathways. The molecular nature of these signals has not been identified in detail making it practically impossible to study CNS development in tissue culture only. This necessitates the use of intact animals for such studies to be truly informative.

Reduction

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

Reduction

Many of our mechanistic studies will be performed using established immortalised cell lines, e.g., P19 mouse embryonal carcinoma cell, PC12 cells, and only verified in primary neural stem cells or animals, thus minimising the numbers of animals used.

Further, we have already generated a triple transgenic mouse strain which carries the deletion of the gene of interest from the cortical neural stem cells and produces the fluorescent reporter, called Green Fluorescent Protein, in all mutant neural stem cells. This will reduce the number of breeding crosses necessary for our proposed experiments and minimise the total number of animals used overall.

To quantify cell numbers, we use multiple brain sections from around 5 animals per experiment. Using litter mates and performing independent experiments in duplicate or triplicate minimizes and controls for inter-animal variation, in the process helping to reduce the number of litters 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 are the animal of choice since they are the only mammalian species that can be easily manipulated genetically with well documented brain anatomy, physiology and function. Moreover, many mouse models resemble various human diseases thus providing an excellent possibility to advance clinically relevant studies.

Most of our transgenic manipulations are not expected to cause discomfort, pain or distress to the mice. Moreover, our initial observations of the transgenic mouse strain we are working with indicate that these animals appear healthy and do not show any signs of discomfort. If unexpected adverse effects should develop (e.g. neurological signs such as tremor, circling behaviour, hyper-activity) the animals will be humanely killed.

PROJECT 2: FINE-SCALE MOTIONS OF BASKING SHARK RELATED TO FORAGING AND COURTSHIP BEHAVIOUR

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

Project Title	Fine-scale Motions of Basking Shark Related to Foraging and Courtship Behaviour
Key Words	movement, foraging behaviour, courtship behaviour, conservation
Expected duration of the project	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;
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;
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):

To understand the foraging and courtship behaviour of basking shark in relation to its migration and local movement.

What 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 species is listed as Endangered in the east North Atlantic (IUCN) and the study is designed to improve our knowledge of basic behaviour of the shark. This in turn will lead to refined management and protection measures for the species.

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

A maximum of 100 basking sharks 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 procedure is not expected to have any adverse effect on the sharks, given past experience. The animals are in the wild and will continue their normal behaviour.

Application of the 3Rs

Replacement

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

Replacement

The study is aimed at helping to conserve the species and information can only be collected using the species to achieve this goal for the species.

Reduction

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

Reduction

The season for tagging is very short and time is needed to collect the tags after deployment, and the tags are relatively expensive. Additionally, for an effective study, the number of sharks needed has been set to a minimum per year for statistical analyses. These factors limits the number of sharks 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 is listed as Endangered in the east North Atlantic (IUCN) and the study is designed to improve our knowledge of basic behaviour of the shark. This in turn will lead to refined management and protection measures for the species.

PROJECT 3: INVESTIGATING DENDRITIC AND SYNAPTIC STRUCTURE FUNCTION RELATIONSHIPS IN NEUROPSYCHIATRIC DISORDERS

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Word limit; 1000 words

Project Title	Investigating dendritic and synaptic structure function relationships in neuropsychiatric disorders.
Key Words	Dendrites, neuropsychiatric disorders, neurogenesis
Expected duration of the project	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):

The structure of excitable cells in the brain (neurons) is intimately related to their function and ability to process information. By looking at the post-mortem human brain some significant changes to the structure of neurons in areas involved in sensory and cognitive processing have been found in patients with schizophrenia, bipolar disorder and autism spectrum disorder. These structural changes and their functional effects are beyond the current resolution of non-invasive human experimental methods, such as MRI, and as such, until recently, investigating their role in disease has not been possible. However, large scale human genetic studies have begun to identify specific genetic changes that markedly increase risk for development of major psychiatric conditions. Disruption of these risk genes in rodent models has recapitulated many of the structural changes observed in the diseased human brain opening up the possibility of studying their role in disease. This project will combine rodent models with cutting edge experimental techniques to investigate the normal function of brain circuits and the mechanisms and pathways underlying disruption of function in neuropsychiatric diseases. Increased understanding of these mechanisms opens the possibility of identifying novel treatments for these individually, societally and economically costly diseases.

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

This project will advance the scientific understanding of basic mechanisms of dendritic function, sensory processing in the thalamus and the mechanisms underlying adult neurogenesis in the hippocampus. These processes are incredibly important to normal human brain function and behaviour but are also disrupted in range of neurodevelopmental and neuropsychiatric diseases. This project has the

potential to identify how human disease risk-associated genes alter these important physiological processes and to identify new potential therapeutic targets for the treatment of these disorders where current treatments are not optimised.

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

We will use a maximum of 750 mice and 750 rats including genetically modified, inbred and conventional strains during the 5 year period 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 protocols we will use in this project subject the animals to a maximum level of 'moderate' severity. We do not anticipate that these procedures will produce significant adverse effects in the animals or result in more than minor stress and discomfort. The majority of animals will only be subjected to 'mild' severity procedures described for breeding and husbandry in Protocol 1 before being used to provide tissue for ex vivo experiments as described in Protocol 3. At the end of experiments animals will be humanely killed to provide tissue for genetic and other analysis to maximise the information obtained from the smallest number of animals possible.

Application of the 3Rs

Replacement

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

Replacement

The processes we aim to investigate in this project depend upon the very precise structure of brain cells and circuits and these cannot be recapitulated in any non-mammalian animal model, in cell culture or *in silico*. Moreover, we cannot study these process non-invasively in the human brain because they are beyond the resolution of existing techniques.

Reduction

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

Reduction

We will employ good experimental and statistical practises to ensure experiments are designed to obtain the best quality reliable data from the minimum number of experimental animals possible. The breeding of animals will be tightly regulated to ensure only animals required for experimental procedures are produced.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 to use rodents as the processes we aim to investigate show similarity to those in the human brain. Rodents represent the lowest sentient mammalian species with sufficient similarity to humans to make the experimental findings valid and translatable to human processes. Rodents also offer potential for genetic manipulation to produce experimental models of human disease and tools for interrogating brain circuits (e.g. optogenetics).

We will also make technical advances to our experimental techniques, using viruses to target DNA to specific cells, that allow us to significantly reduce the numbers of animals used compared with previous approaches.

Animal welfare will be ensured by the high level of training supplied to individual scientists and technical support staff and by working closely with qualified veterinarians.

PROJECT 4: UNDERSTANDING INFLAMMASOME DEPENDENT INFLAMMATION

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

Project Title	Understanding inflammasome dependent inflammation
Key Words	Inflammation, Disease, Injury, Infection
Expected duration of the project	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;
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):

Here we aim to understand the regulation of an organisms inflammatory response to infection or injury. The inflammatory response is how our immune system reacts to a stress or danger. Inflammation in the absence of infection (sterile inflammation), during injury or disease, can often be damaging and is increasingly implicated as a important factor in many human diseases such as Alzheimer's disease, stroke and metabolic diseases. There are particular components of the inflammatory response that are now known to contribute to disease (called the NLRP3 inflammasome) but we do not know fully how this is works and we do not have good ways of stopping its actions. The objectives of this work are to understand how the inflammasome (in particular NLRP3) works, to identify molecules that can stop it 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)?

We aim to develop a better understanding of the pathways regulating inflammasome-dependent inflammation and through this understanding, develop new therapeutic interventions for inflammatory disease. In particular we hope to identify new drugs to modify dangerous inflammatory responses. This work could benefit humans as well as animals where inflammation plays a key role in the disease.

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

Mice (1800) and rats (350) 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?

The severity is moderate and the animals will only experience temporary discomfort. We will cause an inflammatory reaction in the animals by injecting them with agents that mimic an infection or a sterile injury. The animals will experience some sickness and the impact will be similar to what humans experience when sick with an infection and symptoms could include lethargy, fever and reduced appetite. However, the symptoms will usually only last for a few hours before the animals are sacrificed to take tissues for ex vivo analysis. In those tissue we will then look at the expression of immune cells and the agents that they release.

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 vascular and cellular response that cannot be modelled accurately in *in vitro* systems. An inflammatory response also produces symptoms such as sickness behaviour which is due to an interaction of the immune system with the nervous system and the brain. Thus, the whole body is involved in an inflammatory response and as such whole animals are needed to understand this complexity. The proposed studies could also not be undertaken in lower species because they do not show such similarities to humans including their immune system, and importantly they do not have NLRP3, which is a key complex that we are interested in.

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. Whenever we get new data we will always re-do our calculations in order to make sure we are still using an appropriate animal number to achieve our 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

Mice and rats are the lowest vertebrate species that share common pathways to humans with respect to this pathway. We will use well-established methods to cause inflammation without causing severe or long lasting harm to the animals. We understand what doses cause a response in the animals without making them too sick, so we will always use the lowest dose possible to give us an answer. 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.

PROJECT 5: STRUCTURE AND DEVELOPMENT OF THE MAMMALIAN BRAIN

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Word limit; 1000 words

Project Title	Structure And Development Of The Mammalian Brain
Key Words	Cerebral cortex, thalamocortical projections, subplate neurons, hypoxia-ischaemia, development
Expected duration of the project	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;
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):

Our aim is to study the basic steps of brain development (neurogenesis, neuronal migration, development of connectivity) to understand the molecular and cellular mechanisms determining functional cortical circuits with particular attention to the early circuits in the cerebral cortex.

The causes and remedies of a large number of cerebral cortical developmental disorders are not known, but their prevalence in the general population numbers are high [schizophrenia (1:100); autism (1:68); attention deficit hyperactivity disorder (1:30); dyslexia (1:10); childhood epilepsy (1:200); neural tube closure defects; cerebral palsy (2:1000)]. The developing brain is not just a smaller version of the adult brains. The management of developmental conditions requires very different approaches than in the adult. The vulnerability of the developing brain to **hypoxia, ischemia, maternal infection** are still not understood, but the impact on the life on the individual and their family can be devastating. These particular studies could have direct implications on establishing general policies e.g. **vaccination during pregnancy**, with or without particular genetic susceptibility or management of neonatal hypoxia-ischaemia.

Some cortical developmental disorders cause abnormal neurogenesis or cortical neuronal migration and generate smaller (**microcephaly**), some unusually smooth cortical surface (lissencephaly) or unusually convoluted brain (**polymicrogyria**) that has devastating consequences on learning ability. We do not yet understand the basic principles of these cortical abnormalities.

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

Greater understanding of these basic neurobiological problems could contribute to the prevention and treatment of many neurological and psychiatric disorders (e.g. childhood epilepsy, schizophrenia, attention deficit hyperactivity disorder, autism) that affect millions of people of all ages at tremendous cost to the national economy. Understanding the possible dangers during brain development can help with prevention or treatments of several neuronal developmental disorders. We hope to contribute to the basic knowledge base that, in time, shall have a very significant impact on this field, including clinical diagnosis and possible treatment.

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

Mouse and rat are the selected models for these studies. Including breeding of transgenic animals, we expect to use up to 41200 mice (including 18000 embryos older than 14 days of gestation but that may never be born) and up to 3175 rats over the next 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 vast majority of the experiments will rely on collecting tissue for further analysis with minimal intervention (mild). Some procedures will require injections of tracers, genes, and/or pharmacological agents to the developing or adult brains to study the anatomical and physiological effects. In some cases we shall perform neurosurgery to administer substances to the brain, or introduce electrodes to record from the brain. In some of the experiments we shall introduce specific channels into selected neuronal populations that can be then monitored or modulated. Some of these genetic manipulations or pharmacological interventions or perinatal hypoxia-ischemia or maternal inflammation during pregnancy will change the behaviour of the animals, This will be closely monitored and every effort will be made to minimise the pain or suffering (moderate). All animals in our experiments will be killed either under terminal anaesthesia or 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 use of animals is essential; currently we cannot use non-animal alternatives. The questions cannot be answered in human or with cell-line models. Nevertheless, in the future computer models may 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 maximising the information obtained from each animal will use the minimum number of animals. We shall follow the following principles: (i) The experiment should as directly as possible seek to answer the scientific question under study. (ii) Techniques will be used that can maximise the information obtained from each experiment. (iii) Statistical power will be assessed before and in the course of the experimental series.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 rats and mice to perform our experiments because they are the lowest vertebrate group on which the above-described experiments can be performed. The presence of isocortex (sixed layered neocortex) is limited to mammals; therefore the use of fruit fly, zebra fish or chicken is limited to certain specific questions relating to gene interactions and cascades.

The protocols we use are well established and scrutinised by the veterinary and research communities. We shall ensure that we further improve these protocols in the light of new developments in anaesthesiology and surgery as more information becomes available. We have recently introduced “glow checking” as a means of genotyping some transgenic animals to reduce the need for tissue biopsies for genotyping.

We are using state of the art methods to provide anaesthesia to the experimental animals and use refined surgical procedures with aseptic technique to achieve our objectives.

PROJECT 6: DESIGNING OPTIMAL CONSERVATION STRATEGIES FOR MARINE FISH IN BRITISH WATERS

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Word limit; 1000 words

Project Title	Designing optimal conservation strategies for marine fish in British Waters
Key Words	Basking shark, Bluefin tuna, Marine conservation
Expected duration of the project	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.

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 aim of this project is to generate species-specific data to improve the conservation management evidence base for basking sharks and bluefin tuna in UK territorial waters, using electronic tags and autonomous underwater vehicles. For basking sharks, this pertains to increasing information regards breeding behaviour, including mate selection, energy expenditure and response to human activity (e.g. boat traffic, noise and strike risk), for bluefin tuna this pertains to increasing information on the genetic stock of origin and movements both within UK coastal waters and throughout the Atlantic.

What 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 more coherent understanding of the movements and behaviours of these species will improve our understanding of their location-specific behaviours. The benefits of improved knowledge will help minimise human-wildlife conflict, promote population recovery and safeguard the ecosystem services they deliver. These ecosystem services include grazing on plankton (by basking sharks), nutrient cycling and predation that regulates abundance (bluefin tuna, basking sharks). Data gathered also have the potential to contribute to site selection for civil engineering projects at sea and seismic survey operations for hydrocarbon exploration, both of which pose concern for wildlife due to their potential environmental effects, in particular noise. Further, access to these animals will allow us to develop eDNA techniques; these are newly developed techniques that allow water samples to be analysed for the presence of species based the presence of their DNA. This is a non-invasive procedure once developed and will lead to a step change in our understanding of the distribution of species of conservation concern.

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

We expect to tag up to 100 basking sharks with electronic tags. Up to 250 bluefin tuna will be tagged with conventional identification tags. A subset of these will be fitted with electronic tags. The project will be active for 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?

We expect adverse effects to be minimal and mostly associated to the animal handling procedures needed to capture animals and attach tags. The level of all procedures will be mild. Electronic tags will eventually detach from animals that have been captured and tagged and thus setting animals free to the wild. Bluefin tuna receiving only conventional identification tags will be set free to the wild immediately upon release to the sea.

Application of the 3Rs

Replacement

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

Replacement

Fisheries-independent data on the movement, behaviour and distribution of free-ranging marine fish can only be gathered from animal-borne electronic tags, conventional identification tags or from underwater autonomous vehicles. No alternatives exist to gather robust data to inform the development of conservation management strategies for these species of conservation concern.

Reduction

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

Reduction

Ecological, behavioural and management information on basking sharks and bluefin tuna in UK waters is limited. As such, we strive to gather robust data, which necessitates an approach of collecting data until resulting patterns begin to stabilise. Population sizes of these species are also unknown and so our estimates of numbers to be tagged cannot be contextualised, however we feel confident that populations are not so small that our work would have a deleterious impact on population trajectory and that our suggested sample sizes will likely yield information with low levels of uncertainty when being used to infer population-level generalisations about behaviour and movement necessary to develop management plans.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 animals represent species of conservation concern and have been selected as they span the ecosystem from planktivore to apex predator. Each species represents significant conservation challenge to the UK and data on their behaviour and movement are limited yet there is a pressing need to better manage these species for improved conservation trajectories. The project will take all possible measures to minimise harm to animals, from optimising animal handling techniques, through to use of best practice tagging techniques and technology. All equipment is designed to eventually detach from animals with fail safes also employed to minimise chances of tag associated entrapment in objects (e.g. fishing nets).

PROJECT 7: IN VIVO MODELS OF JOINT DEGENERATION

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.

Word limit; 1000 words

Project Title	In vivo models of joint degeneration
Key Words	Osteoarthritis, Inflammatory arthritis, Abnormal loading, knee joint, Drug therapies
Expected duration of the project	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;
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.
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):

Osteoarthritis (OA) is a debilitating condition that affects over 8.5 million people in the UK alone. Joints such as the knee and hip are commonly affected; the disease manifests initially as pain, stiffness and inflammation during the early stages followed by end-stage loss of articular cartilage (which covers the ends of long bones). Articular cartilage is highly important as it protects our joints against the impacts received during daily movements; without this cartilage, the joint is unable to function correctly and this characterises the pathology of OA. Major risk factors demonstrate that mechanical loading influences the start and progression of OA. Mechanical factors associated with protective normal loading (i.e. walking, jogging) and destructive abnormal loading (sports injury, obesity) in humans, and the way in which these factors influence joint function and pain are largely unknown. ***Understanding these links will help identify new targets for therapy and help make decisions on surgery and rehabilitation to improve care of patients and animals with arthritis.***

Specifically, this project will determine how mechanical loading influences the maintenance or breakdown of musculoskeletal tissues. The effect of mechanical load on transgenic mice (i.e. loss/addition of a gene, genetic mutation akin to that observed in the human population) will also be carried out to determine how important specific molecules are in the development of arthritis, and consequently how we may specifically target them to develop a drug therapy for this disease. This will provide important information to help in the understanding of processes that lead to joint diseases in particular OA and also rheumatoid arthritis (RA), as well as aid in the development of new targets for arthritis drug design.

We intend to use three different models, all of which induce an arthritic disease in the animal including:

1) A well-defined non-invasive loading model in living mice and rats that will apply mechanical loading to long bones and joints of one leg under general anaesthesia

while the other leg acts as a control. The model will either recapitulate a loading regimen that mimics long-term degeneration of the cartilage, akin to that observed in human primary ‘wear & tear’ OA, or it will elicit load-induced rupture of the anterior cruciate ligament, akin to that experienced in human post-traumatic OA after a sports injury/accident.

2) A well-defined surgical model in living rats that, under anaesthesia, surgically destabilises the meniscus (a key component in the joint that protects the articular cartilage from excessive loads), akin to that experienced in human post-traumatic OA after a sports injury/accident.

3) A well-defined inflammatory model of arthritis in which this disease is induced in rats, akin to that observed in OA and RA pathology.

Each of these *in vivo* model systems will allow us to determine how the pain and tissue degeneration is instigated in arthritis, thereby providing vital information to further our studies into developing therapies to treat arthritis in both humans and 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)?

This project will further characterise our existing targets and identify new targets to develop new therapies for use in the treatment of arthritic joint disease in both human and animal patients. It will determine points in the disease process where we may be able to intervene to halt progression or even reverse the effects of arthritis e.g. pain and tissue breakdown to improve the patient’s quality of life. The information that we get from these animal studies will enable us to correlate these outcomes with our other studies on human patients suffering from arthritis.

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

Rodents (mice and rats) will be used for the proposed studies. It is envisaged that a maximum of 2400 mice and 950 rats will be used for the remaining arthritis induction protocols over the 5 year duration of the project licence.

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

Experimental protocols are well-established in our group, and therefore the likelihood of adverse effects are low. Furthermore, to reduce the likelihood of this occurring, we have stringent procedures in place to minimise risk/harm to the animals. Two of the protocols are expected to be low severity with minimal discomfort to the animals and the remaining four protocols are expected to have moderate severity as they could induce discomfort and/or distress to the animals. The primary adverse effect is likely to be pain experienced by the animal after the specific procedure and once the

arthritis has developed in the joint; therefore a painkiller will be administered at the time of the procedure and continuous monitoring of animal welfare will be conducted over the period of study. At the end of the study, animals will be humanely culled and joint tissues processed to maximise the amount of information collected and analysed.

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 nature of the project, animal replacement is not feasible. Within the Centre, we are developing explant and cell based models to investigate the mechanisms of joint destruction and protection in specific tissues and use these models wherever possible. However, experiments carried out on isolated cells and tissues alone are unable to recapitulate the biomechanical environment and the biological interactions between the surrounding joint musculoskeletal tissues, and hence the complexities of how the entire joint responds to mechanical load. Furthermore, validation of targeted interventions as therapies for arthritis treatment can only be truly assessed in *in vivo* models where we can directly measure how well they rescue the disease symptoms in the different joint compartments. This can only be addressed by conducting the proposed *in vivo* experiments.

Reduction

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

Reduction

Due to the highly reproducible nature of the invoked pathology in our model systems and in conjunction with power calculations, a group size of 5 mice is required increasing to 8 animals for the intervention studies in order to reach statistical significance and acquire meaningful data. This has also enabled a reduction in the number of control animals required. Our use of non-surgical loading models also eliminates the necessity for sham surgical controls, halving the number of animals per intervention 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

Rodents will be used for these studies as their joint anatomy/physiology sufficiently resembles human. These model systems are well-defined with specific and rapid timeframes to enable therapeutic intervention at the point of injury or in the early inflammatory phase to be performed. Measures are in place to monitor the animals on a daily basis after the induction of arthritis to ensure no adverse effects on their health and well-being. To minimise unnecessary stress and/or discomfort, rodents will be provided with appropriate soft bedding and nesting material to cushion affected limbs and maintain their body temperature during this period. Furthermore, rodents will be provided with additional cage items e.g. tunnels to distract from induced pain and to provide refuge for those rodents with compromised mobility. Also, as routine procedures, rodents will be housed with other cagemates and will have easy access to food and water. Following arthritis induction, rodents will be handled in a more sensitive manner due to the potential discomfort that they may be feeling. At all times we will respond swiftly to the needs of the animals and will routinely assess their well being e.g. weight loss, appearance and lameness. This will help inform on our judgement of appropriate humane endpoints for each individual protocol. All rodents are routinely acclimatised for at least one week prior to the initiation of protocols such that they become familiar with handling, surrounding environment and cagemates. Where appropriate, painkillers are provided to the animals if considered necessary with humane endpoints to minimise distress or suffering.

PROJECT 8: THE ROLE OF INFLAMMATION IN CEREBROVASCULAR DISEASE

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.

Word limit; 1000 words

Project Title	The role of inflammation in cerebrovascular disease
Key Words	stroke, vascular dementia, inflammation, cerebrovascular disease, neurovascular
Expected duration of the project	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;
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):

This project aims to find out how inflammation contributes to devastating conditions of the brain that are a result of disruptions in the supply of blood or function of blood vessels, so-called cerebrovascular disease. This includes stroke as well as vascular dementia.

We aim to find out how changes in inflammation in the brain and rest of the body are involved in the death of brain cells as well as the functional complications (cognitive decline, depression etc) seen in cerebrovascular disease. At present this is poorly understood and more research is needed.

What 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 hopes to find new ways to treat stroke and vascular dementia, conditions that at present have no widely effective treatments.

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

Studies will be mainly in mice though some experiments will use rats. Over the five-year period of the project we expect to use 6900 animals in total. Approximately a third of these (2200) will be for breeding purposes and generation of transgenic animals with the rest (3900 mice/800 rats) being used in 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?

In order to mimic human stroke and vascular dementia we will use experimental procedures to reduce the blood supply to the brain in rats or mice (cerebral ischaemia). This will mainly be done by opening up the neck of animals through a small incision to reveal the carotid artery. This artery is one of the main ways that blood gets from the heart to the brain. Then, using a number of different approaches, we will interrupt or perturb this blood supply. One way of doing this is through the injection of very small particles (or microemboli). These microemboli flow into the brain through the artery and then become stuck in blood vessels that are narrow. Alternatively, we can advance a fine filament (or suture) into the artery which will reduce the amount of blood reaching a large area of brain. We can also physically reduce the diameter of the artery that will reduce the flow of blood to the brain. Another way to disrupt blood flow to the brain is through haemorrhage (i.e. the rupture of blood vessels), and in rodents we can achieve this by directly injecting into the brain very small amounts of substances that cause minor blood vessels to burst. In addition to accessing the main arteries supplying the brain through the neck we can also do it through a small hole in the skull (a so-called craniotomy). For all the techniques described animals will be fully anaesthetised and will receive drugs (analgesics) to minimise any pain due to the surgery that is required. We expect most of the animals to fully recover from surgery and then they will usually undergo some tests of behaviour. These behavioural tests are designed to assess any problems with movement or sensation as would be seen in stroke patients, or memory problems as seen in vascular dementia, as well as other complications commonly reported by patients, including fatigue and depression. None of the behavioural tests are harmful to the animals and often just require observation for a short period in specialised apparatus. Tests can take place a few days or sometimes weeks after the initial surgery. In a few studies we will re-anaesthetise animals and use specialised imaging techniques to look at changes in how blood vessels function in the brain or the amount of brain cell loss etc. Animals may also receive simple injections or have blood samples taken. Clinically stroke, by its very nature, is a devastating disease, resulting in significant mortality and morbidity in patients. In trying to model stroke in animals a balance has to be struck therefore between establishing a valid model and in minimising pain, suffering, distress or lasting harm to the animal. However, as far as we are aware, effects of the stroke itself mainly result in discomfort to the animals, with severity kept to a minimum to ensure no lasting harm. The experimental approach to induce cerebral ischaemia is obviously specific to the experimental studies and it is inevitable that animals will suffer some level of pain due to the surgical procedures involved. At all times it will be our aim to reduce this to a minimum by the use of pain-relieving drugs. At the end of experiments animals will be killed by overdose of anaesthetic and we will take blood, brains and other organs/tissues to investigate various measures that will help us meet our overall aims.

Application of the 3Rs

Replacement

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

Replacement

Studying mechanisms involved in brain diseases such as stroke and vascular dementia is extremely complex. Alongside the death of cells in the brains of stroke and dementia patients, these diseases are characterised by profound changes in behaviour, which it is not possible to study in cells in isolation. The proposed animal studies are complementary to a broad programme of work on stroke/dementia using human samples, isolated cell systems and non-protected model organisms such as zebrafish embryos.

Reduction

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

Reduction

Pathological and behavioural end points proposed in this project are well established in studies of stroke/vascular dementia and experiments are planned based on our own extensive experience or previously published data. We will use the minimum number of animals that can answer the desired scientific objectives and will extract all relevant information in the data by using appropriate statistical analysis. Studies will be designed using the newly released Experimental Design Assistant (EDA) from the NC3Rs (https://www.nc3rs.org.uk/experimental-design-assistant-eda).

We will also consult regularly with qualified statisticians with regard to experimental design and 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

A critical feature of this work is that it is focussed on changes in function of blood vessels in the brain and the supply of blood that result in stroke and vascular dementia – so called neurovascular function.

Neurovascular function in rodents is comparable to humans and animals also develop post-stroke complications that are seen in humans, including depression and cognitive impairment. The proposed studies could not be undertaken in animals with a lower degree of neurophysiological sensitivity (e.g. drosophila, C.elegans)

because they do not show such similarities to humans, and *in vitro* experiments do not allow the study of interactions between different body systems.

We will use previously published methods to induced cerebrovascular disease, the choice of model being dependent on the hypothesis being tested. Models of stroke (both ischaemic and haemorrhagic) are extremely well established in many laboratories across the world and, though there is no 'perfect' stroke or vascular dementia model, those to be used in this project are chosen on the basis of their pathological and behavioural similarities to cerebrovascular disease in humans, which itself is extremely heterogeneous.

All animals will be closely monitored for adverse effects and procedures put in place to minimise these, using very recent guidelines produced by the stroke research community. These guidelines draw on a wealth of experience in modelling stroke in rodents and have been produced through an NC3Rs working group that includes veterinary surgeons and other experts in animal welfare.

Throughout the project we will continually review the literature and engage with colleagues/collaborators to learn of any new refinements to the protocols that could be implemented. In this respect the applicant is a co-author on the publication 'The IMPROVE Guidelines (Ischaemia Models: Procedural Refinements Of *in Vivo* Experiments', the output from a NC3Rs working group on stroke published in full in the Journal of Cerebral Blood Flow and Metabolism.

PROJECT 9: IMPROVING CANCER THERAPY IN ENDOCRINE SENSITIVE AND INSENSITIVE CANCERS

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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.

Word limit; 1000 words

Project Title	Improving Cancer Therapy In Endocrine Sensitive and Insensitive Cancers
Key Words	cancer, imaging, metastasis, drug development
Expected duration of the project	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):

The primary objectives of our translational research programme are to discover and validate new molecular targets and small molecules for anticancer drug development. New chemical entities are initially selected on the basis of *in vitro* and cellular efficacy. Before any lead compounds can be considered for clinical development, it is first necessary to determine their efficacy *in vivo* using appropriate mammary tumour models. Specifically, the clinical potential of new drugs will be assessed by their capacity to inhibit the growth of human tumours which have been induced in animals by the introduction of human tumour cells ("xenograft") in immunocompromised 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)?

The aim of the work is to evaluate new cancer targets and therapeutic agents. These findings will (a) confirm the importance of these targets and (b) develop new therapeutics that will, in particular, prevent or reverse drug resistance in cancer (c) demonstrate the utility/efficacy of new cancer drugs and (d) develop therapeutics jointly with appropriate companion diagnostic(s) to enable use in personalised medicine setting. The results obtained will be used to drive cancer treatment through clinical development of the therapeutic targets and drugs, ultimately towards the evaluation of successful targets/drugs in Phase I/II setting. Moreover, assuming successful proof of principle, we would expect to publish these results in suitable academic peer reviewed journals.

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

Mice Average of 4800animals for 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?

Level of severity: Mild-Moderate Tumours will be induced in animals but the size of tumour needed for this research is unlikely to affect the health of the animals. Adverse effects due to drug administration will be monitored in accordance with NCRI guidelines. Animals will be humanely killed at the end of the experiment and also if there are indications (based on recognised symptoms) during routine monitoring that the animal may be subject to undue pain or distress, in order to minimise suffering

Application of the 3Rs

Replacement

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

Replacement

Development of new anticancer drugs requires evaluation of efficacy in animal tumour models prior to clinical trials. *In vitro* assays such as cell based screening cannot display *in vivo* efficacy of lead molecules. Therefore, *in vivo* work is needed to determine the therapeutic potential of lead molecules.

The rodent xenograft models are widely regarded as the most appropriate and least severe to evaluate new anticancer drugs. Nevertheless, *in vivo* assessment is only carried out following rigorous testing of potential targets and/or drugs using *in vitro* assays and in cell culture model systems.

Reduction

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

Reduction

In vitro cell based screening of targets/compounds for mechanisms of action and efficacy, as well as in vitro pharmacology studies for compounds are carried out in order to limit compounds/targets to only the most appropriate and well-defined targets/compounds. This helps to ensure that the fewest possible number of compounds/targets are taken forward to animals studies and hence reduces the number of animals used.

The following measures have been taken to reduce the number of animals:

- Use of *in silico* and *in vitro* assays in the lead selection and optimisation phase
- Tumour cell lines are stored in liquid nitrogen when not in use; tumour lines are not maintained in animals. Appropriate cell line types with high success rates for our purposes are used.
- We are able to design our experiments according to publishable standard in compliance with NC3Rs ARRIVE guidelines.

In designing each experiment, we have consulted a statistician for advice on the minimum number of animals required to ensure a statistically valid result. Animal model used is well established, providing an efficient and robust system for our research, thereby minimising the numbers of animals that need 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

Choice of species: In-bred mice will be used to reduce experimental variance; majority of the work (~95%) will be in mice but on occasion we may require the larger rat model to enable visualisation of lesions or to obtain more blood for a pharmacokinetic study. Mice will be used in the studies because they are the lowest mammals on the evolutionary tree for which suitable models of cancer are available.

Animals welfare: All animals undergoing recovery surgery will receive routine analgesia as advised by the NVS. Analgesia will be administered for at least 1 day for minor surgery and at least 2 day for major surgery. Tumours needed for research are excised from animals which have been humanely killed. This approach should fit the study's scientific needs while not inflicting undue distress on the mice used.

Adverse effect monitoring: The size of tumours arising from this project are unlikely to affect the health of the animals, but will nevertheless be carefully monitored by non-invasive techniques to ensure the animals do not experience any discomfort. Adverse effects due to drug administration will be monitored in accordance with NCRI guidelines. All protocols will be mild or moderate. Detailed information concerning tumour and animal behaviour under experimental conditions will be recorded and shared with other researchers (as appropriate).

PROJECT 10: CHARACTERISATION OF MODELS OF CARDIOMYOPATHY

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.

Word limit; 1000 words

Project Title	Characterisation of models of cardiomyopathy
Key Words	Cardiomyopathy, heart failure, metabolism, signalling pathways, therapeutics
Expected duration of the project	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;
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):

This project aims to better understand the causes of cardiomyopathy (CM) and heart failure (HF) to help find new effective and safe treatments. These diseases affect more than 1 million people in the UK, but even with the best modern medical therapy, mortality remains very high: up to 40% of those with heart failure will die in the first year after diagnosis.

Heart failure refers to a state where the pumping action of the heart is insufficient to meet the demands of the body. While the early stages of heart failure are generally asymptomatic, it can and usually does progress to cause symptoms which can include: breathlessness, inability to exercise or even walk, swelling of the limbs (oedema) and abdomen, palpitations, loss of consciousness or even sudden death. Heart failure is a complex syndrome with multiple different causes, including coronary heart disease (resulting in heart attacks), hypertension, diabetes, obesity, and valvular heart disease (such as narrowing of the aortic valve). Cardiomyopathies are an important largely genetically inherited cause of sudden cardiac death and requirement for cardiac transplantation or defibrillator implantation in young otherwise healthy individuals. Inherited cardiomyopathies can result in heart failure and are broadly grouped according to the gross appearances of the heart into: those with abnormal excessive thickening of the heart muscle (hypertrophic cardiomyopathy, affecting 1 in every 500 people) which can be severe enough to obstruct blood flow out of the heart; and those that result in marked enlargement of the heart cavity and weakening of its contraction (dilated cardiomyopathy). There is

no cure at present for heart failure or cardiomyopathy and, when very severe, these conditions may require heart transplantation. The current limitations in clinical treatment, particularly the lack of specific tailored therapies, for these disorders largely reflects limitations in our understanding of what causes heart failure and cardiomyopathy, and what are the key drivers of altered heart structure and function are at a molecular and cellular level. This project seeks to address this with the aim of improving therapeutic options and outlook for the individuals with these conditions.

Rodent models of heart failure and cardiomyopathy have proven highly relevant to understanding human cardiovascular disease and have been instrumental in the development of therapies which are now widely used to prolong life, reduce hospitalisation and relieve unpleasant symptoms of heart failure. This project builds on this using several state-of-the-art surgically-induced, environmentally-induced and sophisticated genetically engineered mouse models of heart failure designed to mimic key aspects of human heart failure and cardiomyopathy faithfully in order to uncover the early molecular changes occurring in the heart which underlie its core clinical features. These include the molecular basis of weak cardiac contractile function, abnormal enlargement (dilation) of the heart, abnormal heart rhythm including fast heart rhythms (such as atrial fibrillation or ventricular tachycardia) and slow heart rates (that in humans result in need for early pacemaker implantation), abnormal heart thickening, and excessive scarring of the heart (termed fibrosis) which can lead to dangerous chaotic heart rhythms and risk of sudden death. By carefully investigating the basis of this in mouse models reflecting the human disease, starting from molecular and microscopic cellular changes, progressing to altered physiology and whole organ/body function, we aim to identify new avenues of treatment. These mouse models can then be used to investigate potential new therapies designed to prevent or improve features of HF or CM, with the ultimate aim of translating this to the clinical treatment of 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)?

This project will improve our understanding of the precise molecular and cellular changes underpinning the development and progression of cardinal features associated with different forms of heart failure and cardiomyopathy, including the excessive heart muscle thickening, abnormal cardiac contractility, scarring of heart tissue (myocardial fibrosis), and heart rhythm disturbances. Such knowledge is likely to lead to new drug targets which hold promise for improving symptoms and outlook of individuals suffering from these currently incurable diseases. Critically, this approach has strong potential to lead to specific treatments tailored for specific features of these disorders which can currently only be treated symptomatically or for which no therapy is available at all. This would represent a major breakthrough for patients with these diseases.

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

Mice will be the predominant species used (approximately 3000 mice/year), with a very small number of rats throughout the course of these studies. The majority of animals (over 75%), will be used for breeding and maintenance purposes. Mice have a number of distinct advantages as a species for cardiovascular research: they possess a very similar cardiovascular system to humans, although on a much smaller scale; advanced genetic engineering techniques, based on Nobel Prize winning scientific advances, are widely available which allow the generation of very precise mouse models bearing the same gene mutations observed in humans with cardiomyopathy; microsurgical techniques and careful recovery anaesthesia are highly advanced and standardised techniques used in mice to generate surgical models of heart failure with high clinical relevance to major causes of human heart failure (e.g. narrowing of the aortic valve, or a heart attack).

In the context of what you propose to do to 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 will involve breeding of mice with a precise genetic alteration, most commonly the same mutation that occurs in humans with cardiomyopathy, typically involving a ‘spelling error’ in a single gene among ~20,000 otherwise normal genes in that mouse. Careful study of these transgenic mice is a powerful method to identify new disease pathways that cause CM or HF that may be possible to target with drugs. Given the complexity of human heart failure, not all causes can be modelled adequately by mutating a single gene. Some, for example, require the use of microsurgery (e.g. to simulate the changes that occur in the human heart after a heart attack), or an altered diet (e.g. high-fat diet) to mimic the changes seen in humans with obesity or early diabetes. Experimental mice will generally undergo non-invasive tests of cardiac function using methods identical to that employed routinely in hospital for patients. These include echocardiography (cardiac ultrasound), cardiac MRI and electrocardiography (i.e.an ECG, which records the electrical pattern of the heart). In virtually all cases, mice are carefully anaesthetised to allow these recordings without distress or suffering. Anaesthesia is well tolerated in mice and complications are minimised by the careful use of supportive therapies including heat pads and fluid replacement. Typical imaging sessions lasts 30-40 minutes for echo and up to 2 hours for MRI, while ECG recordings tend to be shorter at ~ 15 minutes. The heart rate and breathing pattern and level of anaesthesia are monitored carefully throughout the procedure and, if required, the level of anaesthesia adjusted accordingly. Blood samples may be taken to test for biochemical markers in some mice. This typically involves using a scalpel to make a very small cut across a surface vein in the tail. A special collection tube is used to collect the blood. Usually no more than a few drops of blood is required. Although a small wound is made, the animal retains full function of the tail and this heals quickly.

If more than one sample is required it is typically taken at least one week later to allow sufficient time for wound healing. Therapeutic studies To test potential new therapies, a small proportion of mice will undergo dosing with drugs likely to be beneficial in treating CM or HF. Drugs are commonly administered mixed in with food or added to drinking water in order to minimise impact to the animal. Injection is also a common way to administer substances when less invasive routes are not suitable (e.g. due to insolubility of drug in water). Routes for the latter include; subcutaneous injections under the skin, usually the scruff off the neck/back, into the abdominal cavity (intra-peritoneal administration) and rarely, intravenous, usually through a vein located in the tail. All animals are monitored carefully daily for physical condition and possible adverse effects of drugs. Surgical models A small number of our animal models will undergo surgical procedures to allow us to investigate common causes of CM and HF in humans. One of these is thoracic aortic banding, a well-established procedure used to replicate aortic stenosis (narrowing of the aortic valve) in humans. To generate this model, an incision is made in the thorax (chest) to allow visualisation of the vessels in the chest cavity under a microscope and a polypropylene thread is used to tie a knot around the transverse aorta, reducing the circumference of the vessel by approximately 70%. This results in the gradual thickening of the heart muscle as it contracts to move blood through the aortic narrowing and eventually progresses over weeks to heart failure. Constriction of a coronary artery on the surface of the left ventricle to mimic ischaemic heart disease (i.e. a heart attack) is another model. To gain access to the artery the chest cavity is opened through a small window between the ribs and the area of interest observed under a microscope. Once the appropriate artery is identified it is constricted with polypropylene thread, resulting in 40-50% ischaemia (reduced blood flow) to the left ventricle. For genetic editing of very specific regions of the heart to produce effects such as altering heart rhythm a glue like substance that carries a genetic component is ‘painted’ onto a chosen portion of the heart such as the atria through a small opening between the ribs. This mode of gene therapy is potentially one which can be applied to humans, e.g. at the time of open heart surgery for the treatment of atrial fibrillation. Mini-pump implantation is a refinement adopted for prolonged administration of drugs to avoid continuous daily injections where possible. This is used for substances that can either cause heart disease (as a non-surgical alternative to the aortic banding model) or as a way to deliver therapeutic agents. This is a minimally invasive procedure where a small incision is made and a specially designed capsule that is filled with the drug of choice is placed under the skin. The surgery takes approximately 10 minutes per animal and recovery is very quick. All animals undergoing surgical procedures will be carefully monitored throughout the course of the study. Even with this, there is a small but defined mortality rate associated with surgically mimicking these conditions in mice: mortality rate typically less than 5% of those undergoing surgery, and less than 1% of total number of mice used). When this does occur, this is usually while animals are still under surgical anaesthesia and insentient. The majority of potential adverse effects following

surgery relate to the development of heart failure, including abdominal swelling, reduced mobility, weight loss and altered breathing rate. The combination of careful monitoring and assessment of animals using sensitive imaging tests even before the emergence of frank heart failure allows any pain and distress to be limited to the minimum. Non-surgical studies Immune system studies: For selected experiments a very small number of mice may undergo radiation treatment. This will result in depletion of cells that control the immune response of the animal. After this the cells will be replaced with ‘donor’ immune cell types from the bone marrow of healthy mice or vice versa to examine the contribution of immune cells to the fibrosis and other aspects of heart failure and cardiomyopathy. Such mice will be carefully monitored after treatment for any signs of discomfort or side effects, which may include lethargy, weight loss and diarrhoea. This procedure is highly standardised and undertaken to rigorous standards, such that the risk of death resulting from radiation treatment is very low. Where feasible, mice may also be treated with substances to alter the function of immune cells in models that mimic CM/HF. Metabolic studies: Some animals will be fed a commercially prepared diet designed to be high in fat and sometimes in sugar content, mimicking features of a ‘Western diet’, a leading case of obesity and diabetes mellitus. Mice on this ‘high-fat’ protocol for several weeks can faithfully recapitulate important features of human diabetes and obesity – both the latter are emerging as global epidemics and major risk factors for developing heart failure. These mice are carefully monitored for symptoms resulting from excessively high blood glucose and weight gain. Analysis of animal metabolic profile can include short-term fasting followed by a glucose tolerance test, regarded as the gold standard test in humans to detect the presence of early or overt diabetes. Food is removed from the cage for a set time, typically lasting 4-6 hours, but with free access to water throughout. After the fasting period, mice are weighed and glucose solution is given by a tube through the mouth or injected into the abdominal space. How quickly the blood sugar level returns to normal after this treatment is assessed by collecting a small drop of blood from the tail and analysing this with a glucose meter over time. What will happen to animals at the end? At the end of an experimental protocol or when a new drug or mechanism of action is being studied, some animals will undergo a final non-invasive imaging or invasive physiological test to determine the level of cardiac function. This can involve surgical procedures that are performed on animals under terminal anaesthesia from which they will not be recovered and are insentient throughout. The latter procedures can include one of; • measurement of the pressures within the heart and aorta by use of a very fine pressure sensing catheter which is inserted into the left ventricle through a major artery in the neck This allows measurement of the heart’s pumping response at baseline and after addition of drugs which normally increase blood pressure. • internal microscopy of the heart, which involves opening the chest cavity to examine the heart using a special microscope to allow the visualisation of certain cell types using specific dyes (e.g. individual heart muscle cells or immune cells). • testing susceptibility to irregular cardiac rhythms, - this entails insertion of a fine electricity conducting probe via the

throat. The probe is then used to apply brief pulses of electrical current under anaesthesia (termed pacing) using a very similar approach to that used routinely in hospitals when patients are tested and treated for abnormal heart rhythms. While breeding mice may be re-used for a period of time while fertile, standard killing methods are used to end all procedures enabling collection of tissues 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 use of live animals is currently unavoidable in studying a disease as complex as heart failure which affects whole organs including the heart, kidney, nervous system, as well as the body's hormonal system, inflammatory response and blood vessels. The complex interactions between these organs cannot be reproduced adequately with isolated cells or computer simulations in isolation although we integrate these approaches in our work. Mice are the simplest organisms for which there are good models of human heart failure and cardiomyopathy and very advanced means of introducing precise genetic modifications. Similar models have been used successfully to develop gold-standard medicines currently used to treat heart failure in humans such as ACE inhibitors and β-blockers.

Reduction

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

Reduction

Use of highly sensitive techniques by experienced research scientists, in conjunction with careful statistical planning of experiments and small-scale initial pilot studies, reduces the number of animals we need to study, whilst providing high quality scientific information. Complementing these procedures are detailed laboratory molecular analyses completed after the lifetime of the animal to identify molecular changes occurring in response to the genetic alteration, to ensure that maximum use

of precious sample material is made which in turn helps to reduce the numbers of animals used. The nature of our experimental studies is such that re-use is generally not feasible, e.g. post-surgery, and is avoided as it would both impact on the burden experienced by that particular mouse as well as potentially compromise the scientific validity of the data provided.

Refinement

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

Refinement

Inbred and genetically altered mice will be used. The vast majority of these are housed socially in the latest individually ventilated cages, often with their littermates with specific number limits per cage to ensure adequate roaming and foraging space, given environmental enrichment, kept in clean conditions with strictly controlled temperature/humidity, and lighting and monitored closely. Use of state-of-the-art non-invasive imaging enables completion of studies in the great majority of mice before the onset of any heart failure symptoms – i.e. the pre-clinical phase. A small number of models involve surgical procedures which mimic human disease, such as induction of heart muscle thickening by creating a surgical narrowing of the main outflow vessel of the heart, the aorta. The micro-surgery involved is highly advanced and standardised to incur minimal suffering to animals, including strict aseptic technique, and diligent attention to intra- and post-operative support sharing much in common with the approach to human surgery. Mice undergoing surgery receive heating throughout and additional heating after the procedure until recovered, very strong pain relief including use of opioid analgesia, ready availability of softened food at floor level and regular nonintrusive monitoring on physical condition during recovery, including when returned to their familiar home cage. Surgical procedures require rigorous training to ensure a high standard of competence in completing the surgery safely and ensuring optimal rapid recovery – both are critical for both the welfare of the mouse and the most valid scientific outcomes and given high priority. Surgery is carried out under aseptic conditions as stated by LASA guidelines, usually with inhaled (rather than injectable) anaesthesia and with specialised equipment (e.g. surgical tools and a ventilator) specifically designed for use in mice. Anaesthesia is carefully monitored throughout surgery to ensure the overall physiological condition of the animal is well maintained during the surgery. Recovery is usually rapid post-surgery with most animals showing typical grooming, eating and behaviour well within 24 hours. Heat and fluid support with

veterinary grade analgesia are given routinely prior to surgery, with further administration if the animal is showing any signs of pain. To reduce the stress to animals associated with surgery extra enrichment is provided in the form of chewing blocks, additional housing, more straw bedding and forage mix. Animals are checked regularly after these procedures by the surgeon, usually three times a day for five days after the procedure, followed by twice a day monitoring to assess the welfare of the mouse and ensure early detection of clinical signs that suggest the onset of heart failure. Any animals which do display any signs of distress at this stage are rapidly and humanely euthanised and cardiac tissue collected.

PROJECT 11: DOPAMINE FUNCTION IN BEHAVIOUR: LINKS TO SCHIZOPHRENIA AND ADDICTION

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.

Word limit; 1000 words

Project Title	Dopamine function in behaviour: links to schizophrenia and addiction
Key Words	Addiction, Attention, Dopamine, Motivation, Schizophrenia
Expected duration of the project	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):

The aim of the program of work is to identify the behavioural and neurochemical contingencies determining dopamine release in the main dopaminergic pathways in the context of normal behaviour, and of disturbed behavioural in mental disease.

The specific objectives are:

1. To determine what features of behavioural stimuli drive activity of these dopamine neurones.

2. To assess the role of drugs affecting dopamine function on this activity and on behaviour.

3. To identify pathways impinging on dopamine neurones involved in controlling behaviour, and identify the neurotransmitter(s) involved

4. To identify changes dopaminergic neurone function in models of psychiatric disease, including schizophrenia.

5. To determine the mechanism of long-term adaptive changes in dopamine systems related to effects of addictive drugs, and to long-term changes in schizophrenia

What 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 from these studies will give us a better understanding of the role of the dopamine systems in controlling behaviour and how other neurotransmitter systems modulate dopamine release. This is important in the context of understanding neural

mechanisms controlling normal behaviour, and also in revealing dysfunctions underlying abnormal function in psychiatric disease. The focus on models of schizophrenia and addiction should help elucidate abnormal neuronal function in these conditions. Thus the studies will not only address fundamental neuronal mechanisms controlling behaviour, but also provide a better understanding of the origin of psychiatric disease, possibly opening up much needed novel therapeutic strategies for their treatment.

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

All the experiments will use rats, We would anticipate using approximately 800 animals over the course of the 5 years of the license.

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

We expect moderate severity to be experienced by around 40% of our animals, accounted for by animals which receive recovery surgery (approx 20% of total) and those receiving aversive stimulation (approx 20% of total). For animals receiving surgical intervention, aseptic surgical techniques and established aftercare procedures refined over many years, coupled with peri-operative analgesia means that animals' suffering is kept to a minimum. For animals receiving aversive stimuli (e.g. mild footshock, loud noise), we use the minimum required to elicit the desired responses. Therefore, although there is some immediate short term discomfort, this is transient, and no long-term adverse effects are expected. Animals undergoing procedures which do not involve either recovery surgery or aversive stimulation (approx 60% of total) are expected to be of mild severity, producing some transient behavioural changes, with little suffering. No long term adverse effects are expected. All animals will be humanely killed at the end of the procedures, and the brains will be taken for further analysis using in vitro or post mortem analytical techniques

Application of the 3Rs

Replacement

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

Replacement

Studies investigating neurotransmitter mechanisms underlying behaviour necessitate using unanaesthetised, behaving animals. However, we adopt a layered approach to experimental design, where initial studies are done in vitro. Recent advances in our lab using fast cyclic voltammetry (FCV) in brain slices in vitro, mean we can now use in vitro rather than in vivo methods for investigating basic brain neurochemistry, thus replacing moderate severity procedures with non-regulated or mild severity (where

drug pretreatment is required) procedures. Whole animal in vivo studies are used only where there is a necessity for keeping neuronal networks intact (only local netwarks remain intact in slice preparations) to investigate how different brain areas interact, and in experiments in which we measure changes associated with behaviour.

In addition, we are actively looking into using un-protected animals (e.g. Planaria flatworms) for some of our studies. However, at present we do not have appropriate non-mammalian models for the human conditions we are studying.

Reduction

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

Reduction

Where feasible, we now use FCV in vitro for experiments looking at the basic effects of drugs on transmitter release. This approach enables a substantial amount of data to be collected from a single brain, thus contribution to a reduction in overall numbers of animals used.

In addition, developments in the methodology of FCV, optical imaging, and MRI allow repeated recordings from each animal. Thus the techniques used in the in vivo experiments generate large amounts of data from each animal, and incorporate longitudinal experimental designs which allow within-subjects comparisons (mostly factorial ANOVA), thus reducing the number of animals used over more 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

Rats have been shown in the past to provide a good animal model of motivational systems, providing the least neurophysiologically sentient species appropriate for these types of study. Many of the studies on which the current research program is based were performed on rats, and therefore a wealth of information exists on which to base theories under scrutiny.

We now use FCV in vitro where we are looking at the effects of chronic drug treatment on subsequent neurotransmitter function. Either naïve animals (non-regulated) or animals receiving drug dosing in vivo (mild severity), are used, with the neurochemical recording done in vitro. This refinement means that these animals no longer undergo in vivo neurochemical measurement (moderate severity).

Where possible, in vivo experiments are carried out using anaesthetised animals, with procedures on conscious animals used only where behavioural stimulation and/or responses are to be employed, and where we are studying neural network activity which is likely to be affected by anaesthesia. Where possible we use behavioural procedures relying on animals' innate behaviours, and using non-aversive stimuli; we are also actively investigating procedures to further reduce the impact on the animals. In all cases the procedures used are the minimum severity level required to produce the required effects.

Aseptic surgical techniques and established aftercare procedures have been refined over many years: this, coupled with improvements in peri-operative analgesia means that animals' suffering is kept to an absolute minimum.

Established procedures used routinely in our lab adhere to ARRIVE guidelines in the design, execution and reporting of the experiments

PROJECT 12: IBV: ATTENUATION AND VACCINE DEVELOPMENT

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.

Word limit; 1000 words

Project Title	IBV: attenuation and vaccine development
Key Words	chicken, IBV, coronavirus, pathogenicity, vaccine
Expected duration of the project	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;
No	(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):

Infectious bronchitis virus (IBV) causes a respiratory disease in chickens. It causes economic losses and is a risk to food security as infected chickens do not gain as much weight or produce as many good quality eggs as uninfected chickens. IBV is prevalent throughout the world and there are many different strains of the virus circulating. Currently available vaccines are not able to protect chickens against all of these strains and have the potential to revert to a more virulent form.

This project aims to study the replication of IBV in chickens and generate novel, more effective and stable vaccines against the disease. The objectives of this project are:

1. To determine the pathogenicity of IBV strains. A major objective of our work involves the identification of regions of IBV that can be modified to make the virus less pathogenic for chickens. The only way to determine whether the virus modification has resulted in reduced pathogenicity is to infect chickens, the natural host of IBV.

2. To determine the protective efficacy of IBV vaccine candidates. The ability of potential vaccine candidates identified in objective 1 to protect chickens against challenge with a virulent strain of IBV will be investigated.

3. To analyse of the role of antibodies in protection against IBV. Our fourth objective is to determine whether chickens lacking the ability to produce antibodies are still able to produce a protective immune response against IBV.

What 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 different strains of IBV replicate and cause disease in chickens will facilitate the development of better vaccines to control the disease. This will benefit poultry farmers and the UK economy as economic losses due to IBV

infections will reduce. Better vaccines to control IBV will also improve the welfare of commercial chickens.

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

This project will last five years. In this time, approximately 500 chicken eggs will be used to grow IBV and 1750 chickens will be infected with IBV to study the replication in the natural host.

In the context of what you propose to do to 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 our experiments using chickens involve the development of vaccines ultimately for the benefit and protection of chickens against disease and are carried out in environmentally controlled experimental animal facilities. We keep animals under regular observation and use non-invasive measurement of clinical signs of infection. IBV mainly causes clinical signs very similar to the common cold in humans; a few days of snicking (akin to sneezing), watery eyes and wheezing. Chickens are expected to recover from respiratory disease within ten days and will experience a maximum of moderate disease severity, most will only experience mild disease. As we need to analyse virus growth and disease pathology in different organs, all chickens will be humanely euthanised 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

Chickens are the natural host of IBV and most virus strains will only grow in chickens or eggs. As the overall objective is to develop new live IBV vaccines we have to inoculate chickens with our viruses to determine whether the modifications we have introduced make the viruses less pathogenic and therefore safe for vaccine use. The potential vaccines must also be tested to establish whether they are able to protect chickens against infection with IBV. This can only be carried out in chickens, the natural host for IBV.

Reduction

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

Reduction

A statistician has been consulted regarding the proposed number of chickens to be used in the trials to ensure that an appropriate number is used to generate meaningful results. The number of birds per group at each time point has been selected to guarantee statistically relevant results for the assessment of protection and pathogenicity based on many years of experience. Guidance will be taken from the recently published meta-analysis of the required sample size in vaccination-challenge experiments with IBV and the NC3Rs Experimental Design Assistant.

Refinement

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

Refinement

Chickens are the natural host of IBV. Most strains of IBV only grow in chickens and eggs. Any potential vaccines designed to protect chickens against IBV must be tested in chickens. Understanding the replication of IBV in chickens facilitates the design of better vaccines against IBV.

Chickens and eggs infected with IBV will be monitored closely to assess disease progression using non-invasive methods and will not be allowed to experience severe suffering caused by IBV infection. The birds will be housed in groups with access to fresh food and water with varied environmental enrichment at all times.

PROJECT 13: CHEMISTRY AND BIOLOGY OF NOVEL BONE GRAFT SUBSTITUTE MATERIALS

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.

Word limit; 1000 words

Project Title	Chemistry and Biology of Novel Bone Graft Substitute Materials
Key Words	Osteoporosis, Bone graft substitute, Ovariectomy
Expected duration of the project	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;
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 main objective of this study is to use a rat model to study the effectiveness of a new bone graft substitute (BGS). BGS are natural or man-made materials that are used to replace bone in patients with diseased or damaged bone. The project license will focus on osteoporosis, a disease that is characterised by bone loss in women due to oestrogen deficiency following the menopause. The bone loss that is seen in osteoporosis substantially increases the risk of fracture in these women. Using a rat model of bone loss following surgical removal of the ovaries (ovariectomy), we will determine whether new BGS materials can be used to replace the bone that is lost in osteoporosis.

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

The data from this study will support an application for regulatory approval for human clinical trials of a new bone graft substitute for use in women with bone loss and skeletal fragility caused by post-menopausal osteoporosis. Although the BGS materials to be tested have been shown to be effective in patients with normal bone, their effectiveness when used in diseased bone is untested. One of the key questions that regulators will need to see answered is how the material behaves in osteoporotic bone. It would be extremely difficult to secure ethics approval to undertake a clinical trial to inject AGN1 (or other BGS) in patients with osteoporosis because the injection procedure in humans will require a surgical procedure; without some evidence of efficacy in osteoporotic bone, it is very unlikely that ethical review board approval could be secured. This rat study will form the foundation of the preclinical data that will be needed to confirm efficacy in osteoporotic bone, paving the way for subsequent human clinical trials. Additionally, since most women with

osteoporosis are now treated with drugs called bisphosphonates that partially block bone loss, it is important that our rat work includes a comparison between the effects of the BGS materials in untreated osteoporotic bone and in bone that has been treated with bisphosphonates.

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

The first study under this license will evaluate a calcium sulphate bone graft material, and this work will require 108 skeletally mature female rats over the next two years. Additional studies, using other bone graft substitutes alone or in combination with cells, are anticipated in the future. Some of these experiments may focus on combining bone-forming cells with the BGS materials; others will make use of stem cells that will be collected from the patient, grown in the laboratory (to increase the number of cells available for transplantation) and then mixed with BGS and implanted in the bone defect in the rat. These additional studies are under planning and can only be initiated when additional grant funding has been secured. Each subsequent study on a BGS material is expected to require approximately 100 rats and we anticipate testing at least three additional variants of BGS (or GBS plus cells) during the 5-year lifetime of this project license. In total, we would expect to use a total of up to 400 rats under 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?

This study will use surgically prepared female rats that have undergone surgical removal of both ovaries (ovariectomy, or OVX), performed by the commercial supplier of the animals. 4 to 6 weeks later, when animals have fully recovered from surgery, the rats will be delivered to our institution, where they will begin weekly treatment with subcutaneous doses of either alendronate (a drug used to treat osteoporosis in women) or a placebo (saline). 12 weeks after OVX, when the bone loss has been fully established, rats will undergo surgery to create drill hole defects in the bottom end of the left and right femurs. The defects will be filled with the new BGS material and the animals followed for times ranging from 2 hours up to 6 weeks. At each time point, groups of animals from each treatment group will be killed and tissue samples collected for analysis of (a) the chemical changes in the BGS over time, and (b) the bone response to the BGS over time. In some animals, bone markers (fluorescent labels that bind to bone, providing a way of quantifying new bone formation in the living animal) will be injected into the animal in order to allow quantitative assessment of bone turnover through histological evaluation of bone specimens collected at the time of euthanasia. These labels are well tolerated by animals and will allow us to determine how much new bone forms in and around the BGS materials. The most significant potential adverse effects are expected to be post-operative pain, lameness and an increased risk of fracture due to the creation of bone defects. Post-operative pain and lameness would be considered moderate

severity so drugs that will provide pain relief will be administered. Fractures are a potential complication of any surgery involving bone, but the risk will be minimised through the use of careful technique. If a fracture occurs during surgery when the animal is anaesthetised, the animal will be killed without being allowed to recover consciousness.

Application of the 3Rs

Replacement

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

Replacement

We make use of non-animal alternatives wherever possible, including cell culture models for studying the effects of bone graft substitutes on isolated bone cells, as well as for evaluating the effects of simulated body fluids on the chemistry of the biomaterial. However, none of these laboratory systems recapitulates the interactions between the implant, bone, bone marrow and immune system that develop in the living animal.

Reduction

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

Reduction

This is a pilot study to determine the time course of changes in implant chemistry and bone microstructure following implantation of bone graft substitute materials. Sample numbers are based on best available data from the literature. Within each experiment, we maximise data collection from individual animals by using serial non-invasive imaging and blood tests, allowing us to obtain a number of data readings from the same animal rather than using one animal per measurement, thus reducing the number of animals 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

The rat is recommended by the Food and Drug Administration, the governmental agency that regulates the approval of new drugs and treatments in people in the United States as the preferred preclinical model for studying therapies for osteoporosis. The focus of this study is to better define early changes in implant

chemistry and relate them to the bone response around the implant. The surgery involves the lower part of the thigh bone and from previous work in rats we expect that animals will tolerate the procedure well. Pain relief will be provided to all animals during and after surgery and thus any pain effects are minimised as far as possible. The study is also limited to 6 weeks since this is the time period during which we anticipate seeing the most scientifically informative changes in chemistry and biology in and around the implanted material.

PROJECT 14: CANCER-SELECTIVE LABELLING AND BIOMARKER DISCOVERY

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.

Word limit; 1000 words

Project Title	Cancer-selective labelling and biomarker discovery
Key Words	Cancer biomarkers, Methods development, Cancer-selective labelling, Treatment monitoring
Expected duration of the project	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;
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):

If detected early enough, cancer patients would have a much better chance of receiving proper treatment.

One of the reasons why there are currently very few blood-based tests for detecting cancer is that there are no appropriate experimental technologies to directly link molecules such as proteins to cancer cells: it is impossible to unambiguously determine which blood-based proteins originate from the tumour itself. This greatly limits the utility of existing animal models of human cancer for discovery and development of diagnostic and prognostic biomarkers with relevance for human disease.

Our research mission is to develop novel cancer-selective protein labelling methods in mouse models of cancer in order to facilitate the discovery of cancer-relevant proteins biomarkers that ultimately have diagnostic, prognostic and predictive value in human disease. Using cell-selective protein labelling methods in conjunction with established mouse cancer models will have multiple advantages including **(1)** it will greatly facilitate the analysis of cancer cells in the natural context of other cell types in the tumour microenvironment and **(2)** enhance the discovery of circulating protein biomarkers as any identified labelled protein in the circulation can unambiguously be linked to the tumour cell compartment, and most likely **(3)** improve the translation success of findings in animal models by overcoming the inherently correlative nature of present biomarker discovery approaches.

What 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 strongly believe that combining cell-selective protein labelling with a range of established mouse models of different cancer types will enhance the probability of translating identified cancer-specific biomarkers into the clinic and help determine cancer stage, monitor disease progression, and provide indications of treatment efficacy

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

5150 mice (including 2000 breeding mice) and 540 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?

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. All tumour-bearing animals will be closely monitored and will be killed should clinical indications develop, such as loss of condition, a greater than 15-20% 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 moribund. 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

Any new cell-selective labelling approach will be first tested on cells growing in cell culture.

As identification of cancer-relevant biomarkers is intimately tied to complex factors of living organisms (e.g. blood perfusion, lymph drainage, tissue architecture etc), we need *in vivo* animal studies to identify biomarkers that both make it to the circulation of animals (sensitivity) and are relevant for the disease studied (specificity).

Reduction

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

Reduction

Animal use will be minimised by using the smallest possible experimental groups in pilot studies. A power analysis may then be used to determine how many more animals are needed to reach statistical significance in a larger animal cohort setting.

Animal use will also be minimised by acquiring multiple real-time samples such as blood or urine during the experiment and/or through non-invasive imaging of tumour development parameters in a single mouse, reducing the numbers 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

Genetically engineered mouse tumour models are becoming increasingly important as systems in which to test new drugs and in which to develop new methods for detecting cancer early. The program proposed here will provide new avenues to enhance the utilization of these mouse models for better understanding of the non-genetic underpinnings of cancer and for the discovery of new disease biomarkers.

Xenografted rats will be used for method development as larger blood volumes can be continuously sampled from rats compared to mice and thus enabling assessment of labelling over time in the same rat, ultimately saving the number of animals needed for successful method development.

We have optimised the procedures to minimise potential pain, suffering or distress, and enhance animal welfare. Animal suffering is minimised by appropriate use of anaesthetic and analgesics.

PROJECT 15: INVESTIGATING HORMONE SECRETION AND ACTION IN METABOLIC DISEASES

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.

Word limit; 1000 words

Project Title	Investigating hormone secretion and action in metabolic diseases.
Key Words	Diabetes, Metabolism, Hormone, Pancreas, Obesity
Expected duration of the project	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):

415 million people worldwide and 4 million in the UK live with diabetes, which is defined by elevated blood glucose (hyperglycaemia). >90% of cases are type 2 diabetes (T2D), which is characterised by defective insulin secretion from pancreatic islet beta cells, dysregulated glucagon secretion from islet alpha cells and insulin resistance. T2D is often associated with obesity, poor diet and a sedentary lifestyle.

Hormones released by the pancreas, gut and fat tissue play a key role in regulating whole body metabolism, yet we do not understand many of the basic molecular mechanisms controlling their release, action, stability and clearance from the circulation. The goal of this project is to investigate: 1) normal islet and gut endocrine cell function; 2) the signalling role of lipid metabolism in nutrient sensing and hormone secretion; 3) how islet and gut endocrine cell function adapts (or fails) during health and metabolic disease; 4) drug targets and mechanisms that could be used to enhance, protect, restore or mimic islet and gut endocrine cell function during metabolic 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 health burden of living with diabetes is substantial: complications and co-morbidities such as cardiovascular disease, nerve damage (neuropathy), kidney disease (nephropathy), blindness, limb amputation, depression and dementia all contribute to the reduced quality or life and reduced life expectancy. In addition, the financial burden on healthcare systems is significant: £10billion per year (10% of the annual NHS budget) is spent managing diabetes, and the loss of productivity in the workforce due to ill health is substantial. As UK and global populations are facing an

obesity and diabetes epidemic, research into this area is a medical, economic and ethical priority. Our project will make substantial progress towards better understanding, treating, and managing metabolic disease in humans. Moreover, we will generate models, tools and expertise to support other research efforts in the metabolism and endocrinology fields.

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

We will exclusively use mice for our studies. Over the 5 year period outlined in this project license application, we estimate that we will generate 7000 mice under our breeding protocols (protocols 1-2), the majority of which will be transferred to our study protocols (protocols 3-5). Under protocols 3-5 we estimate we will study 5900 mice to enable us to achieve our scientific aims.

In the context of what you propose to do to 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 undergo procedures to test their ability to control blood glucose levels and other aspects of metabolism. Most of the tests are regarded as of mild severity, as they involve little more than a single injection and several small blood samples being drawn from the tail. Some interventions/studies are regarded as of moderate severity due to the need for recovery surgery (e.g. to implant a minipump to administer a peptide), but are not expected to impart any lasting pain or discomfort to the mouse. Our models of diabetes are regarded as moderate due to the potential for overt diabetes and complications (although this is rare). The tests listed have been refined over many years to cause the least disturbance to the mouse possible, whilst gaining suitably robust data to answer our research questions. It is important to remember that stress or pain will impact on metabolism and therefore confound our experimental data sets: therefore, there is a strong scientific as well as ethical rationale for us to avoid inducing stress or pain. Where multiple tests are required upon the same mouse, sufficient recovery time between tests will be allowed. At the end of the protocol all mice will be culled by schedule1 or terminal procedure (under appropriate general/terminal 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 essential that our research project is carried out using mice, as there is simply no alternative. Glucose homeostasis and hormone secretion, action, stability and clearance are regulated by a myriad of circulating factors (each with their own

pattern of systemic regulation) and the contributions of multiple organ systems, which cannot possibly be recreated in vitro: therefore, these processes must be investigated in a living mammal to ensure our results are physiologically relevant.

It is neither practical nor ethical to conduct these interventional experiments in human volunteers and we have opted to study mice (see reasons under refinement below). Notwithstanding this, we will always replace in vivo experiments with in vitro experiments where possible, such as testing expression or basic function of a gene/protein, or optimising drug dosage (as depicted in the experimental flow diagram above). These data will then inform the subsequent in vivo testing which will establish physiological relevance.

Reduction

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

Reduction

Mouse numbers used in experimental protocols will be reduced to the minimum required to achieve our experimental goals. We have taken a number of steps to achieve this:

1. Ensuring technical competency of researchers
2. Appropriate experimental design and power.
3. Using rigorous and robust controls:
4. Efficient and appropriate breeding strategies.
5. Systematic tissue collection and banking (for future lab projects).
6. Sharing of samples, resources, models and 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

We have opted to study mice due to 1) they are a good model of human metabolism and endocrine function; 2) there is a wealth of existing data on mouse physiology; 3) there are a large number of genetic tools available; 4) mice breed readily and quickly.

Our mouse models and techniques have been selected as they: (i) are relevant to understanding human metabolic disease (e.g. diabetes, obesity etc.); (ii) can be used to address basic biological questions about the normal regulation of whole body metabolism, blood glucose, body weight and hormone secretion; (Iii) have the least impact on the animal's welfare, compatible with our scientific objectives.

Our monitoring protocols will take into account the potential for pronounced diabetes to occur, even though this is not expected with the models we have selected.
Our protocols have been refined over many years, ensuring that studies will be conducted with the least disturbance to the mouse possible, whilst gaining suitably robust data to answer our research question.

Recovery surgery (moderate severity) will be conducted under appropriate general anaesthesia, with local analgesia, under a sterile field and by a trained researcher, to minimise the potential for infection, stress or pain.

PROJECT 16: THE ROLE OF IRON IN IMMUNITY, ANAEMIA AND METABOLISM

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.

Word limit; 1000 words

Project Title	The role of iron in immunity, anaemia and metabolism
Key Words	Iron, Hepcidin, Infection, Anaemia, Immunity
Expected duration of the project	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):

The project is divided into three major objectives:

1. Understanding regulation of systemic iron control: Iron is required for carrying oxygen in red blood cells, for generation of energy, and for the synthesis of DNA by enzymes, and for many other biological processes. Iron deficiency impairs these important activities, whereas too much iron is toxic. How the body is able to maintain an appropriate amount of iron, and how these processes go wrong in anaemia and in disorders of iron overload, are not well understood. A hormone called hepcidin is important to maintaining iron balance but how synthesis of hepcidin is controlled is not fully understood. The basic biology of iron homeostasis represents a key scientific unknown, and correcting defects in the process would address unmet clinical needs.

2. Iron in infection pathogenesis and adaptive immunity: We want to understand how infections, most notably malaria, are affected by changes in the amount of iron available in the body – as microorganisms need iron to grow. Correspondingly, white blood cells that fight infections need iron to function effectively, but how iron is important for white blood cells is not known. We want to understand how changes in in iron concentrations affect blood cells and defence against infection.

3. Development of iron-modulatory therapeutics: We want to develop and test treatments for infections, inflammation, anaemia and iron overload disorders that are based on manipulating the activity of hepcidin.

What 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 planned programme of work will bring benefits in the following ways: 1. Increased basic understanding of iron physiology that will inform our other objectives involving infection and immunity. There will be benefit to scientists and clinicians

through publication, conference presentation. Because of the similarity between mouse and human iron regulatory systems, the information will contribute to understanding of human physiology and how it is altered in diseases that are associated with too much iron (haemochromatosis, thalassaemia) or not enough iron (anaemia). 2. Discovery of basic mechanisms of malaria, communicated via publication and conference presentations, will benefit the research community looking for new ways to combat this important infection. Findings will help to inform future anti-malaria strategies and help to treat malarial anaemia. 3. Data on how iron influences white blood cells and response to immunization are of direct relevance to optimising vaccine design in e.g. developing world context for children and pregnant women who are frequently iron deficient. This work will complement other studies providing observational and trial-based assessment of the role of iron in human immune responses and will also benefit researchers investigating basic aspects of how the immune system works. 4. Identification and validation of new treatments will lead to patents, publications, and potentially medical benefit to sufferers of highly prevalent iron-related disorders.

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

Mus musculus – mouse. We estimate that we will use 20,000 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?

For all of our objectives, we will typically administer substances or use strains of mice that enable manipulation or perturbation of iron control. Most of these treatments, over the typically short timecourses used, will have mild or sub-clinical effects. However, for each objective, some of our treatments that are necessary for understanding the iron response to such conditions may cause transient suffering, defined as moderate in severity - in practice, since our work relates in part to the impact of iron on infection and associated inflammatory response, this means mice may experience for a short time 'fever' type responses including: lethargy, reduced appetite, a degree of weight loss, ruffled fur. In Objectives 1 and 3, the non-infectious, but inflammatory substances that we administer will induce this transient ‘fever’ type response which is expected to affect hepcidin production and may cause anaemia; we may also administer substances that lead to transient development of anaemia, which may cause the mice to become lethargic, although in many cases no sign will be apparent. For Objective 2, we will investigate the involvement of iron control on the development of infections, primarily mouse models of malaria, but also specific bacterial and viral infections. The malaria strains used are non-lethal, but the mice are expected to experience effects of the infection while the parasites are proliferating, and before the mice begin to control the infections, they may transiently display signs such as reduced mobility, a loss of appetite, a degree of weight loss, and ruffling of fur, until the infection is controlled, when the mice will recover. The

bacterial and viral infections, if left unattended, would be lethal – however, when these infections are used, we will ensure careful monitoring to ensure that mice will not progress beyond clinical signs such as those described above, at which point they will be killed humanely. Also in Objective 2, we will investigate how iron affects the development of immune responses. In this case mice will be given immunisations, which again may be associated with some inflammation. All of our mice, whether generated as part of breeding programmes, or whether used in experiments as above, will be humanely euthanized at the end of the protocols.

Application of the 3Rs

Replacement

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

Replacement

Our project is to investigate how iron balance is maintained, how defects in iron balance cause disease and how iron interacts with infection, immunity and other aspects of metabolism. Control of iron (iron homeostasis) and immunity are both multi-organ systems. For example in iron homeostasis, anaemia is sensed by the kidney, which then sends a signal to the bone marrow to increase production of red blood cells, the bone marrow also instructs the liver to decrease synthesis of the iron hormone hepcidin, and low levels of hepcidin allow the absorption of iron from the diet and the release of iron from the spleen. The released iron increases iron availability for red blood cell production so that more red blood cells can be made, correcting the anaemia. Clearly such layered and multifactorial processes cannot be accurately modelled in their entirety in vitro, or indeed in lower animals. Often there are no *in vitro* alternatives that enable understanding of complex physiology, and likewise models for human iron disorders that involve defects in these systems are best achieved using a whole mammal organism that closely resembles humans in aspects of iron and immunity. Therefore, mice are the most suitable animals for these studies.

Reduction

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

Reduction

Prior to experimental work, statistical power calculations will be performed allowing the use of the smallest number of animals needed to provide satisfactory data. We will continuously evaluate and update our statistical approaches and group sizes based on experience of the desired effect size and observed variation.

The number of excess mice generated through breeding will be minimised by constant and careful monitoring of breeding programmes. Specifically with respect to our mouse line in which the gene encoding the iron hormone can be inducibly deleted, our breeding strategy ensures that each animal that is generated can be used in breeding or experiments, reducing 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

Choice of species

Mice are the most characterised species for detailed immunological and iron-related analyses. Furthermore many human iron-related disorders are well-modelled in mice. Because of the numerous different immunological tools available, mice can be used for detailed characterisation which is not possible in other organisms and they also provide the opportunity to study the role of particular genes (eg those involved in iron handling) and iron diets in the induction of an immune response and protection against infection, by using genetically modified mice with defined, or inducible, gene modifications.

Choice of models

We will use transgenic mice that lack, or can be induced to delete, genes that are well conserved between mouse and man and that exert control over iron homeostasis, synthesis of hepcidin, and response to iron-induced toxicity. For infections, we will use models of malaria that best mimic the aspects of human malarial disease that we are most interested in, namely the liver-stage of infection, anaemia, and the generation of parasite forms that transmit the disease from infected hosts back to mosquitoes. We will also use bacterial and viral infections that closely mimic human disease. Lastly we will use models of immune responses that will allow us to investigate how iron concentrations influence the biology of white blood cells and the ability of the immune system to fight infections.

General measures to minimise harms

1. REDACTED we are able to minimise the number of blood samples such that blood is taken at the most informative time points post-vaccination depending on the type of immune response we are measuring (e.g. antibody versus T cell response).
2. By using *inducible* hepcidin deletion we will minimize animal suffering by decreasing the amount of time mice accumulate iron.

3. We will not use a traditionally-used preparation termed Freund's adjuvant in order to induce inflammatory signals in our studies, as this may cause significant pain and severe inflammatory reactions; it can be replaced by safer alternatives for our studies.
4. Mice will be monitored daily and as infections progress, the frequency of monitoring will increase to ensure undue suffering does not occur. Humane endpoints will be constantly re-assessed and refinements implemented to minimise systemic illness while maintaining the scientific quality of the study.
5. Malaria studies employ mouse strain / *Plasmodium* species combinations such that the infection is non-lethal and self-limiting.
6. To minimise distress due to administration of substances, we will keep to minimal volume limits will and use fine-gauge needles and anaesthesia to reduce harms.
7. Where multiple repeated doses of a substance need to be administered over a longer timeframe, the use of devices called osmotic minipumps which slowly release the substance and eliminate the need for multiple injections will be considered and used where appropriate.

PROJECT 17: IMMUNITY INFLAMMATION AND CANCER IN THE DANIO RERIO (ZEBRAFISH)

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.

Word limit; 1000 words

Project Title	Immunity inflammation and cancer in the Danio rerio (zebrafish)
Key Words	immunity, inflammation, cancer, zebrafish
Expected duration of the project	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):

While immunity and inflammation are essential steps in the repair of a damaged or infected tissue they can also become detrimental if they fail to resolve, yet the reasons behind a non-resolving response are far from understood. The aim of our research is to understand the mechanisms that underpin inappropriately sustained inflammation and in the longer term to identify targets that might be relevant for therapeutic intervention. We use zebrafish as a model organism as they have very similar immunity and inflammation to humans, but they allow visualisation of cellular interactions and molecular processes in a variety of situations that we have designed to mimic those that humans experience.

Since inflammation is involved in numerous disease systems this has involved us under the previous licence to develop a number of models that we aim to further investigate.

During this project we will focus on four major areas of research:

- basic understanding of the immune system. This is essential to tackle the problem of complex inflammatory diseases;

- developing infection models that resemble those of human diseases. Zebrafish are naturally susceptible to a bacterial infection that resembles that of human TB as well as some viral diseases and these will allow us to investigate the mechanisms of disease development.

- developing chronic inflammatory disease models in zebrafish brought about by agents that we may come across in the environment – e.g. different dietary components (for intestinal disease) and particulates (for lung disease)

- continue to develop models of cancer in zebrafish

What 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 By studying cellular and molecular processes involved in inflammation we will be able to identify targets for intervention. Our models will help identify measurable indicators of the animal's biological state which themselves can be used to map disease progression with the potential for eventual use in drug discovery.

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

We aim to use as much as possible, zebrafish embryos that are not regulated under ASPA. We will also use larval and adult fish, including for breeding in order to maintain our genetically modified colonies. We estimate to use a maximum of 48,300 fish 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?

Our experiments will involve treatment of animals with special diets for example followed by live imaging under anaesthesia, after which animals will be humanely killed. Various organs will thereafter be collected for molecular analysis with an overall severity classified as moderate. One of our projects involves blood stem cell grafts to study the effect on the fitness of stem cells under inflammatory conditions. For this we will aim to refine a classical and widely used protocol that relies on the survival of the animal post transplantation as a measure of success or failure of the technique. To compare this classical method to our new method we will need to perform some experiments and follow survival. This will therefore involve a small number of animals that will classify as severe. If we successfully develop a refined protocol, this will be shared with the community and this should reduce the number of severe procedures in other labs

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 immunity and inflammation including the study of complex physiological diseases and potential drug development screens which cannot be fully replicated in an invertebrate model or *in vitro* systems. It is therefore necessary to resort to the use of live animal experimentation. This will enable the mechanisms that are involved in the development of normal and disease states to be understood.

In previous years we have done a great amount of our research on rodent systems and looking at alternatives to achieve our objectives, we found zebrafish to be highly suitable as a model.

Wherever possible experiments will be performed first in zebrafish embryos (<5d) for example to establish drug toxicity doses. We will use zebrafish >6dpf (protected by the act) only when necessary.

Reduction

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

Reduction

A large proportion of our work will be carried out using zebrafish embryos and larvae at non-sentient stages (<5dpf).

- For our fish maintenance program fish are bred regularly to monitor their fertility, once fish are no longer at their peak of fertility they will be humanely killed.
- We will only generate new genetically modified animals if those needed are not already available.
- Statistical tests will be undertaken for each experimental procedure to determine the minimum number of animals required to reach statistical significance, depending on the range of data expected. Importantly we will make use of all information gained during our previous projects to optimise our group sizes.
- Whenever possible we will perform longer term studies with multiple sampling or imaging per animal in order to reduce the number of animals used per study. We have made significant steps in this area under the previous PPL.
- We consistently follow the ARRIVE guidelines.
- To increase the quality of our data, we pay particular attention to keeping our husbandry conditions as stable as possible (e.g. same food regimen, same water quality, same rearing 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

Since the goal of this project is to provide information with potential relevance to humans, the use of vertebrates rather than invertebrates is essential. This is because invertebrates do not possess an adaptive immune response and therefore are not suitable for the study of a complex immune system resembling that of

humans. Zebrafish possess both an innate and adaptive immune response and have the same of types of immune cell as humans. There is good evidence from our own as well as other studies that it is a relevant immunological model.

Embryos develop rapidly *ex utero* and are optically transparent which permits non-invasive analysis of observable physical features of the body and internal organ systems in a short space of time. Tumour growth and spread can be followed within a few days compared for example with weeks or months in mice.

A large proportion of our work will be carried out using zebrafish embryos and larvae at non-sentient stages. When sentient zebrafish are being used, any procedures liable to cause distress will be performed under general anaesthesia. We aim to refine anaesthesia/analgesia methods during the course of this work, but this will be done with support using combinations of drugs in order to minimise distress. Where there is potential for adverse effects the fish will be regularly monitored and will be killed using a humane method if they show any of the recognised symptoms indicating distress.

One aim of our study is to study the effect of inflammation on blood stem cells. Historically, the success of grafting such stem cells is measured by the survival or death of the animal. This project is currently funded by the NC3Rs with the motivation to develop an alternative for animal survival as the assay end point thereby refining this protocol. We will this share protocol if successful with the community.

PROJECT 18: PATHOPHYSIOLOGY OF CEREBRAL VASCULAR DISEASE AND ALZHEIMER'S DISEASE

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.

Word limit; 1000 words

Project Title	Pathophysiology of cerebral vascular disease and Alzheimer's disease
Key Words	Dementia, Alzheimer's Disease, Vascular Disease, Causes, Treatments
Expected duration of the project	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;
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.

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):

There are currently estimated to be 850 000 people living with dementia in UK (2016) with an annual cost to the UK of £26bn per year. Alzheimer's disease and vascular disease are the most common causes of dementia and the numbers are expected to rise with an increasing aging population. They cause significant human suffering including to families and carers as the diseases are often protracted over many years. We still do not understand what causes these diseases and there are no treatment options. Thus we require relevant animal models that mimic better the complexity of the human diseases in order to understand better what causes the disease and to identify targets to test and develop effective 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)?

There are several benefits that may arise from this project. We will aim to provide more detailed information on the causes leading to vascular and Alzheimer's disease. Following on from these discoveries we will aim to develop and test potential drugs in the most relevant models and determine whether they can delay or halt the progression of disease. We work closely with clinical colleagues who are ideally placed to undertake follow-on early clinical trials of any promising therapies. The information generated from our research will be published and be communicated through presentations at relevant scientific conferences to alert the research community of our findings. We have close interactions with members of the public, including dementia carers or families, through our funding and we will also communicate our findings to those individuals.

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

We will use rodents, predominantly mice and some rats. Over the 5 year period we would approximately expect to use 8000-12000 rodents with the majority of these numbers due to 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?

Since we are studying models of progressive degenerative disease relevant to cerebral vascular disease and Alzheimer's disease we expect the models will mimic the human conditions and adverse effects can include disabilities and behavioural changes similar to the human disease. Therefore there will be adverse effects on some the animals in these models. For the majority of studies we will be studying the less severe (mild/moderate) severity limits and it will be rare that we will study rodents that have severe adverse effects. These adverse effects will be limited by the use of careful endpoints during the studies. We will aim to study multiple endpoints in the same animal such as changes in molecules and brain pathology alongside measures of memory. All animals will be routinely and clinically evaluated for any signs of adverse effects throughout the work protocols. Whenever possible, measures to revert the adverse effects will be applied throughout the duration of each protocol (e.g. increase of animal temperature via heated chamber, supplemental/soft foods to encourage eating). All animals will be culled humanely at the end of the experiment. All brain tissue will be 'banked' for either analysis of brain pathology or molecules for future use.

Application of the 3Rs

Replacement

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

Replacement

Alzheimer's disease and vascular disease are complex and can only be investigated using animals. Cells in isolation cannot develop the type of pathology, amyloid plaques and tangles, that are found in models of Alzheimer's disease. Additionally both Alzheimer's disease and vascular disease may be influenced by blood flow. Blood flow is absent in cell model systems. There is a complex interplay between the many different cells in the brain and regulation of blood flow that cannot be studied in any other model. Animal experiments are critical to identify what causes disease; the time at which the brain changes occur (also essential for knowledge about drug administration) and also provide considerable insight into the development of new drugs.

Cell models may be useful to examine the cellular responses to brain injury (albeit in the absence of blood flow) and these models can make contributions to our knowledge. We have used these in the past in parallel with our *in vivo* models and will strive to use these if deemed appropriate.

Reduction

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

Reduction

The majority of our animal numbers will be due to the breeding and maintenance of colonies. We will maintain our rodent colonies using as few animals as possible to generate sufficient numbers for studies without being wasteful. We will use the lowest number of animals required to give meaningful, statistically relevant results. We will 'bank' tissues to use for multiple measures. In addition in some studies we will conduct longitudinal studies in the same animal which also reduces the numbers 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

We aim to study relevant animal models that mimic aspects of human diseases, Alzheimer's disease and vascular disease and the risk factors that may influence their progression. Over the last years we have sought to develop models that are more akin to human disease. We have significant experience with these models and have over time refined our endpoints and strategies to reduce adverse effects wherever possible. We train researchers to the highest standards to be able to monitor and study rodent models of disease, to identify adverse effects quickly and to respond to these accordingly. We have rigorous monitoring procedures in place to minimise any adverse effects.

PROJECT 19: TARGETED THERAPIES TO MODULATE INFLAMMATION IN ALCOHOL-INDUCED INJURY

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.

Word limit; 1000 words

Project Title	Targeted therapies to modulate inflammation in alcohol-induced injury
Key Words	Liver, alcohol, hepatitis, therapy, inflammation
Expected duration of the project	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;
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):

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 pentoxyfylline 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. 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

PROJECT 20: UNDERSTANDING INFECTION PATHOGENESIS TO GUIDE THERAPIES

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.

Word limit; 1000 words

Project Title	Understanding infection pathogenesis to guide therapies.
Key Words	Infection, Immunity, Pathogensis
Expected duration of the project	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;
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):

Infections remain a major cause of death worldwide. The latest available numbers from the World Health Organisation (WHO) shows that infections accounted for 3 of the top 10 leading causes of death worldwide with a total of 6.1 million deaths annually. Even in the UK lung infections are still in the top 5 leading causes of deaths each year.

Infection control is a battle between the body's immune system and the ever evolving pathogen. Over the last 10 years we have seen a sharp rise in pathogens that are resistant to antibiotics due the overuse of antibiotics. These pathogens are not just resistant to one or two antibiotics but we are seeing the emergence of pathogens that are resistant to multiple antibiotics. This is made worse by the fact that there have been very few new antibiotic products that have been developed over the last 10 years.

We will be studying bacteria, viruses and parasites and the objective of this project is to understand and define the mechanisms involved in the host / pathogen interaction and to develop novel therapeutic approaches, or to develop new vaccine candidates.

What 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 strongly believe that the work described in this proposal will both improve our understanding of infection related illness and help identify new targets for new therapeutic approaches such as vaccines. We will share the data we generate with other researchers around the world through scientific publications and open access research databases. Any new mouse lines we generate will be shared with other research groups.

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

We will use mice, including genetically altered mice. The number of animals will be 10,000 mice used over 5 years. 5,000 of the mice will be used in breeding procedures which have a ‘Mild’ severity limit, and 5,000 will be used in 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?

We will infect mice with bacteria, viruses and parasites so that we can study the effects of these infections. Mice will be injected, given the pathogen orally, or the pathogen will be administered into the animal’s nose. Sometimes, housing the animal with another infected animal, or using bedding from a cage where an infected mouse has been housed will be enough to infect another mouse. We will take poo, take blood samples and images to find out how the infection progresses. At time points we will cull animals, remove organs and count pathogen numbers. We will immunise some mice which have an infection by injection and see if the injection works to protect the animals against the pathogen. Some animals will be irradiated to remove their immune cells and then given immune cells of either a wild type mouse or the immune cells of mouse where a gene has been deleted. This allows us to know that if a particular gene knockout cannot control infection that this is due to the immune cells of that mouse and not the rest of the tissues as only the immune cells will be gene knockout and the rest of the mouse will be wild type. As we are infecting the mice we expect to see a certain level of adverse effects. However we have a lot of experience with the infections we use and we monitor the mice using weight loss and other clinical signs on a daily basis. The level of severity for the infection procedures on the licence is Moderate and we have established defined humane end-points. All animals will be humanely killed at the end of the experiments and we will remove tissues to count the pathogen or measure immune responses.

Application of the 3Rs

Replacement

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

Replacement

We only use live mice when we cannot use alternative approaches (cell based assays for example). It is not possible to monitor all aspects of the interactions between the different stages of a real infection process meaningfully outside of the whole animal.

Reduction

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

Reduction

As we are an experienced team with a lot of experience in the infections we use, we will draw on the knowledge and data we already have to design our experiments using the minimum number of animals, and to get the most information from each animal as possible.

We work closely with statisticians who help us with the experiment design and advise on the correct number of animals to use.

The results of the research will be published in scientific journals and presented at scientific conferences so the data can be used by other scientists

We will make resources (data, animal lines) available to researchers to reduce the number of animals that might be used in similar 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 for experiments as they represent an appropriate mammalian model to study host-microbe interactions and serve as an invaluable pre-clinical model for therapy development. Mice can be genetically manipulated to mutate genes relevant to human disease susceptibility and there are immunological reagents available to monitor the host response to microbe interactions. We closely monitor the mice on a daily basis for signs of illness and suffering, scoring for clinical signs of illness such piloerection (raised fur), hunched gait and loss of mobility, along with weight loss. Our animal facility uses a sophisticated database to track the health status of every animal and alert us to when the animals are showing any signs of feeling unwell.

PROJECT 21: DEVELOPMENT AND FUNCTION OF THE ZEBRAFISH INNER EAR

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.

Word limit; 1000 words

Project Title	Development and function of the zebrafish inner ear
Key Words	Zebrafish, Inner Ear, developmental biology, deafness, vestibular disorders
Expected duration of the project	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;
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):

Our overall aim is to understand how the inner ear—the organ of hearing and balance—develops in the embryo, and the genetic and environmental causes of deafness and vestibular (balance) disorders. Vestibular problems place a large burden on the health services: dizziness and loss of balance, for example, are thought to contribute to falls and fractures in the elderly. In addition, the causes of both rare inherited diseases affecting balance function and those that are relatively common (e.g. Menière's disease) are poorly understood. We study different genetic strains of zebrafish, some of which develop with specific defects in the inner ear. The benefit of this work is to increase the scientific knowledge base for how the vertebrate ear develops, and to identify approaches for better diagnosis and treatment of inner ear disease in humans, including the development of novel therapeutic drugs.

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

This project will advance our scientific knowledge and understanding of the developing inner ear in vertebrate embryos. Data generated will help to build the knowledge base and increase understanding of gene function and the regulation of molecules that act as messengers or signals within and between cells as the ear develops. Our work contributes to the branches of science known as developmental biology (the study of the changes that occur as a fertilised egg develops into an embryo and finally a mature adult) and sensory neuroscience (the study of our senses, including hearing, balance, sight, touch, smell and taste). Findings from the work described here will support existing and future research programmes and funding proposals in these areas. Beneficiaries include our collaborators (including

biologists, mathematicians and engineers), other zebrafish researchers, and others working in similar fields. Our genetic data will be of interest and benefit to clinicians and patients (e.g. in the development of genetic diagnostic tests); a number of our genetic strains form direct models for human disease. We have recently established a collaboration with a commercial company who will be using our data to develop drug discovery programmes. In the longer term, there may be potential benefits for patients with hearing or balance loss, disorders of the nervous system, or cancer.

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

Most adult fish are used for breeding purposes to generate the embryos that are needed for experimental work. For each gene that we study, we require a breeding stock of 80-120 individuals to provide embryos for experimental analysis. Most lines are kept as stocks where 50% of the fish are genetically normal, and 50% are carriers for a mutation of interest. As carriers, these fish usually develop normally and are healthy, but when bred together, they generate embryos that can develop with abnormalities in the ear, which we use for our research studies. Overall, we expect to raise ~5000 adult fish annually that carry mutations or transgenes. At the end of their breeding life (at ~2 years old), fish are killed humanely. This approach ensures that we have a steady supply of embryos for our research experiments, most of which are carried out during the first five days of development (below the age of protection). We estimate a further 600 fish per annum will be used to generate new genetic lines, and 100 per annum will be used for behavioural studies. Overall, we expect to use 23500 fish carrying mutations or undergoing procedures over the five-year period 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?

Some genetic strains may develop with mild behavioural problems, such as hyperactivity, circling or loss of balance. Fish are generally kept as mixed sex shoals, but for some experiments, will need to be isolated for a few days at a time or paired for mating. This can be stressful for the fish, and will be minimized by returning fish to the group tank as soon as possible. DNA samples may be taken by taking a small biopsy of the fin under anaesthetic. Fish recover well from this procedure and regenerate the fin in a few weeks. Any fish showing signs of general ill health due to aging (e.g. listlessness, failure to feed, tumours) 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

Development of the inner ear involves interactions between multiple cell types, and complex three-dimensional shape changes, to generate the intricate structure of the adult inner ear. It is not possible to recapitulate all these events using cultured cells. The inner ear is a vertebrate-specific structure, so non-protected animal alternatives do not recapitulate all the features that we wish to study. Most experiments will be done on embryos under the age of protection. A few experiments will use juvenile or adult fish.

Alternative model systems include the chick and mouse, but these are of higher neurophysiological sensitivity than the zebrafish, and do not have some of the advantages that the zebrafish can offer (e.g. ease of imaging and drug treatments). The zebrafish is therefore a very suitable complement to mammalian or bird models for the types of studies that we wish to perform.

Reduction

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

Reduction

We keep sufficient numbers of adult fish to ensure a steady supply of healthy embryos for our experimental work. The numbers of adults are under constant review to ensure that they meet this demand but do not exceed it. For behavioural work with adult fish, we will use power calculations to estimate the group sizes required to achieve statistical significance. Wherever possible, we will employ in vitro methods (e.g. cell culture), together with mathematical and computational models, alongside our in vivo experimental work.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 an excellent model system for these studies. It breeds well in captivity, and the embryos are optically transparent, meaning that internal structures such as the inner ear are easy to see. We can manipulate gene function in a number of ways. Cells can be labelled with harmless fluorescent markers for live observation. The fish embryo (below the age of protection) is also ideal for drug discovery, as it is small and permeable to chemicals that can be added directly to the water.

Fish are kept under a high standard of welfare, with daily checks on fish health and water quality. All new experiments require an Individual Study Protocol, discussed with the Named Animal Care and Welfare Officer, who will advise on refinements that can minimise welfare costs. Pilot experiments, using a few animals in the first instance, will be used to test the feasibility of new approaches. We will aim to refine experimental design wherever possible, for example by testing the genetic make-up of animals at the embryonic stage to avoid raising unwanted genetic strains beyond the age of protection.

PROJECT 22: EVALUATION OF COGNITIVE FUNCTION IN ANIMAL MODELS

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.

Word limit; 1000 words

Project Title	Evaluation of cognitive function in animal models
Key Words	Rat, Behaviour, Cognition, Schizophrenia, New drug treatments
Expected duration of the project	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):

This work will continue to develop animal models for studying cognition, mood, motivation and social behaviour deficits of relevance to those that occur in human psychiatric illness.

Neurodevelopmental disorders (NDDs) including schizophrenia and autism spectrum disorder (ASD) remain poorly managed, present a large personal burden to the sufferer and a major economic burden to society. Brain disorders cost €141 billion per annum in the UK, with a total 2010 cost for psychotic disorders of €16.7 million (Fineberg et al. 2013). A major factor contributing to this high cost is lack of effectiveness of existing drug treatments.

Existing drugs are not effective enough and have unpleasant side effects reducing compliance. Better treatments are therefore required, particularly for memory and social communication in schizophrenia. Critical for the development of improved treatments is improved understanding of the cause and biological basis of such disorders which can only be achieved through carefully validated 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)?

Humans will benefit from the project in a variety of ways. Schizophrenia reduces the quality of life for patients and carers dramatically and has a large economic cost. Improved treatments have not been successfully developed due to a lack of understanding of the biological basis of these diseases due to limited animal models. For any disorder, prevention is better than cure and early treatment is more successful than late treatment. Our work aims to identify new treatments for debilitating aspects of the illness, memory, mood and motivational disturbances with

reduced side effects and improved effectiveness over existing medication. This will limit the detrimental effects to the patient's social, academic and home life. The benefits will be both in quality of life for patients and carers and economic in terms of restoring function in patients who can then work and make a contribution to society. We also aim to improve understanding of the mechanism(s) by which new treatments (drug and non-drug e.g. exercise) produce their beneficial effects on cognition and aspects of negative symptoms, mood, motivation and anticipation of reward. Animals (in our lab and others) will benefit from development of improved, food-rewarded tests based on naturalistic behaviour.

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

We use rats and use less than 600 per year, we use them for as long as we can so that we minimise the number 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 level of severity is expected to be moderate due to a brief surgical procedure to implant osmotic mini-pumps for administration of a clinically relevant dose of antipsychotic drugs. Only minor, short term, brief behavioural effects of our drug treatments are anticipated as these interact with brain systems. These may include increased or decreased activity and/or increased repetitive movements. Behavioural techniques are generally not stressful and can, in certain cases, be considered enrichment for the animals. Environmental enrichment and wheel running are examples of the methods we use. At the end of the study, or as part of the experimental procedure (e.g. to perform detailed assessment of brain changes induced by the interventions) rats will be killed humanely and quickly by a Schedule 1 procedure.

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 mimic brain and behaviour interactions in cell systems, and whole animal experimentation is therefore vital to obtain a greater understanding of mechanisms and to test effectiveness of new molecules. This work must use whole animals as behaviour is a central feature of the project. Thus effects of new drugs on complex behaviour patterns may only be studied in whole animals and not using in vitro preparations, although CNS studies can also be conducted *ex vivo* and *in vitro*.

To date there is no suitable alternative to the use of whole animals for behavioural research that doesn't involve human subjects.

However, wherever possible the whole animal studies will be accompanied by isolated culture systems and using samples from patients. If any relevant non-animal alternatives become available during the course of the project, we will implement these in our studies.

Reduction

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

Reduction

Reduction

We will minimise the number of animals by re-using the same animal in different behavioural tests, and testing more than one drug in the same animals. Numbers will be further reduced by repeated measures designs where possible. So far this has reduced our animal usage by approx. 40% All these repeat studies will only be conducted following an extensive examination of the animal by the Nominated Veterinary Surgeon. We have consulted a statistical expert and conducted power calculations to ensure we have minimum number of animals for maximum 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

Rats are a popular choice because of the detailed existing knowledge of their brain structure and function and its similarity to that of humans. The rat has been chosen as a subject for the present work for several reasons. Primarily much is already known about memory and brain mechanisms controlling complex behaviours in rats. We have extensive experience of studying behaviour in rats, all our current tests are validated for rats and our tissue analysis systems are validated using rats. Rats are larger than mice and more suitable for brain imaging and recording studies. We have refined our techniques in the continued review of our current work. REDACTED Welfare is critical for successful experiments and we have extensive experience in behavioural analysis of rats which we will continue to use in order to ensure that all our rats are subjected to the minimum adverse events and that undue stress is minimised at all times, particularly when rats are handled and drugs are given. Specific on-going refinements include: reduced use of food restriction, increasing

use of naturalistic tasks involving enrichment eg wheel running and environmental enrichment. The use of smell and digging media to guide rats' behaviour, different grades of sandpaper to run along, improved handling and dosing techniques, including reduced restraint for blood sampling.

PROJECT 23: PRODUCTION OF ANTISERA AND IMMUNE CELLS

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.

Word limit; 1000 words

Project Title	Production of antisera and immune cells
Key Words	Antiserum, immune cells
Expected duration of the project	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;
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.
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 purpose of this licence is to allow production of immune sera or cells that will be used in research or diagnosis where these are not readily available from other sources.

There are a number of key areas within the Establishment that have a requirement for polyclonal antibodies or immune cells that can bind to specific disease-causing agents such as bacteria or viruses or to particular fragments or components of these agents or the toxins that they might produce to cause disease in the host. These areas are either in the field of developing and assessing vaccines in developing new diagnostic 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)?

This project will provide immune serum or cells that are essential components of diagnostic tests used to identify infectious agents in samples taken from patients such that a specific disease can be diagnosed. In addition, such immune assays can be used to assess whether immunisation has been successful in terms of raising immunity in an individual or population as part of a vaccination programme. Immune responses seen in animals can also inform on vaccination strategies such as the best route of administration or the number of boosts (if any) required to establish effective immunity.

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

Over the five years of the project it is anticipated that we will use no more than 5,000 mice, 100 rats, 500 guinea pigs, 100 rabbits, 10 goats REDACTED

In the context of what you propose to do to 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 Protocol 1 has been classified as MODERATE in recognition of the fact that the occasional use of Freund's complete adjuvant (FCA) may induce localised granulomas or even ulceration at the site of injection. However, it is anticipated that that majority of animals will not receive FCA and so will not experience severity greater than MILD. FCA will not be used in Protocol 2 and so is classified as MILD. As we seek only to immunise animals to raise immune responses using methods that are similar to those used in human medicine the majority of animals will not experience severity above MILD. Where an immunisation is conducted via the intradermal or subcutaneous route this may cause localised inflammation, slight swelling or even scabbing as it does in humans. In our experience this causes little or no distress or discomfort to the animals and lasts only for a few days. At certain time points before and after immunisation, a small blood sample will be taken to see how the immune response is developing and this will help to determine whether a further boost is needed to give the required level of antibody or immune cells. In the case of macaques, wherever appropriate and with the advice of the Named Veterinary Surgeon that the general state of health and well-being is likely to be fully restored, animals may subsequently be issued to suitable authorised Project Licences i.e those allowing re-use of animals and having a severity limit of Moderate or lower. In line with Section 14 of A(SP)A, 1986 any animals transferred will have undergone minimal procedures with sedation only used to immobilise animals for blood sampling or vaccination. In most cases, however, due to the value of blood samples and tissues from immunised animals, they will be exsanguinated under terminal anaesthesia in order to maximise the number of samples available for future tests and to reduce the need for further use of animals.

Application of the 3Rs

Replacement

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

Replacement

Many diagnostic tests that are essential in identifying the agent that is causing a disease rely on the ability of antibodies to bind to either the agent itself or to a particular component that the agent produces. Although these tests are conducted "*in vitro*", that is without the use of animals, it is necessary to produce the antibodies

specific to the agent by immunising animals. Animals such as rabbits are frequently used for this purpose and are usually immunised in the same way that humans are immunised to stimulate the immune system to produce antibodies in the circulating blood. Typically the viral or bacterial agent is killed or a small but immunogenic component of the cell wall is extracted to provide the material for immunisation. This is frequently mixed with a substance called an adjuvant that serves to stimulate the immune system so that a definite response is ensured. Adjuvants are frequently used for human vaccines and are thus required to be non-toxic when given by the appropriate route.

Reduction

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

Reduction

The number of animals used will be the minimum required to produce the volume of antiserum and/or number of immune cells required and will depend on the species chosen and the nature of the assay. Wherever possible any samples not used immediately will be archived so that further assays can be conducted in future without further production in animals. For evaluation of production or research vaccines where immunogenicity is the key readout, numbers of animals will be minimised by the application of statistical methods based on the reproducibility of the vaccination protocol and the assay. Every opportunity will be taken to reduce the number of animals used as protocols or assays become more refined in the light of experience or technical improvement.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 chosen will be selected as the most relevant to produce adequate quantities of antiserum containing antibodies of the highest specificity and avidity. Consideration will also be given to the antigen/infectious agent/toxin and application of the product when deciding on the most appropriate species.

Protocols used will follow the Home Office guidance: “Antibody Production, Principles for Protocols of Minimal Severity.”

Where blood samples are taken protocols will follow the guidance : First Report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, 1993, *Laboratory Animals* 27: 1-22.

For the conduct of any procedures an assessment will be made on the need for sedation to reduce stress and this will be appropriate for the species and type of procedure.

Wherever possible, animals will be housed in compatible social groups and given environmental enrichment appropriate for the species. In the case of macaques this will include continued housing within one of the colonies managed on site by the Establishment and maintenance of peer group structure wherever possible.

PROJECT 24: FUNCTIONAL GENOMICS FOR HELMINTHIASES

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.

Word limit; 1000 words

Project Title	Functional genomics for helminthiases
Key Words	Parasitic disease, Schistosomiasis, Trichuriasis, Genetically modified parasites, Host-parasite interaction
Expected duration of the project	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):

Approximately one fifth of humans are infected with at least one species of parasitic worm, including flatworms and roundworms such as schistosomes and whipworms, respectively. Infections cause long term disease amongst many of the poorest people in the world, hence research involving these parasites has been neglected. There are few drugs to treat these infectious diseases, and drug resistance is a growing concern. Genome sequences for many parasitic worms is now available but the identities and functions of most genes are unknown. The **overall goal** of this project is to understand how genes drive the basic biology of neglected helminth infections using molecular tools combined with genome analyses. Specifically, we aim to study the worm genomes, how they are organised and regulated. By identifying genes that underlie important aspects of infection and disease, we aim to discover exploitable vulnerabilities. To do this, technology needs to be developed; in particular, we will develop molecular tools that will allow the function of genes to be determined. Our approach is to grow parasites in mice to produce the material needed for the analysis of all parasite genes. To understand how parasites infect or develop, host and parasite genes will be disrupted.

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

With this project we expect to understand two of the most common and devastating parasitic worms that together infect almost 1 billion people worldwide. The purpose is to decode their genomes, provide information on how their genes drive their biology and how infections develop. This is fundamental information that will aid the discovery of new targets for drugs or vaccines.

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

Approximately 2056 mice will be employed per year, i.e. 10280 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?

Mice will be infected with parasitic worms (schistosomes and whipworms) by various approved routes: by placing the tail in water containing the parasites, injections into the abdomen or using a tube passed through the mouth (oral gavage). They may have tissue removed from their ear for genetic analysis, and blood. They may be also treated with approved drugs to be used in mice by different routes: in diet or drinking water, by oral gavage, subcutaneous, intraperitoneal, and intravenous. Mice may be irradiated to remove their own immune cells in order to study the function of immune cells from other mice of different genetic make-up that they receive by injection. Some of the procedures will be carried out under anæsthetic as appropriate, for instance during infection gas anaesthetic will be used. Animals will be humanely killed at the end of the procedures. For all the protocols employed in this project the severity limit is moderate. Infection with parasitic worms may result in a loss of condition, such as swollen abdomen, hunching, piloerection, and weight loss which will be closely monitored. However, mice are not expected to present any adverse effects with the number of input parasites per mouse used in this project, and the time the animal remains infected before the experiment ends. However, any mouse displaying up to three adverse effects, i.e. from 1 to 3, will be humanely killed using 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

Infecting live vertebrate animals, i.e. mouse, is currently the only way to maintain the complete life cycles of schistosomes and whipworms in laboratory conditions and obtain critical developmental stages for this project. To date, there are no currently alternatives as no one has managed to mature parasitic worms *in vitro*. However, extensive *in vitro*/ *ex vivo* experiments that complement and/ or inform *in vivo* studies will be performed, including culture and genetic manipulation of parasites to study the function of genes selected by *in silico* data-mining using open-access databases developed by us and others. In addition, thorough surveys in databases for alternative approaches will be constantly performed.

Reduction

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

Reduction

REDACTED We will consult with statistician in our team to design experiments ensuring (1) the use of minimum number of mice to achieve the scientific aim of the experiment; (2) reproducibility; and (3) the control of sources of variability, to obtain the most reliable information from each experiment. In addition, optimisation of the life cycle maintenance, *in vivo* imaging of reporter-expressing parasites, and the development of cryopreservation protocols for parasites, will allow the reduction of the overall number of animals. Finally, our findings will be published in scientific journals following the ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines), presented at scientific conferences, and accessible via open-access resources.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 refined animal model for the infections we will study in this project. It is fully permissive to the infection, i.e. they can be infected and mirror the infection in humans, which is critical to understand the interactions between the parasite and the host. During the course of this project measures to minimise the suffering will be taken by: (1) assuring refinement of housing and care; (2) carefully considering needle gauge, keeping volumes and doses to the minimum necessary, for injection of substances; (3) reducing the duration of experiments where possible; (4) reducing the risk of aggression by using female mice where possible, using littermates, and selecting appropriately designed refuges in the cage. Some progress in refining mouse-infection procedures has already been made, e.g. we have developed a refined protocol for infection of mice with parasites that reduces the level of stress and suffering of the animal by allowing the delivery of gas anaesthetic while controlling the body temperature. Mice will be closely monitored on a daily basis for signs of illness and suffering. We have access to a state-of-the-art animal facility, staffed by highly trained and dedicated animal technicians and managers, that employs a sophisticated database to track the health status of every animal and alert when there is a loss of condition.

PROJECT 25: IMMUNE DEVELOPMENT AND REGULATION

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.

Word limit; 1000 words

Project Title	IMMUNE DEVELOPMENT AND REGULATION
Key Words	Immunity, B cells, Selection
Expected duration of the project	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):

We are trying to understand the fundamental processes by which the body's immune system normally develops the ability to fight infections but avoids causing the sort of "friendly fire" that sometimes leads to autoimmune diseases like rheumatoid arthritis and insulin-dependent diabetes. We are interested in how the immune system responds effectively to infections and how it can be made tolerant to our own bodies. We want to know if it is possible to use antibodies to inactivate immune cells, as a way of treating autoimmune or inflammatory 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 expect better understanding of how the immune system responds to infections, why it fails it some patients and how we might modify in a range of diseases. One outcome of our research will be an understanding of how to improve the treatment for cancer by triggering a mild form of friendly fire to tumours and new ways to turn off immunity in diseases like rheumatoid arthritis. We expect to be able help families with rare forms of immune deficiency and our study of these diseases may ultimately inform the design of vaccines.

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

All our experiments will be performed in mice. We expect to use approximately 40,000 mice in 5 years.

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

95% of the experiments will involve breeding mice and the majority will be classified as being below threshold or causing only mild distress. Some mice will be immunised and rarely mice may be challenged with self-limiting viral infections, such as influenza, to check their immune response. Some mice will also be irradiated to

destroy the immune system, followed by rescue with donor cells. Some will be used to model inflammatory disease and bone marrow transplantation. These experiments will be classified as 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 are particularly interested in how the T and B cells that orchestrate the immune response attack infectious particles and how these cells are regulated. Discovering how this happens is a challenge because most of the immune response occurs in inaccessible parts of the body like the spleen, so many of the key advances in this field have been made in mice. Although we will do many of our experiments using cells in vitro, the complex interactions of cells that occur can only be studies in vivo. The mouse and human immune systems are very similar and share conserved regulatory pathways; and the mouse genome is very similar to the human, meaning that it is possible to translate findings in genetics between both species.

Reduction

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

Reduction

We will use the smallest possible group sizes that are still informative for a thorough statistical analysis and logical progression to the next step. Advice is sought from experts in statistics to support these aspects of the programme and we use blinding and randomisation to reduce bias whenever this is possible. We are very careful to reduce variation by keeping the mice together and ensuring that they are genetically identical. Several of the new approaches reduce the need for as much breeding as before, such as freezing unused embryos and sperm.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 laboratory mouse is the species of choice for studying immunology and the least sentient model with high similarity to humans at the genetic and protein level. It is the ideal mammal for genetic studies where animals need to be generated rapidly.

We are careful to minimise the distress to animals from the administration of drugs or other substances, including immunisations. We monitor the animals carefully and more frequently than daily if necessary. The mice are co-housed whenever possible and provided with enrichment material. Mice undergoing surgery are given adequate analgesia before and after the operation and special diets and increased access to water, when required. Surgical procedures will use appropriate aseptic techniques and antibiotics will be used to treat infections.

Split dose irradiation is used to focus DNA damage on the dividing cells in the bone marrow and reduce the need for higher doses of irradiation that would cause damage to other tissues. We will use the best available models of disease to gain the most information and cause the least possible distress to the animals.

PROJECT 26: STUDIES OF INFECTIOUS DISEASES

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.

Word limit; 1000 words

Project Title	Studies of Infectious Diseases
Key Words	Infectious diseases, Pathogenesis, Treatment, Resistance, Micro Organisms
Expected duration of the project	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;
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):

This project aims to:

- Understand the mechanisms microorganisms use to cause infection and disease, the host responses to infection and the host attributes giving rise to susceptibility to infectious diseases.
- Use the knowledge gained to develop ideas and targets for diagnosis, treatment and vaccination.
- Test the efficacy and biological activity of drugs and vaccines against infectious diseases.

Infectious diseases are major causes of death and injury, worldwide, placing huge burdens on health resources and inhibiting socio-economic development. The fight against them relies on the twin approach of diagnosis with treatment and vaccination but currently there can be serious deficiencies with these. The central approach is to use infection models with mutated microorganisms and/or animals, in which one or more component has been deleted or altered. The parameters of disease or colonisation that occur with the mutants will be compared with those obtained with a wild-type animal or microorganism. The models also will be used to study new vaccines and therapies and to study the genetic basis of susceptibility to infection. These approaches continue the successful strategy REDACTED. The project will focus on the following infectious diseases: pneumonia, tuberculosis, sepsis, listeriosis, meningitis and its sequelae and intestinal 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)?

Scientific knowledge will advance by determination of how clinical, cellular, humoral and biochemical aspects change over time after infection and by determining the role of selected genes and gene products in the behaviour of pathogens and their host during infection. Humans and animals can derive practical benefit from identification

of new targets for diagnosis, treatment and vaccination and by testing new treatment and vaccine strategies. We can expect benefits to be derived in pneumonia, tuberculosis, sepsis, listeriosis, meningitis and its sequelae or intestinal infections.

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

The mouse will be the usual choice but sometimes it will be the rat if: i) a larger animal is required, ii) established models are in rats or iii) reagents only are available for rats. Up to 10350 mice (including up to 6000 bred under this licence) and 400 rats will be 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?

Following infection, animals may develop signs of disease, moving progressively through piloerection, hunched, lethargic and moribund before death, but the normal end-point will be when the animals become very lethargic, being immobile unless prompted, when they will be humanely killed by a Schedule 1 method or immediately placed under terminal anaesthesia to obtain cardiac blood. It is not the intention that animals become moribund or die from the infection. The overall severity limit is Moderate but two Protocols covering 200 mice are Severe because the animals may stay at or go beyond the very lethargic stage. In one of these Protocols we will test the efficacy of new treatments aimed at combating the toxicity that sometimes explains the signs and symptoms of the infectious disease. Such treatments are likely to be given in alongside antibiotics and to see an effect beyond that seen with antibiotic alone and to mimic the human situation it may be necessary to let the animals reach the very lethargic stage, before beginning treatment. The other Severe Protocol is for the rare situations where severe adverse reaction to a vaccine candidate is observed. In this situation, it is our experience that animals move very quickly to a moribund state. Thus it is inevitable that animals will move to that point during these experiments and it will be necessary for the animals to reach that point if we are to determine the mechanism of toxicity. All animals will be humanely killed by a Schedule 1 method 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

Some characterisation of host-microbe interactions can be made using *in vitro* and *ex vivo* systems and we make full use of these to develop hypotheses. This type of work will continue but using animals is fundamental for complete understanding

because the full complexity of the host responses or the interaction of the bacterium and host during infection and disease cannot be reproduced *in vitro* or *ex vivo*. Thus, only with animal models of disease can complete information be obtained on the host response to treatment and immunisation, dynamics of bacterial colonisation and clearance from tissues. Animal models are crucial for investigating the pathophysiology of the infectious process because it is feasible to do studies that are ethically impossible in humans. In these models it is possible to study and manipulate the pathology during the whole course of the disease process, whereas in humans, study is often possible only at the terminal stages of disease, post-mortem, or at best only after the disease process has begun.

Reduction

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

Reduction

REDACTED The need to use the minimum number of animals required to achieve significant results is recognised but also that to do experiments with too few animals, and thus obtain inconclusive data, is wasteful. The typical design will be a time course experiment and experience and statistical analysis of data, shows that groups of 5-10 animals per time point are needed. Sometimes, an end-point design is used in which the time to a predetermined end-point or the number of animals reaching the end-point is determined. For these experiments we know that group sizes of 10-30 are required depending on the experiment; e.g., the smaller group is suitable for virulence comparison whereas the variability of vaccine experiments can dictate a group of 30.

Where feasible, *in vivo* imaging and telemetry can be used to follow the infection. These offer the possibility of following infection longitudinally in individual animals, thereby reducing the number of animals by removing the need for groups of animals at each point of a time-series 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

The mouse will be the usual choice, being by far the most widely chosen animal for the study of infection and host responses and providing very reproducible models of infection. Furthermore, there are many in-bred mouse strains available, as well as strains with defined mutations for study of disease processes. Furthermore, the genetics of the mouse is the subject of a global research effort, including making

knock-out strains in every mouse gene Additionally, the extensive background of existing experimental murine data is a very valuable resource.

Sometimes the rat is a better choice a larger animal is needed or because established models are in that animal or because reagents are available only for rats. The rat is also useful when it is desirable to test if an observation is applicable across differing infection models, e.g. in studies of new treatment.

Our disease sign scoring system minimises welfare costs. This system has proved exceptionally reliable and it is sufficiently sensitive such that only vary rarely is an endpoint is missed. However, collection of data can be refined, e.g. using telemetry for continuous real-time monitoring or by *in vivo* imaging to follow infection progression in individual animals. Although these involve an additional procedure on an animal, the quantity and quality of data can be markedly increased and less animals need be used.

Where surgery is required good operating theatre practise and aseptic techniques will be followed. When necessary, analgesia will be given as directed of the Named Veterinary Surgeon.

PROJECT 27: FUNCTIONAL ANALYSIS OF THE SRF NETWORK AND RHO-ACTIN SIGNALLING

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.

Word limit; 1000 words

Project Title	Functional analysis of the SRF network and Rho-actin signalling
Key Words	immunity, cancer, transcription, signalling, cytoskeleton
Expected duration of the project	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):

Physiological, developmental, or environmental signals change cell behaviour predominantly through alterations in how genes are expressed, a process controlled by specialised proteins called transcription factors.

The SRF transcription factor network controls the immediate response to two major cellular signalling systems. The Ras-ERK pathway controls cell proliferation and differentiation, while the Rho-actin pathway controls cells' physical integrity and governs their dynamic interaction with the environment. These signalling systems play major roles in how cells proliferate and interact, for example during the response to infection, and in cancer development and spread.

The aim of this project is to elucidate the role of the SRF network and the Rho-actin pathway in immunity and cancer development.

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

The project will develop new insights into how cells adhere to each other, move through tissues and around the body, and how their growth is controlled. In studying how these processes contribute to immunity and cancer, the work may identify new potential strategies or molecular targets for therapeutic intervention.

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

Based on work we have carried out under our previous licence, the number of mice we expect to breed around 25,000 mice over the next 5 year period, and expect to use around 2500 of these in the various experimental 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 majority of mice will be used for breeding, or culled for recovery of tissues and cells for analysis, and not subject to further experimentation. Mice will be culled by Schedule 1 killing at the end of experimental procedures. Some animals will experience adverse effects through tumour development, arising as a result of inherent genetic predisposition, cell transfer, or carcinogenesis. The severity in these experiments, which will be conducted in accordance with NCRI guidelines, is not expected to exceed moderate. Some animals will be subject to complex procedures, such as bone marrow transplantation, a procedure classed as moderate. All experimental animals are closely monitored, and instances where it appears that this level is likely to be exceeded, animals will be immediately culled. For some methods of tissue sampling or processing, animals maybe culled under terminal general anaesthesia.

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 aspects of cell behaviour that we are interested in can be studied in tissue culture. This will give us mechanistic information which will allow us to establish hypotheses about the behaviour of cells in the intact animal. We have considerable expertise in cell culture assays, and will use them in place of animals wherever possible.

Nevertheless, the development and function of the immune system, or cancer development and spread, are long-term processes that in which different cells interact in a dynamic three-dimensional environment, and respond to signals emanating both from their immediate neighbours and distant tissues. Simple tissue culture models cannot recapitulate such complex events, and the use of animals for some studies is therefore essential.

Genetically modified mice provide a valuable source of specialised cell types for study in culture in the laboratory. Use of "primary" cells is more informative than use of established cell lines, whose adaptation to growth in culture frequently alters cell behaviour. Recent advances in techniques to specifically modify genes in cells cultured from normal mice will allow us to increase the scope of our culture models and bypass the use of genetically manipulated mice.

Reduction

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

Reduction

Use of new genome editing technologies to generate mouse strains will reduce the amount of breeding required. We will also use these techniques to modify genes directly in cells cultured from normal animals, increasing the scope of our culture experiments, and ensuring that only the most informative mutations are tested in animal experiments.

Wherever possible we will use the mice that we breed to produce multiple data outputs, and/or cells for culture studies. We will exploit bone marrow transplantation to maximise the information obtained from complex genetic crosses. In our cancer studies we will minimise animal numbers used by tracking tumour development using scanning 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

Mice are most appropriate for our studies because of the ease with which they can be altered genetically. Their biology is close enough to that of humans to allow the generation of mouse infection and cancer models that closely reflect either specific human disease states or specific cellular interactions that occur in the human setting. We will be using models that have been extensively studied by others, which will maximise the information to be gained from our experiments.

All the work will be performed by highly-trained personnel and undertaken under conditions in which animal welfare is a priority. Animals will be monitored frequently for signs of distress.

PROJECT 28: IMMUNITY AND RESISTANCE TO DISEASE IN RUMINANTS

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.

Word limit; 1000 words

Project Title	Immunity and Resistance to Disease in Ruminants
Key Words	Vaccines, Immunity, Infection, Mycobacteria, Genetics
Expected duration of the project	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;
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):

Diseases in cattle have a significant impact on animal health and welfare, and impact on the economy. Two important diseases caused by Mycobacteria are bovine tuberculosis (TB) and Johne's disease (a gut condition). The bacteria that cause these diseases can also infect people leading in some cases to TB or possibly to Crohn's disease which affects the human gut.

In order to control these diseases in cattle we need to develop new vaccines or ways to identify infected animals so we can treat or remove them from farms. Developing new vaccines or diagnostic tests requires us to understand the immune response that can lead to immunity (versus disease) and also to understand why some animals respond differently to each other (the role of host genetics).

REDACTED We need to understand this in greater detail so that we can provide information that will, in the future, help to make better vaccines or tests for diseases and also perhaps to breed resistant animals.

Therefore, the major aim of this project is to understand how these Mycobacteria interact with components of the immune system and how this differs when cattle are vaccinated before being infected. This will help us define targets to work with in the future. We can use animals known to have different genetics to help determine whether we can breed for resistance. We will carry out a small number of experiments with other pathogens (parasites, bacteria, viruses) for comparison.

Key objectives are:

1. Understand how Mycobacteria, vaccines and their components interact with cells of the immune system in the laboratory (using blood and tissue derived cells)
2. Assess the response to vaccines in animals where we can look at the cells as they move away from sites where the vaccines are injected (using surgically prepared animals)
3. Examine immune responses in animals following injection of vaccines or infection with pathogens (mainly Mycobacteria)
4. Compare responses of experiments to those of animals that are identified as having disease on farms

What 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 determining how immunity can be stimulated by vaccines, what cells and mechanisms are involved we will provide helpful information for a number of scientific studies looking at the development of new ways to control Mycobacterial diseases (of cattle but we will also influence studies of related human diseases). We expect within the next 5 years to use the information to begin to work with companies to provide information that will allow them to develop new vaccines or tests for disease; this will include the registration of at least one new product and a patent. These studies are ultimately aimed at producing effective vaccines, diagnostic tests and other control measures against diseases of livestock that have significant impacts on animal health and the economy. These will mostly occur within the next 5-10 years.

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 200 cattle. Cattle that are used as blood donors will be re-used, with each separate use recorded as one procedure: therefore, the numbers of recorded procedures will be much higher (estimated at >3500 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 animals used will be used for blood sampling only; this is a mild procedure with no expected adverse effects. These animals may be kept alive at the establishment (with the agreement of a vet) and may be re-used on procedures if appropriate and licenced. 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. A small number of these animals may be culled by an overdose of anaesthetic drugs or by captive bolt. Some animals will undergo surgical procedures that will be performed under general anaesthesia; animals will be given pain relief following the surgery. Animals will have a small tube inserted into a lymphatic vessel (these carry cells from the tissues) that exits from the closed surgical site. This tube is attached to

a bottle in which we will collect fluid and cells. We will inject vaccines into the skin above the surgical site and measure changes in the cells and fluid. We will check the animals carefully for any alteration in their behaviour (e.g. alertness, tiredness), food and water intake and breathing. If we see alterations animals will be treated by a vet as appropriate. A small number of these animals may later be given a pathogen which could cause disease. We will monitor them as above. If the monitoring shows changes we will treat the animals to clear the infection. Surgically prepared animals may be housed individually: this reduces the chance that the tubing will be pulled out. However, all calves will be able to see other animals and be housed next to each other. At the end of these experiments we will kill the animals as above. Other animals will be injected with vaccines and/or be infected with bacteria that could cause disease. Infection will be either by delivery of bacteria into the lungs using a flexible tube, or by giving bacteria in food/milk to allow them to reach the gut. However, we use doses of bacteria and timescales that result in mild effects (if any at all) and clinical disease is not seen in these animals. These animals will also be blood sampled. All of these animals will be monitored for alterations in behaviour as detailed above. Where we see changes a vet will be consulted and animals may be treated or culled by the same methods as above.

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 the use of cattle. There are no good alternatives that can be used: mice can be infected with Mycobacteria but they do not have the disease outcomes as cattle and cell lines are not available to use in the lab. However, where we can we will use cells derived from blood and tissues to mimic responses that occur within animals. For example, we can use new technology to generate ‘mini-guts’ in the lab to look at the early interactions of the bacteria that Johne’s disease with the gut or we can use blood derived cells to understand how TB causing bacteria ‘hide’ from the immune response and cause infection/disease.

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

Cattle are the natural hosts for the pathogenic organisms being studied and no accurate models are available. Vaccination and challenge studies will enable us to pinpoint critical factors associated with the induction of immunity, or the progression to infection. We can define both immune and genetic factors enabling well defined experiments to be carried out.

The majority of procedures will be of mild severity. Following surgical procedures analgesia will be provided and we have refined the post-operative care that calves receive; for example, we now only inject an anti-coagulant once a day for 3 days which reduces stress to the animals (who might previously have become needle-shy with 2x daily injections for up to 28 days).

All animals undergoing infectious challenge will be monitored at least twice daily using well-defined clinical scoring criteria. This will allow us to continually monitor the wellbeing of the animals to minimise harm.

PROJECT 29: PROTEOSTASIS: MECHANISMS, DIAGNOSIS AND TREATMENT OF DISEASES OF PROTEIN HOMEOSTASIS.

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.

Word limit; 1000 words

Project Title	Proteostasis: mechanisms, diagnosis and treatment of diseases of protein homeostasis.
Key Words	transgenic and knockout mice, disease models, therapy, diagnostics, amyloidosis, amyloid
Expected duration of the project	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;
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):

Proteins are very large, complex molecules that carry out many different functions in the body. Examples include haemoglobin, which carries oxygen in the blood, and antibodies, which fight infection. All proteins are made within cells and some, like haemoglobin, remain inside cells. Others, like antibodies, are secreted from cells and circulate in the bloodstream. Each protein is made of a long chain of amino acids, which folds into a very precise structure. Sometimes, proteins don't fold properly and, rather than circulating freely, they can clump together and get stuck within tissues. Not only does this prevent the misfolded proteins from performing their normal functions, they can also cause the affected organ (e.g. the kidney or the heart) to fail. In some diseases, misfolded proteins can be toxic. For example, in Alzheimer's disease, this prevents nerve cells from communicating properly with each other and eventually kills them.

Diseases of protein folding are difficult to diagnose, and patients often don't receive their diagnosis until they are very ill. Furthermore, in most cases, the best treatments currently available do not stop the disease from progressing, but only treat the symptoms of the disease. Thus there is a pressing need for better methods for diagnosis of diseases caused by protein misfolding, and for treatments to prevent the accumulation of misfolded proteins.

Test tube experiments have provided us with many insights into protein misfolding, but they do not mimic the vastly more complex situation that exists in the body. There are some promising possible treatments that are emerging but, in many cases, there is no suitable animal model in which to test potential treatments.

The aims of this project are to investigate reasons why proteins misfold and accumulate in the body. This will be achieved by breeding mice with different genetic alterations that alter the way the body processes proteins. The choice of experiments will be guided by results of test tube experiments that are being conducted in parallel. Identification of key factors that normally prevent accumulation of misfolded proteins in the whole animal will help us to devise new approaches to treating the diseases and, at the same time, will provide us with animal models in which to test possible diagnostic methods 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)?

This project will allow the continuation of a productive ongoing programmes of research into life-threatening diseases, the main focus of which are the understanding of disease processes, and the creation of new or improved models of protein misfolding diseases. The availability of these models is essential for further work (to be done under different project licences) to develop improved methods to diagnose disease and evaluate disease progression, and to develop and test new potential treatments. We have very close links with leading clinicians, and findings that have arisen from work undertaken under previous licences are now being translated into the clinic, including in major clinical trials sponsored by a large pharmaceutical company. As well as providing basic scientific knowledge, we fully expect that findings emerging from the work to be undertaken under this licence will lead, at least, to further clinical research. We anticipate that the results of this work will lead, in the medium term, to changes to clinical practice and hence to patient benefit.

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

All of the work undertaken will employ laboratory mice, the majority of which will be genetically altered. The total number of mice used is expected to be fewer than 22,000, 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 large majority of the animals will be used for breeding to maintain existing genetic alterations and to produce mice that have particular combinations of genetic alterations. Most of these mice will experience no adverse effects over and above those inherent in normal, best practice husbandry. A small proportion of the mice will experience transient discomfort on one or a small number of occasions (e.g. from injections or collection of blood samples). A small minority of the mice to be used will experience moderate adverse effects, e.g. following surgery to re-implant manipulated embryos; appropriate steps will be taken to alleviate such adverse effects e.g. by providing pain relief. For the most part, the mice will be killed

humanely when no longer required or if necessary, because of ill-health. Some mice will be provided to other projects for use in experiments to be conducted under the authority of different project licences.

Application of the 3Rs

Replacement

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

Replacement

Although increasingly sophisticated cell culture and computer models are being developed, none individually or in combination are capable of adequately modelling the enormous complexity of the whole animal. Work in non-protected animals is suitable for many lines of research, but there are many others for which they are unsuitable. The work to be undertaken under the authority of this licence will be limited to those areas of research where there are no suitable alternatives.

The research questions supported by this licence involve multiple cell types and multiple organs. The animal experiments follow extensive work in test tubes, which provides very important direction for the animal experiments. In some cases, findings from animal experiments are expected to provide key information that will provide direction for further test tube experiments .

Reduction

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

Reduction

The number of animals to be used is mostly determined by the need to breed mice that have specific genetic alterations, including combinations of different genetic alterations. The breeding programmes will be designed carefully to minimise the numbers of mice that need to be bred, for example by breeding through more generations than the minimum possible; this is particularly relevant when combining different genetic alterations in individual animals.

Wherever possible existing genetically altered stains will be sourced from other laboratories, rather than generating new strains. When the generation of new strains is required, we will use the most efficient method compatible with the requirements of the experiments; we have a long track record of successful and efficient production of new genetically altered mice.

The recently developed CRISPR technology allows the production of some classes of genetically altered strains with greater efficiency, thereby requiring the use of fewer animals. Similarly, the high efficiency of this method can allow the

simultaneous production of multiple different genetic alterations in a single step, in contrast with separate production of each individual alteration and multiple generations of breeding. CRISPR will be used where appropriate.

Some strains of mice will be of use for more than one research question. Wherever possible, material collected from individual animals will be used to satisfy the needs of as many different research projects as feasible (in particular, there is opportunity for control animals to feed into different experiments). As far as possible and when appropriate, material will be collected for analysis from mice that have been used for breeding, rather that breeding animals specifically for analysis.

Where genetic background is not a key issue, breeding will use F_1 hybrid mice, which are less prone to infertility and have superior breeding performance to inbred 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

The laboratory mouse is by far the most versatile vertebrate species for research using genetic techniques. The field is mature, with comprehensive resources freely available, including many thousands of different genetically altered strains, knowledge of the complete sequence of the mouse genome, and many others. The techniques of genetic manipulation are very well established, and continue to be improved, and the techniques that will be used will be those that are the least severe that are compatible with the scientific purpose. For example, rather than using the traditional abdominal approach, vasectomy will be performed using the less invasive scrotal approach. New genetically altered lines of mice will be carefully monitored, and end-points will be applied in order to avoid unnecessary suffering.

Where possible and compatible with the scientific aims, the breeding will be performed to generate the mice that are required in such a way that genetic testing is minimised (and with as little excess as possible).

Husbandry practices will be kept under constant review and best practice will be followed, including the provision of environmental enrichment, e.g. tubes, houses, mirrors and running wheels. Millet seeds strongly encourage foraging behaviour, and will be provided when appropriate, e.g. to reduce anxiety when mice are being inspected. When possible, female mice that are past breeding age will be provided as companions for male mice that are unavoidably individually housed.

Where genetic background is not a key issue, breeding will use F1 hybrid mice as these are healthier than mice of inbred strains.

PROJECT 30: PRECLINICAL RESEARCH OF NEW THERAPIES FOR FILARIASIS

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.

Word limit; 1000 words

Project Title	Preclinical Research of New Therapies for Filariasis
Key Words	Lymphatic filariasis, Onchocerciasis, River blindness, elephantiasis, veterinary filariasis
Expected duration of the project	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;
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):

Filariae are thread-like parasitic worms that infect humans and animals. Filariae cause the disabling diseases, river blindness and elephantiasis. In dogs, filarial infection causes heartworm disease, which is potentially fatal. Heartworm can also cause disease in humans. Filariae induce disease when the worms die in the body, provoking an inflammatory reaction and collateral damage to the eyes, skin, lymphatic system or heart vessels. Over 150 million of the world's poorest populations suffer with filarial disease. Veterinary filariasis is spreading in America and Europe due to the effects of climate change.

Whilst there is no vaccine to protect humans or animals against filariasis, there are drugs that work to block the spread of infection between people or prevent infection in dogs. These drugs are donated by pharmaceutical companies and are distributed frequently to large populations in order to reduce filariasis or even eliminate it. This approach has failed to eliminate disease in certain situations. Two important reasons for this are: 1. There are signs that the filarial parasites have developed drug resistance and 2. People can suffer a range of inflammatory adverse reactions to current drugs causing reduced adherence. Drug resistance is also a problem for preventative treatment of heartworm in dogs.

Another problem with current drugs used to stop the spread of filariasis is that they do not alleviate suffering of an estimated 40 million elephantiasis patients.

Our aims are to develop new drugs that are safe and can cure filariasis with a single administration of up to seven days. We will also research the events in the body that trigger elephantiasis and test drugs that may block these disease-causing pathways. We will test whether co-treatment with anti-inflammatory drugs may alleviate the

worst adverse reactions to current treatments. Finally, we will develop new research models that can be used to replace, reduce or refine anti-filarial animal testing.

What 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 Successful applications for ‘Investigational New Drug’ status will mean that the most promising new anti-filarial drug candidates can then be tested in ‘first-in-human’ studies to evaluate safety. Clinical studies could then extend into patient populations in Africa to fully evaluate efficacy. Ultimately, the goal of full registration of a new cure for filariasis may stem from the necessary preclinical work in this project. Such a drug cure may dramatically reduce the current timeframe to achieve elimination of filariasis, potentially within the ambitious 2030 target set by the United Nations as part of the Sustainable Development Goals. The project will also provide preclinical evidence to justify repurposing of existing, affordable registered drugs for the treatment of elephantiasis pathology or adverse reactions to current treatments of filariasis. This evidence base will be used to support grants for clinical trials to assess the clinical benefit of repurposing such drugs for human filariasis. Finally, the project will attempt to develop new preclinical models of heartworm. If successful, the models will be made available to veterinary health companies to test new treatments for heartworm. The project may also use these new models to assess whether the drugs under development for human filariasis will also be effective against veterinary filariasis

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

We will use approximately 500 gerbils and 3500 mice over the duration 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?

It is necessary to experimentally infect animals with filariae both to generate parasites to screen early stage drug candidates outside of the body but also to test the efficacy of the most promising drugs within animals. Most infection procedures will not cause overt signs of ill health in the animal. Some infection procedures are designed to induce a degree of disease or inflammation in order to test whether therapies modify these pathologies. It is possible some ill health may arise in a minority of the animals on these procedures. Animals will be carefully monitored and where this becomes apparent to a degree of ‘moderate severity’, which includes subdued behaviour and/or weight loss, the animal will be humanely destroyed. All animals at the end of the study will be humanely destroyed.

Application of the 3Rs

Replacement

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

Replacement

Filarial parasites have a complex life cycle and require a mammalian and insect host to develop and reproduce. Filariae do not survive long-term and are not reproductively active in culture outside of the body.

Drugs are absorbed, distributed, metabolised and excreted by the body in a complex dynamic which cannot be precisely emulated by replacement techniques. This necessitates using animals to determine how drugs mediate pharmacological effects on filarial parasites.

Assessment of filarial disease requires a whole organism approach to appropriately model the complex effects of multiple cell types and pathological tissue changes at the site of inflammation or infection.

However, we will first test drugs in cell culture and molecular assays and use a predictive mathematical model to predict efficacy; only those compounds meeting strict criteria for efficacy, pharmacokinetics and safety will be will be advanced for testing on animals.

Reduction

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

Reduction

We have used existing data to assess the minimum group sizes needed to test with a degree of statistical rigour whether the drug compounds work against filariae or filarial disease.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 mammalian group that are susceptible to filarial infection.

Experimental durations are constrained to limit the extent of disease and avoid overt signs of ill health. However, it is possible some ill health may arise in a minority of the animals. Animals will be carefully monitored and where this becomes apparent to

a degree of ‘moderate severity’, which includes subdued behaviour and/or weight loss, the animal will be humanely destroyed.

In invasive procedures where a degree of pain is expected, the procedure will be undertaken with general anaesthesia and appropriate painkillers to minimise suffering. We will aim to reduce the total number of invasive procedures by evaluating and adopting non-invasive alternatives, including bioimaging.

PROJECT 31: ANTIBODY, BLOOD PRODUCTS AND TISSUE ON A SERVICE BASIS

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.

Word limit; 1000 words

Project Title	Antibody, blood products and tissue on a service basis
Key Words	Antibody, Polyclonal, Monoclonal, Pre-Treatment
Expected duration of the project	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;
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 objective of this licence is to:

1. Provide a service for the production of biological material in the form of monoclonal or polyclonal antibodies and/or blood products for the medical research community and diagnostic manufacturing industry within the UK.

2. Provide a pre-treatment service for the preservation of cells, tissue and organs. For example, in the preparation of Cardiac Myocyte isolation.

Antibodies are produced by the immune system of a living organism and play an integral role in Biology in terms of their ability to fight infection by a host of organisms deemed foreign to self, their ability to detect life threatening diseases (fora and flora), and their use as critical tools in the areas of medical research, diagnostic technology and therapeutic medicine development.

Heart failure is a serious condition in which the heart does not pump blood around the body at the usual pressure because it has become too weak or stiff.

The most common causes of heart failure are a heart attack, high blood pressure (called hypertension)or diseases of the heart muscle that cause weakness (called cardiomyopathy). As we age, high blood pressure causes heart failure. The Tissue harvest pre-treatment service will enable the assessment of some scientific questions relating to fundamental research which cannot be addressed without the use of animal tissue. For example, the use of primary rat cardiac myocytes to assess drugs that could improve excitation-contraction coupling, a process that

enables the chambers of the heart to contract and relax. Rats will be treated with anti-coagulants prior to tissue harvest. This procedure will prevent the formation of blood clots within the ventricular chambers, in order to obtain appropriate samples 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)?

Antibodies routinely help scientists to research the function of both health and abnormal cells in disease, by the detection of proteins within the cells at various stages of its development. This is routinely utilised when researching disease and its prevention. In the field of diagnostics antibodies play a critical role in the detection of disease in the clinical environment. This can allow the rapid diagnosis of life and environmental threating diseases. Heart failure is a common, costly, and potentially fatal condition. In the 2014 it was the second most common cause of death, 27%, in the UK. The disease was the reason for 10% of emergency hospital admissions in 2014. The number of hospital episodes has been increasing in all UK nations in recent years. Information gained could contribute towards lower these percentages.

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

Mice 280 (5 years) Rats 250 (5 years) Guinea Pig 70 (5 years) Rabbit 170 (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 production of custom antibodies requires the use of live animals, to which a substance, called an antigen, is introduced to produce an immune response. To assist in the development of an immune response to an antigen a substance known as an adjuvant can be used in conjunction with the antigen to assist in the further stimulation of the immune system and subsequent production of antibodies. Some adjuvants which are very effective in stimulating an immune response can cause tissue reactions in the animals at the site of injection, therefore the use of these adjuvants is carefully controlled, with any reaction being closely monitored. Subsequent blood samples will be taken from an animal in order to test the level of antibodies being produced within the animal. These blood samples will be taken from an appropriate collection site on the animal such as veins/arteries and as such can (but rarely) lead to the formation of bruising and slight skin damage. Upon reaching a desired level of circulating antibody to an antigen an animal will be moved forward for exsanguination where animals are given an anaesthetic from which they are not

allowed to recover and their blood is collected to provide the antibodies. When this has been done the animal is humanely killed and further tissues may be collected for scientific use. Although significant adverse signs within any animal used for the production of antibodies are not expected full veterinary attention will be provided should there be any unexpected consequences of any procedure carried out. For the preservation of cells, tissue and organs rats and mice will be treated with various substances, for example, hormones and anti-coagulants prior to being killed. The dose levels to be administered should produce no toxic effect to the animals. Only transient pain at the point of injection is expected. Injection sites will be monitored for any signs of infection (rare). All procedures conducted by well-trained and experienced licensed animal technicians with the ability to handle animals sympathetically. All animals used under the authority of this licence are subject to well defined humane endpoints, which if experienced will result in the animal being removed immediately from the study.

Application of the 3Rs

Replacement

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

Replacement

At the time of writing this licence there are no alternative (non-animal) methods for the production of blood serum containing a wide variety of antibodies to various targets or the production of specific antibody secreting cells.

Alternative systems for pre-treatment, for the preparation of Cardiac myocyte isolation were developed to help reduce the use of animals (embryonic cell lines, stem cell-derived myocytes, computer modelling), none of these methods can supply cells that resemble the genotype and phenotype of adult cardiac myocytes well enough.

Reduction

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

Reduction

The use of appropriate species and methodology will ensure the production of better quality and a higher number of antibodies, therefore reducing the number of repeat production programs required where use of additional animals would be needed. Our expertise and experience in this area allows us to provide guidance on best practice from the beginning, this includes the selection of appropriate species based on the

substance to which the antibodies are to be raised against, the way in which the substance will be introduced to the host and the schedule of inoculations to be followed. With all of these considerations we can ensure that the minimum number of animals are used for each and every project undertaking.

Refinement

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

Refinement

A variety of species (Rabbit, Guinea Pig, Rat, & Mouse) will be made available for this project. Selection of a specific species will be made following the careful consideration of a number of factors, both ethical and scientific. Our experience in this field allows us to make ethically sound decisions based on knowledge and expertise as well as ensuring the highest levels of care and attention are afforded to all animals utilised in this project. Our production protocols are designed with the principles of minimal severity and are under constant review to ensure best practice is followed at all times, whilst also keeping abreast of new and refined techniques/technology utilised in this field.

PROJECT 32: CHANGES IN THE FUNCTION OF THE AUDITORY SYSTEM FOLLOWING HEARING LOSS OR THE INDUCTION OF TINNITUS

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.

Word limit; 1000 words

Project Title	Changes in the function of the auditory system following hearing loss or the induction of tinnitus
Key Words	Auditory system, tinnitus, hearing loss
Expected duration of the project	2 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;
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 aims of this project licence are to advance our understanding of how the brain processes sound, how this processing is affected by hearing loss and tinnitus, and to test potential treatments for tinnitus.

What 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 from this research are to guide future treatments for both the communication problems associated with hearing loss and tinnitus. Even people with mild and moderate hearing loss, where they can hear most of the relevant sounds in their environment, experience serious communication problems in noisy, social environments. This can lead to social withdrawal and isolation and may even contribute to other problems such as dementia. Modern hearing aids do not effectively address these communication problems. Unfortunately we do not understand why people experience such problems. We hope to uncover these problems by directly examining how the neurons in the brain which process sounds are affected by hearing loss. Making significant progress in this respect would enable hearing aid development to target these problems, whereas currently they cannot. Currently there is no effective cure for tinnitus. Progress in treating tinnitus is hampered by the fact that the mechanisms behind it are essentially unknown. This project could advance this understanding, but it also seeks to test several treatments which could ameliorate the symptoms. Thus we will test two classes of drugs that we believe may reduce the symptoms of tinnitus.

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

140 adult guinea-pigs over two 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 be exposed to loud-sounds in one ear, while deeply anaesthetised, in order to give them mild to moderate hearing-loss or as a means to induce tinnitus. The anaesthesia prevents any pain but the long period of anaesthesia, during which hearing thresholds are measured before and after noise exposure, will be stressful. Occasionally an adverse reaction or complications during the long period of anaesthesia may mean that an animal has to be humanely killed before it has recovered. Some of our animals will develop tinnitus which may be mildly distressing. We will seek to abolish the symptoms of tinnitus by trying out candidate drugs but these drugs may have unexpected adverse effects in a few individual animals and produce a fall in blood pressure or imaginary flashes of light in the retina. Many recordings from neurons are made under non-recovery anaesthesia, so they remain unconscious and cannot feel pain throughout the procedure. Some animals will undergo surgery under general anaesthesia to implant recording electrodes (similar electrodes have been implanted in humans for medical reasons), so we can measure the responses of neurons to sound when animals are awake. This important step allows us to monitor the responses of their brain as tinnitus develops. The brain has no pain receptors but the scalp does and pain killers will be given during and after surgery. The maximum level of severity expected is moderate, but this would only be for short periods. For most of the time animals will experience little or no discomfort. We will maintain the highest standards of care during these experiments, for ethical reasons and to ensure that data are of the highest possible quality. At the end of the experiments 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

The responses of neurons to sound can only be studied in an intact brain connected to the ears. Likewise tinnitus is a conscious percept that almost certainly results from an interaction between the intact ear following hearing loss, and the consequent changes in the processing by the brain. This means it is not possible to conduct these experiments in vitro. Where possible, we use computer simulations of the auditory system in place of animals. This is to some degree possible for cochlear responses, for which fairly accurate models exist, and we do this. However for most regions of the brain which process sound, we still need to record from real neurons.

This project is complemented by other work which does not use animals – development of computer models, non-invasive studies in humans.

Reduction

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

Reduction

Where possible (i.e. for the cochlea) we will be using computer simulations instead of experiments. These models are also used to develop our experimental paradigms for when we record from the brain – this reduces the number of pilot studies required to refine the experiments. We also adopt the best and latest technology for recording (allowing us to record from many neurons at once). When comparing the activity of neurons in the brain or the behaviour of animals, with and without tinnitus, we perform power calculations to determine the minimum number of neurons we need to record from or animals we need to dose with a drug in order to show a statistically significant effect. Thus we don't perform more experiments than is necessary. By only deafening one ear it means that we can compare the activity of neurons on the deafened and non-deafened sides simultaneously in one animal instead of needing two animals – one deafened and one not.

Refinement

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

Refinement

Guinea-pigs are an ideal animal as although small, they have excellent low-frequency hearing. This is necessary for understanding how sound in the human speech frequencies (which are low) is processed. Mice, and rats have poor low frequency hearing, which we know operates quite differently. The physiology of fish, amphibians and birds are quite different to mammals, and difficult to relate back to humans.

All involved in this program of research place great emphasis on the welfare of the animals. They live in a state of environmental enrichment, of social interaction, exercise and stimulation. We carefully monitor the health of our animals and always seek the help of the Named Veterinary Surgeon whenever there are any concerns. He maintains close oversite of our surgical procedures and prescribes drugs such as pain-killers or antibiotics administered to support animal welfare. The procedures themselves are developed specifically to minimise the suffering of pain, distress or discomfort. Recovery surgery is all performed in a specially designed surgery suite of three rooms with state-of-the-art equipment including an operating microscope

designed for human surgery and with full aseptic precautions using a team of at least two highly trained staff along with a trainee. Terminal experiments are also all performed with two people and aseptic precautions including sterile gown, drapes and instruments. If any animal shows unusual levels of distress or looks as though it will suffer from a prolonged period of more than mild discomfort, it will be humanely killed with an anaesthetic overdose after the responsible staff have been consulted. This ensures both high standards of welfare and valid experimental results that can contribute to the understanding and treatment of auditory disability.

PROJECT 33: ROLE OF AUTOPHAGY DURING TUMORIGENESIS

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.

Word limit; 1000 words

Project Title	Role of autophagy during tumorigenesis
Key Words	therapy, brain, tumour, cancer, autophagy
Expected duration of the project	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;
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):

Autophagy, or self-eating, is a cellular degradative mechanism that is frequently activated during tumourigenesis. How autophagy affects tumour progression and formation is controversial. The aim of this project is to understand how autophagy influences tumour biology and can thereby be targeted during treatment. To do so, autophagy will be specifically inhibited genetically in various models of cancer with emphasis on glioblastoma, the most common and aggressive form of brain 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)?

The outcome of this project will not only enhance our understanding of the pathological relevance of autophagy, but it is also hoped to have direct clinical impact on treating disease particularly aggressive cancers.

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

The project requires the use of approximately 11000 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 main adverse effect of this project is tumour formation which will be induced in a way to closely resemble what is observed in the clinic. Mice will be monitored closely for signs indicative of tumour formation and will be culled immediately to minimise their suffering. Veterinary surgeons and experienced animal care staff are always available for advice and help since the welfare of the animals is of major concern to us. All mice are culled 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

Various aspect of the tumour biology, such as tumour microenvironment, hyperplasia and metastatic potential, can strongly influence tumour growth and response to treatment in patients. While some of these aspects can be mimicked using in vitro studies using cell culture systems (e.g. anchorage independent growth in soft agar, oxygen and nutrient withdrawal, and transwell migration assays), accurate modelling of tumour growth cannot be recapitulated using non-animal systems. This is especially important when studying the process of autophagy which is frequently activated under many of the conditions observed in vivo.

Reduction

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

Reduction

We will utilise mouse models that ensures highest tumour incidence. Pilot experiments will help optimise the number of mice used and time points. The experimental group size will be determined in order to obtain sufficient numbers of tumours of a measuable size suitable for statistical analysis. We will use genetic systems to introduce genetic alterations (e.g. inhibition and/or activation of cancer-associated pathways) in postnatal animals that avoid the need for breeding thereby reducing mice numbers (breeding of different genetic backgrounds results in variable offspring genotypes some of which are not suitable for experimental 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

We have chosen mice to use as they are the animals that are most similar to humans that are readily amenable to genetic modification – this means that, for us, they are the best model to answer our questions about cancer development and therapy. Of specific research importance are cancers of unmet clinical need including agressive brain tumours. Researchers have developed the most refined models to study brain tumours and patient-derived gliomas are one of the few human cancers for which we can isolate, culture, and genetically manipulate primary cells as well as their genetically "normal" counterparts.

The protocols are designed to generate as much information as possible from as few mice as possible with the least harm possible. They are optimised to obtain efficient

tumour formation with minimal surgical intervention and animal discomfort. Anaesthetics are used for any procedures that would be expected to cause temporary discomfort. We are experienced in most of the protocols listed and know them to create minimal suffering. Where we intend to perform a protocol that we are less familiar with, we do so in consultation with the veterinary surgeons so that suffering can be minimised.

PROJECT 34: SENSITIVITY OF THE LENS EPITHELIUM TO IONISING RADIATION AND CATARACTOGENESIS

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.

Word limit; 1000 words

Project Title	Sensitivity of the lens epithelium to ionising radiation and cataractogenesis
Key Words	Cataract, Lens epithelium, Lifespan
Expected duration of the project	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;
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):

To investigate the effects of low dose ionising radiation on the lens of the eye at clinically and occupationally relevant doses to humans, using multiple strains of mice including sensitive ones, to establish the lowest dose at which changes leading to cataract can be detected. We aim to identify specific mechanisms and pathways involved in the early stages of cataract progression, using low doses of ionising radiation for occupational and clinical dose comparison, as well as using a highest dose of 2 Gy for long term studies and endpoints as a positive control not only for evidence of early biological changes, but as a control for the limited lifespan of a mouse. We hope to identify a dose response and to compare this with limited data from 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)?

The aim here is to further understand what biological events lead to radiation-induced cataract and also to develop a rapid, non-invasive method to analyse humans so that data on cataract risk in exposed individuals can advise future radiation protection guidance and shielding. Also at what threshold, if any, should these be applied? The research team working on this project licence have already established the foundations for analysis of both early radiation-induced changes (studying the lens epithelium for a number of changes) and long term effects (using live lens imaging). The expected outcome from this project to understand further early influences of ionising radiation on the damage response of lens cells, as well as subsequent effects on cell growth and division. As mentioned above, preliminary results suggest an effect at lower doses that previously thought, an area we will focus our research on.

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

Mice. Approximately 2,800 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?

The overall radiation dose levels are fairly low (maximum dose 2 Gy), however with animals followed for lifespan and the use of sensitive mice, a greater level of incidence of mammary tumors will likely occur, including in un-exposed mice. Daily health checks (including weekends) are performed on all animals, allowing for early identification of sick animals, and allowing time for humane termination. Imaging of the lens is non-invasive, with procedures lasting under one minute per observation. Some stress is expected during restraint of conscious animals. Mice under anesthetic during observations would also incur stress on induction and recovery from the anesthetic. Non-recovery after anesthetic is unlikely due to the short term nature of the procedure. Therefore the severity limit of this Project Licence is moderate.

Application of the 3Rs

Replacement

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

Replacement

The lens of the eye is an extremely difficult tissue to recreate *artificially* using cell lines. The epithelial cells that maintain and populate the lens are in various states of cell cycle and their maintenance is tightly controlled, with distinct regions of cells based on their different roles. Cells in culture to not demonstrate this behaviour, and show an abnormal response to DNA damage such as that caused by radiation. In addition, it is very difficult to obtain human lens samples, and difficult to isolate the correct cells from human organs to produce stable and uncontaminated cell lines.

Reduction

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

Reduction

Group sizes of animals used at each dose and time point, including controls are determined based on statistical calculation by our research team statistician to ensure our findings will be significant. Eight lenses per dose and time point is optimum. The use of inbred mice reduces inter-individual variability, with previous research identifying C57BL/6 and BALB/c mice to have particularly low variability

compared to other similar strains, hence their use. We aim to make use of tissues besides only the lenses to maximize experimental output. Various organs and blood lymphocytes may be taken for comparison by some of our collaborators to maximise scientific gain and output from killings, and we are aiming to establish a technique for histology of the lens where multiple endpoints can be analysed together. Once established this technique could further 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

The lenses of rodents and primates are very similar to that of humans. Rodents are a proven useful model for the study of human cataract development, and the use of the mice to assess early changes in the lens which are likely to be indicators for cataract formation is well established. We will use sensitive strains of mice as a positive control, as these will be more susceptible to cataract induction following radiation. As the life span of mice is significantly shorter than that of humans, sensitive strains are more likely to develop cataract and can be related to human studies. REDACTED therefore we will use a maximum dose of 2 Gy to ensure cataract formation and maximum useful data is collected. After eye dilation, animals will be kept in the dark up to one hour post-procedure to ensure full recover with minimal stress.

PROJECT 35: DIRECT INTRACRANIAL DRUG DELIVERY FOR THE TREATMENT OF NEUROLOGICAL DISEASE.

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.

Word limit; 1000 words

Project Title	Direct intracranial drug delivery for the treatment of Neurological Disease.
Key Words	Drug delivery / Neurological Disease / Convection Enhanced Delivery
Expected duration of the project	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;
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;
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 aim of the work conducted under this licence is to undertake developmental work and preclinical evaluation of treatments delivery directly into the brain by Convection Enhanced Delivery (CED) for the treatment of tumours and neurodegenerative conditions. The work is an essential step in advancing and improving the treatment given to patients within the NHS.

The specific objectives of the studies are to:

1. Optimise the design of catheters used for drug delivery.
2. Develop methods to enable drug distribution to be visualised using established medical imaging systems.
3. Assess the effectiveness of treating brain tumours by CED .
4. Assess the effectiveness of treating neurodegenerative conditions by delivery of therapeutic agents by CED.

What 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 drugs are prevented from entering the brain due to the low permeability of the blood vessels that pass through it, a feature know as the 'blood brain barrier'. As a consequence, the range of drugs that can be used to treat conditions affecting the brain is more limited than for other regions of the body and often restricts the effectiveness of treatment. Convection Enhanced Delivery (CED) is a method of delivering drugs to the brain that has been developed to overcome the restrictions imposed by the blood brain barrier. The purpose of the work is to enable new treatments and advances in the process of CED to be tested in order that they can be progressed into clinical trials within the NHS. A major focus of the work is to

assess the effectiveness of CED for the delivery of drugs to treat incurable brain tumours and neurodegenerative diseases. In so doing the work will facilitate the rapid translation into the clinical setting of advances in treatment for currently incurable brain diseases.

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

Over the five years duration of this licence we estimate use up to 1500 rodents and 100 pigs and 60 sheep. Some rodents may have genetic alterations that cause them to develop neurological defects.

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

Convection Enhanced Delivery (CED) involves surgery conducted under general anaesthesia. Following surgery animals will be given analgesics to minimise any pain. Animals may receive further anaesthetics to either repeat the treatment or to enable medical imaging to be performed in order to evaluate the effectiveness of the treatment. These procedures are classed as moderate. In all cases the animals are expected to make a rapid recovery and return to normal behaviour within 24 hours. At the end of the study the animals will be humanely killed and tissue samples taken to determine the outcome of the study. Whenever models of progressive neurodegenerative disease are used humane endpoint criteria will be set to ensure that the animals are killed as soon as it becomes clear that disease is developing.

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 work conducted under this licence is to facilitate the transfer of advances in treatments delivered by Convection Enhanced Delivery (CED) into the hospital setting. In order to achieve this aim the safety and efficacy of these approaches must be first shown in a relevant animal model. It is not possible to undertake these studies in a non-protected animal as the work can only be performed in an animal with a functional brain. Rats are the smallest laboratory animal suitable as the brains of mice are too small to allow meaningful data to be obtained. Prior to progression into human trials confirmatory studies will be undertaken in pigs, because even the brains of rats are far too small to enable the procedure in humans to be replicated in a meaningful manner. Prior to undertaking any *in vivo* studies extensive *in vitro* and *ex*-vivo testing will be undertaken to determine the safety and efficacy of our approach and only

developments shown to be effective and safe will progress into animal studies. These tests will include assessing the distribution of the agent being delivered using agarose gel brain phantoms and/or the brain tissue of animals killed by a Schedule 1 method. In addition the safety and efficacy of all novel therapeutics will be assessed using a tissue culture system and only those shown to be safe and effective will progress to *in vivo* studies. Our *in vitro* testing includes a programme of work utilising a Microfluidic test bed for the fast prototyping of nanoparticle distributions in tumour-like environments and the data generated in these studies will be used in the development of a computational model of drug distribution within tumours.

Reduction

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

Reduction

In order to minimise the number of animals used, at each stage of the project, the experimental protocol will be designed with carefully defined objectives and the group sizes kept to the smallest number needed to enable statistical analysis.

Whenever possible, duplicate infusions will be made into both sides of the brain. This enables quantitative comparisons between control and treatment using the same animal thereby minimising variability and ensuring that the number of animals used is kept to an absolute minimum. The number of animals in each group will be determined using power calculation based either on data from previous studies or pilot 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 aim of the work conducted under this licence is to facilitate the translation of advances in CED catheter and treatments into the hospital setting. Consequently the techniques used for CED throughout this study have been refined in line with best clinical practice, as used within the NHS, and the volumes and rates of infusing drugs kept to levels known to cause negligible tissue damage. For all disease models clear endpoints have been set that will ensure that prompt action is taken to minimise suffering. All surgical procedures are conducted under a deep plane of anaesthesia all animals will be given analgesics before they recover to provide post-operative pain control. In all case animals undergoing surgical procedure are expected to return to a normal state within 24 hours. Preliminary studies will be conducted using rats.

PROJECT 36: SAFETY EVALUATION IN DOGS AND PIGS

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.

Word limit; 1000 words

Project Title	Safety Evaluation in Dogs and Pigs
Key Words	Non-clinical, Safety, Dog, Pig
Expected duration of the project	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.
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);

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):

This project contributes to the safety evaluation of new medicines, agrochemicals or other chemicals to which humans are likely to be exposed, by investigating their toxicology and metabolism in two non-rodent species (dogs and pigs).

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

New medicines have the potential to provide new or improved disease treatments; new chemicals/agrochemicals have the potential to increase or protect food production while minimising safety risks to consumers and/or adverse effects on the environment. Before potential new medicines are administered to humans, or new chemicals/agrochemicals used in the environment, their safety must be evaluated. This testing is a mandatory legal requirement and provides information on risks to patients and consumers. At present there are no alternatives to animal use that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.

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

Dogs 5400, Pigs 4700 over 5 years. These estimates are based on historical usage under previous projects with the same overall aims, and on anticipated trends in regulatory and scientific requirements for safety/toxicity data in non-rodents.

In the context of what you propose to do to 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 dosed by the intended/likely route of human exposure (for example oral administration, injection, infusion or inhalation), and observed regularly to monitor appearance, behaviour and clinical health. Some animals may undergo a surgical procedure under general anaesthesia, eg placement of a deep vein catheter for intravenous infusion, or implantation of a monitoring device or minipump.

Investigative procedures carried out in these studies are similar to diagnostic procedures that might be used medically to monitor progress of a human patient and include, for example, collection of blood and urine samples for laboratory investigations, or ECG monitoring to assess heart rate/function, or examination of the eyes using an instrument similar to those used by opticians. Other more unusual tests might include assessment of retinal function, taking small samples of tissue under general anaesthesia, collection/examination of body fluids such as tear fluid or semen, collection under general anaesthesia and examination of lung washings or spinal fluid, body temperature by rectal thermometer. A minimal degree of restraint or confinement may be required for some procedures. Where appropriate, positive reinforcement training (using treat rewards) is used to encourage co-operation in (and minimise any stress of) handling/procedures. Environmental enrichments appropriate to the species, including provision of suitable bedding materials, toys, exercise and socialisation periods, are used within the animal facilities. Some animals may be used on procedure on more than one occasion (re-use); such re-use is limited and strict criteria are applied, eg veterinary examination indicates that it is appropriate to do so. Some animals (dogs only) may be re-homed via the establishment's rehoming scheme if it is in their best interests, but most animals are humanely killed at the end of the study to allow detailed examination of the organs. Most animals are expected to experience no, or only mild, adverse effects during the course of the study such as slight weight loss. A small percentage of animals may show more significant adverse effects, such as more marked weight loss, reduced activity, vomiting or tremors. No animals would be expected to die or to suffer prolonged adverse effects as a result of the procedures, and where necessary early humane end-points are applied, under veterinary guidance as necessary, to prevent this; such end-points might include interventions to discontinue dosing, or to provide supportive treatments, or if necessary to humanely kill the animal.

Application of the 3Rs

Replacement

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

Replacement

Testing pharmaceuticals, agrochemicals and chemicals to which humans are likely to be exposed is a mandatory legal requirement and provides information on the risks to people of such exposure. At present there are no alternatives to animal use that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.

Non-animal testing methods and computer modelling are used in combination with animal studies to inform study designs and assist in understanding of potential toxicity but cannot yet replace studies in animals.

We maintain a constant awareness of regulatory guidance and ensure that where non-animal methods exist which fulfil the regulatory requirement they are used in preference to animal studies.

Reduction

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

Reduction

Each study is designed to use the minimum number of animals necessary to achieve its objectives, drawing on scientific knowledge of the test item to be evaluated and of the animal model, on statistical considerations and on recognised guidelines on regulatory study design. Where appropriate, additional investigations may be included in a study in order to avoid the need to conduct a separate study with more animals. In some cases, re-using animals in a second study instead of using a new batch of animals is possible, and may reduce the overall welfare cost as well as 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

There is a scientific and regulatory need for safety/toxicity data in non-rodent species such as dogs or pigs to supplement rodent data and enable a complete risk assessment. We use pigs in preference to dogs wherever possible; dogs are only used where necessary to achieve the study objectives, ie when the pig is unsuitable (for example due to species-specific differences from humans, confounding pharmacology or toxicological responses, or practical limitations due to anatomy or physiology).

All procedures are subject to ongoing assessment and technique improvement and we participate in cross-company working parties on best practice. Animals are regularly reviewed for general health and veterinary staff are on call at all times to assess any adverse events and provide supportive care and treatment as appropriate.

Treatments shown to be effective in rat studies will be further assessed in pigs as the small size of a rats brain does not permit clinically meaningful data on drug distribution to be obtained.

PROJECT 37: MECHANISMS CONTROLLING CALCIUM DYSHOMEOSTASIS IN MALIGNANT HYPERTHERMIA SUSCEPTIBLE MICE

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.

Word limit; 1000 words

Project Title	Mechanisms controlling calcium dyshomeostasis in malignant hyperthermia susceptible mice
Key Words	Malignant Hyperthermia (MH), Heat stroke, Volatile anaesthetic, muscle, Calcium dyshomeostasis
Expected duration of the project	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;
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):

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.

PROJECT 38: REGULATION OF THE IMMUNE RESPONSE TO TUMOURS AND PATHOGENS

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.

Word limit; 1000 words

Project Title	Regulation of the immune response to tumours and pathogens
Key Words	Tumour, Cancer, Immune, vaccination, flu
Expected duration of the project	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;
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 aim of our project is to understand how tumours and pathogens escape from the immune system and to develop new immunization tools to combat these diseases. We are using the mouse tumour models in order to address therapies in a wide range of human tumours in clinical trials that we are about to initiate. The treatments that we develop in mice will be used in treatments for Melanoma skin cancer, Lung cancer, colon cancers and bladder cancer.

Cancer:

White blood cells of the immune system have the ability to kill tumour cells and protect the body from cancer. However, tumours can fight back; as tumours grow and try to avoid the body's immune system they change their surrounding in order to promote their own growth. In doing so, the tumours can suppress the immune system. For example, tumours can consume the nutrients in their surrounding area in order to starve invading immune cells. Tumour cells can also display molecules on their surface that instruct the immune cells to stop working. It would therefore be great if we could combine ways of boosting the immune response against cancer while at the same time prevent the tumours from fighting back.

Influenza (flu) virus epidemics:

Most vaccines against influenza virus rely on generation of antibodies in the body of the vaccinated person. These antibodies are proteins that adhere to the surface of the virus and neutralize it. Unfortunately, the surface-characteristics of influenza virus changes very rapidly and vaccines that generate antibodies to one influenza virus rarely protect people to a new influenza virus that has changed characteristics.

In addition to antibodies, the immune system has killer cells that can recognize, and kill, flu-infected cells thus preventing the virus from spreading and therefore eliminating the disease. This part of the immune system is slightly less susceptible to changes in the flu virus because it recognizes parts of the virus that change less frequently. This means that generation of an immune response to one flu virus is more likely to protect the person against other viruses. Vaccines that work best to induce such immune killer cells are “attenuated” viruses; they are alive but their ability to cause disease is disabled. The key is to develop a virus that is attenuated sufficiently so that it does not cause disease but it is viable enough to trick the immune system to think there is a real infection. We have developed such a vaccine and believe that it is safe enough not only to immunize in the normal injection route but also as an inhaled vaccine into the lungs where it is much more effective at raising a protective immune 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)?

1)Discovering new compounds that can be used in immunization to jump-start the immune system. 2)Identifying and understanding the ways that tumours fight back against the immune system. 3)Discovering compounds that can subdue the tumours’ ability to suppress the immune system. 4)Test new antitumour treatment before applying them to people. 5)Develop new vaccines against influenza virus.

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

Approximately 7,200 mice will be used per year over a five-year period. More than a third of these will simply be used for breeding and maintaining the many strains that are required for this research. The rest will be used to address many aspects of the aims described above

In the context of what you propose to do to 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 immunized by injections or inhalation of a vaccine, together with antibodies and/or drugs. The mice will then be injected with tumours or infected with viruses to test whether the vaccine treatment can protect them. In the majority of cases mice will only receive a few injections, and a few blood samples will be taken Tumour models: i) In most tumour experiments, tumour cells will be injected under the skin. Such tumours are not expected to cause harm since they do not metastasize or interfere with the animals’ natural behaviour. ii) Some tumours are injected into the bloodstream of the mouse. The development of these tumours is monitored by periodical blood sampling. The danger with these tumours is that they may get lodged in essential organs and disrupt the animals’ normal physiology. However, based on our knowledge of these tumours this is a very rare event, but the mice will be carefully monitored for signs of abnormal behaviour and appearance

during such experiments. iii) In some experiments melanoma cells are injected into the blood and 2-3 weeks later the appearance of tumours in the lungs is examined. These experiments are not allowed to exceed the time limit of 3 weeks. Very rarely animals may present problems breathing and when that happens they will be removed from the experiment and culled to prevent suffering. In addition, some of the mice that have tumours will be injected with a light-emitting compound. The mice will then be anaesthetised and their bodies scanned in an imaging machine. Since this is done under anaesthesia, the procedure will not stress the animals. However, repeated anaesthesia carries a small risk of anaesthetic death and we minimize this by carefully monitoring the animal's breathing rate and making sure that it is completely recovered before repeating the procedure. Influenza model: Infecting mice with influenza virus makes them ill. If the mice do not receive immunization they may lose up to 20% of their weight within 5-7 days. If this happens the mice will be humanely killed to prevent further suffering. It is necessary to produce this disease in order to see whether our vaccines provide effective protection. Other pathogens Some mice will be infected with other non-lethal pathogens such as vaccinia virus, attenuated adenovirus, or non-pathogenic strains of bacteria. Animals will experience symptoms equivalent to the common cold as the immune response kicks in, but these are all infectious organisms that are cleared within a few days, so symptoms disappear within 24-48 hours. Animals experiencing symptoms such as lack of movement or problems with breathing will be killed humanely. Irradiation In few instances animals will be irradiated to kill their blood cells and then replacing the cells with cells from another animal (bone marrow transplant). Animals which are irradiated in this way experience some discomfort as evident from a lack of grooming. Animals are also more susceptible to infections during the 3 weeks after irradiation and so are given antibiotics to prevent infection. During this time animals are kept under close watch to intervene if their health deteriorates. Injection of Immuno-modulating substances. In several cases, animals will be administered compounds that stimulate or inhibit the immune system. These may cause a short term (24-48 hours) general ill health that will resolve within that time. Animals will be observed closely for signs of ill health and will be humanely killed if symptoms persist. In some instances, the application of substances will be intranasal (done under short term anaesthesia), which may affect the animal's breathing rate. Animals are observed closely to make sure there is no immediate adverse effect. The same is true of intranasal administration of infectious organisms (attenuated flu vaccines).

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 our work is done without the use of animals. This is possible by using samples that we obtain from patients undergoing surgery or as part of the analysis of samples from patients in clinical trials. We also use cell lines that we grow in the laboratory conditions (in vitro) and allow us to some extent to mimic physiological behaviours.

Experiments that are done in mice are only done where they examine questions that can not tested outside a live organism.

The animal experiments are often designed on the basis of information that we assembled from computer analysis of published information and from data that we obtained from the laboratory (in vitro) studies.

We chose mice as an animal model to experiment with because mice are the lowest vertebrate group in the evolutionary tree from which suitable models of immune responses are available.

Many of the experiments are carried out in genetically altered mice that allow us to identify the role of specific genes in generating an immune response. At present, such genetic alterations have only been prepared in mice.

By using mice, we are also building on a vast body of information that has accumulated from laboratories around the world so that we can focus on our questions without having the "re-invent the wheel" by haveing the use less well studied organisms.

We use in vitro methods as far as possible, but these methods cannot reliably mimic the multi cellular environment that determines the action of the immune cells or neutralizing antibody.

In order to examine the usefulness of vaccination methods in the setting of the complex interactions between the tumour and the many types of immune cells, we have to use a whole organism.

Reduction

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

Reduction

Experiments in animals are done only after all other options have been exhausted.

For some T-cell activities we have in vitro assays which can be used to screen reagents such as antibodies and enzyme inhibitors, before we use them in vivo. This is done in order to whittle the number of reagents down and reduce the number of mice that are used.

We use the minimum number of mice that allows us to gain a clearly identifiable effect so that experiments do not need to be repeated. No animals are used that do not add to the conclusions we reach. To achieve this we minimise the number of animals that are bred. We consult statisticians in order to determine the minimal number of animals that need to be used for each experiment in order to get a statistically significant result. We also coordinate with our colleagues to make sure that mice that are not required by us can be used by others and vice versa.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 choosing an animal model we decided to use mice for several reasons, the first being that the immune system of mice has been extensively studied and well defined and this allows us to build our research on top of a large body of knowledge.

Another reason is that the immune system of mice is more similar to that of humans compared with the immune system of less evolved animals. Mice are therefore the best balanced-choice for research that is both well-defined and applicable to human disease. Furthermore, the research tools for analysing mice are much more developed compared with any other animal model.

The design of the experiments with animals takes careful account of the welfare requirements of animals. In the case of mice this means housing these social animals in groups and providing them with environmental enrichment such as tubes to tunnel through, objects to chew on, and nesting material. Mice are housed in individually ventilated cages with woodchip bedding, and an ample supply of food and fresh water. Mice are inspected at least once every day by people who are trained to identify changes from their normal behaviour.

During experiments, we use volatile anaesthetics, delivered by inhalation, which act more quickly and disperse more rapidly than injectable anaesthetics. This reduces the anxiety which slow recovery from anaesthetics may cause. Where necessary we treat the mice with painkillers (analgesics) either before a procedure and/or following a procedure.

Some experiments are expected to cause ill health, for example when we give the animals influenza virus in order to assess the potency of new vaccines. In most cases, flu symptoms will last a limited period until the immune system eradicates the virus; however in mice that do not get the vaccine treatment the health of the mice may deteriorate, in which case the animals are humanely killed to prevent suffering.

In procedure where adverse effects are a possibility, we use preventative measures to eliminate any anticipated problems. For example: Animals that are irradiated for the purpose of bone marrow transplantation are given antibiotics for three weeks while their immune system is regenerating. In other experiments, when tumours are implanted on animals, a location is chosen that is least disruptive for their normal behaviour. Normally this is in the rear end of the back where any tumour growth would not interfere with the movement of the mouse and where there is plenty of loose skin underneath which a tumour can grow without generating pressure on any other organ. In experiments where increased dehydration is anticipated, subcutaneous saline injections can be used as a supportive measure.

In order to reduce the animals anxiety, the minimum possible number of injections are used, the smallest possible volumes are injected and the least invasive route of injection is chosen.

Where anaesthisia is used, we try to combine several procedures under the same anaesthetic duration.

When surgery is involved, we will use appropriate aseptic techniques, monitor the animals before during and after the procedure and pay special attention to the husbandry of animals while they recover.

PROJECT 39: MUSCULOSKELETAL FORMATION AND REGENERATION

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.

Word limit; 1000 words

Project Title	Musculoskeletal formation and regeneration
Key Words	zebrafish, muscle, wounding, immune system, regeneration
Expected duration of the project	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;
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):

This project aims to understand how muscle develops and is repaired. It is important to understand this as muscle injury or weakness occurs in a wide variety of diseases or health-related conditions, including muscle dystrophies, obesity or diabetes, cancer and during ageing. Repair and maintenance of muscle involves muscle stem cells interacting with a number of other cell types. As it is not possible to examine how stem cells respond to injury or chemicals in people, it is not known which molecules and genes are important for regulating their responses.

Objectives of this project are therefore to:
1. identify molecules and genes that control muscle stem cells during development and regeneration
2. understand how cells of the immune system are important for regeneration of muscle
3. to understand how diseases and physiological conditions in humans affect muscle 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)?

The benefits of this project are that it will provide a better understanding of how muscle stem cells respond to specific molecules and how changes to the immune system can affect muscle repair and strength. This is important for designing approaches to overcome muscle weakness in ageing, chronic disease or during wound healing. By knowing how muscle stem cells and immune cells talk to each other and which molecules are important it will be possible to design interventions to promote better muscle health in such contexts.

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

This project will use zebrafish larvae and where necessary adult fish, for testing how cells form muscle during development and after injury. Experiments will be designed to use the minimum amounts of animals. This will be through experimental design to test several variables simultaneously and by performing pilot experiments to confirm the conditions are optimal for acquisition of data. Approximately 50,000 animals are expected to be used over the course of this project.

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

Procedures that will be used in this project will involve breeding adult fish to obtain embryos, then using these to test how chemicals, genes or bacterial infection affect the ability of cells to form muscle during development and after injury. Adult animals will be exposed to different diets, genes or chemicals to show how this affects muscle formation. This will be assessed by fluorescent microscopy to follow cell behaviour, in which animals are anaesthetised and placed under a microscope. Muscle strength will also be evaluated by measuring the ability of the fish to swim. Adverse effects from these procedures might include developmental abnormalities, a failure to effectively repair muscle or general ill health because of chemical, genetic or bacterial exposure. If the animal is clearly suffering and this cannot be treated it will be humanely killed. End points used will ensure all procedures do not lead to more than moderate suffering of animals. All animals will be killed at the end of the protocol unless they are to be used for breeding in other projects under another project license.

Application of the 3Rs

Replacement

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

Replacement

To model muscle formation and repair it is necessary to use living animals, as many cell types, including circulating cells of the blood communicate with each other in a complex, poorly understood manner. This means tissue regeneration cannot be easily investigated using cells in culture.

Reduction

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

Reduction

Experiments will be designed to use the minimum amounts of animals that allow us to test by statistics. This will be through using a experimental design to test several variables simultaneously and by performing pilot experiments to confirm the conditions are optimal for acquiring the data needed to achieve our goals.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 a good choice to examine tissue regeneration, as they are very good at regeneration and so unlikely to endure prolonged suffering during the wound healing process. Further, embryonic and larval stage animals are mostly transparent, so allow us to observe cells in living animals by microscopy. This is crucial for achieving our goal of understanding how cells behave and testing the genes that control this cell movement.

We will mostly use embryonic or larval staged animals for experiments that cause injury to animals. This will lead to less suffering, as earlier stages are far less affected by the procedures we aim to perform and do not have complex behaviours seen in adult fish, indicative of complex neural circuits.

PROJECT 40: NOVEL TREATMENTS TO TARGET VASCULAR REMODELLING IN PULMONARY HYPERTENSION

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.

Word limit; 1000 words

Project Title	Novel treatments to target vascular remodelling in pulmonary hypertension
Key Words	pulmonary arterial hypertension, proliferation, inflammation, metabolism, imaging
Expected duration of the project	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):

Pulmonary arterial hypertension (PAH) is a devastating disease that affects the blood vessels in the lungs and leaves sufferers feeling breathless and exhausted. Patients usually die of right heart failure. Current treatments target only the symptoms and prognosis is very poor. Once diagnosed with PAH, a person has a 30 per cent chance of dying within three years. We aim to develop more effective treatments that can tackle the cause of the disease, such as proliferation, inflammation and metabolism, as well as establish tools for assessing the therapy, with the hope to halt or reverse the diseased lung blood vessels and delay the progression of the disease.

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

Pulmonary arterial hypertension (PAH), though uncommon, leads to substantially reduced quality of life and reduced life expectancy. This research proposal will provide new opportunities to understand how pulmonary hypertension develops, assess potential treatments for restoring normal pulmonary vascular structure and function in pulmonary hypertension, and establish imaging tools for assessing response to therapy, aiming to develop novel drugs to tackle this serious disease.

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

Rat 6500 and Mouse 6500 in total 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?

• Animals will be place in an isolator with low oxygen levels for a period of time (maximum 4 weeks) or a toxin extracted from plant, monocrotaline, will be used to induce the lung vessel injuries and set up the pulmonary hypertension model for our study. Animals placed in low oxygen isolator or given monocrotaline may not gain weight at the same rate as their controls. Animals will be weighed regularly after the three weeks post exposure to low oxygen or monocrotaline in-jection time-point. • Animals that undergo surgery lose weight and wound infection might occur. Post-operatively antibiotics and analgesics will be given. Any animals that are not fully recovering from the sur-gery signalled by (i) a loss of 15% body weight from baseline (pre-surgery), (ii) abdominal breathing with cyanosis, e.g. blue nose and paws. (iii) isolation or are very still, and (iv) on handling react violently, vocalise or remain very still will be killed by a Schedule 1 method. • During non-invasive imaging protocols, a reduction in body temperature and dehydration may occur with longer duration scans (over 30 min). To ensure that any weight loss remains within a moderate limit, usually 15-20%, body condition and/ or weight will be closely monitored between each occasion. The body temperature is maintained by a heated bed. Following scanning, the hydration status of the animals will be assessed by tenting the skin around the scruff of the animal. Fluid may be given as a solid hydrated media eg Transgel or as injections eg isotonic glucose saline, as advised by the NVS. • Blood sampling: If necessary, haemorrhaging will be controlled by application of a haemostat (silver nitrate or ferric chloride) or by applying digital pressure until haemorrhage stops. • To prevent unknown side effects from treatment, we will keep a close observation of animals under treatment protocol. • Animals exhibiting any unexpected sickness signs will be killed (Schedule 1), or in the case of individual animals of particular scientific interest, advice will be sought promptly from the local Home Office Inspector. • Animals with altered immune status will be maintained in a barrier environment thereby minimising the likelihood of com-promising health. • Any animal will be immediately killed by Schedule 1 method if it shows signs of suffering that are greater than minor and tran-sient or in any way compromises normal behaviour.

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 objectives is to identify agents that might have therapeutic value in the treatment of PAH. Studies in cell culture alone are not sufficient to provide confidence of a therapeutic effect *in vivo*. This requires an integrated assessment of the pharmacology of the drug and studies in animal models

are an important and recognised pre-clinical step. At this time, there is no recognised simulation that will replace *in vivo* testing in animals.
- No treatment currently in use has been shown to affect structural remodelling of blood vessels in patients. This is challenging as lung biopsies are dangerous in patients with PAH. Non-invasive imaging using PET is attractive. But the signals detected using PET tracers need to be validated and this can only be done by comparing the information obtained by *in vivo* imaging with the histology obtained from the same animals at autopsy. Once a PET tracer signal is validated, it can then be used to follow drug response in patients with some confidence.

Reduction

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

Reduction

- Conduct experiments to be able to publish according to the ARRIVE guidelines [https://www.nc3rs.org.uk /arrive-guidelines] and will use randomisation, blinding etc. where appropriate and avoid biases.
- Initial experiments will use human and rodent pulmonary artery smooth muscle cells in culture for selection of effective agents and establish dose.
- Randomise the animals and run the experiment blindly by separating tasks (dosing, phenotyping and analysis) to particular individuals, hence avoiding bias.
- Appropriate power calculation will be applied.
- Seek advice experimental design and data analysis from Statistical Services Units.
- Apply longitudinal and multi-modality experimental protocols to all the *in vivo* studies where possible to reduce the number of animals used, e.g. co-ordinating the timing of hemodynamic measurements with imaging assessment and tissue sampling experiments to collect the maximum data from each experimental animal (thereby using each animal as its own control) while minimizing any distress.
- Seek professional support for ensuring any breeding of GA lines is as efficient 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

- The rodent models of pulmonary hypertension described in this proposal are recognised internationally as informative for the development of new therapeutic agents for PAH. These models have their limitations and results

have to be interpreted in the light of this but all current therapies for PAH have been examined in these models and so there is a benchmark against which to judge the potential value of new agents.

- The proposed chronic hypoxia and monocrotaline models of pulmonary hypertension involve the use of both rats and mice and are the best characterized models currently available. Chronic hypoxia represents the least severe form of pulmonary hypertension but does not demonstrate marked vascular remodelling and so is limited as a means of investigating the effects of drugs on vascular cell turnover and survival. Monocrotaline is given as a single injection but causes progressive pulmonary vascular endothelial damage, leading to pulmonary hypertension. This appears after 6 to 8 days and pulmonary pressure rises progressively to a new level at 24 to 28 days. Animals usually tolerate this with little evidence of illness for the first 3 to 4 weeks, but then develop progressive dyspnoea, ruffled fur and weight loss and die at around 42 to 48 days. Animals will be assessed regularly for signs of ill health and killed humanely when they show signs indicative of a poor prognosis to avoid unnecessary suffering. We have now more than 10 years experience with the monocrotaline model and the mortality is <1% within our experimental setting (within 5 weeks).
- All animals will be housed in groups where possible, with appropriate environmental enrichment and fed according to current institutional 'best practice'. The efficacy of treatment will be assessed by measuring the change in pulmonary artery pressure, the change in right ventricular mass, the degree of vascular remodelling and, in the case of monocrotaline, time to ill health – defined by agreed criteria (animals will be assessed daily for signs of ill health and killed humanely to avoid unnecessary suffering).
- By developing and promoting the PET and MRI imaging we will be able to assess the early stage of cell proliferation and vascular remodelling in both hypoxic and monocrotaline models.

PROJECT 41: DEFINING IMMUNE INTERACTIONS IN THE SKIN IN CANCER IMMUNOTHERAPY

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.

Word limit; 1000 words

Project Title	Defining immune interactions in the skin in cancer immunotherapy
Key Words	Skin, Antigen presenting cells, Cancer, Immunotherapy, Haematopoietic stem cell transplants (HSCT)
Expected duration of the project	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;
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.

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 skin acts as a physical barrier to protect the inside of the body from damage, and to prevent water loss, but it also contains lots of different white blood cells that are needed for immune responses. Our skin is constantly exposed to cuts and knocks and it is important that the white bloods cells are ready to activate immune responses against any micro-organisms that enter these wounds, and which could cause disease. Our work is focused on a specialised group of white blood cells called antigen presenting cells (ACP) that detect foreign invaders and communicate this information to T cells, which are the immune cells that kill infected cells.

Cancer is caused by our own cells that become changed so they can grow without control. APC and T cells are educated so they do not normally recognise our own cells, but they can be taught that cancer cells are a problem, and need to be destroyed. This approach is called cancer immunotherapy, and has led to some very successful new treatments in the last 5 years. The skin is a very attractive site to target cancer immunotherapies because of the high number of APC, and their ability to activate T cells. However, T cells that are activated by treatments to kill tumour cells sometimes also attack healthy cells in the skin, and other places, and this leads to a disease called graft-versus-host disease, of GVHD. At its worst, GHVD can lead to the complete breakdown of the surface of the skin and patients can die from dehydration, and GVHD is a major clinical problem in the success of cancer treatment. In our laboratory we have developed a mouse model of GVHD that closely mirrors the disease seen in patients. Using this model we have shown that even after the GVHD is better the skin of mice is changed and the APC are different from healthy skin.

This programme of work aims to:

1. Work our how APC and T cells talk to each other in the skin to cause GVHD, and therefore find ways of preventing this interaction to stop GVHD.

2. Investigate what the effect of GVHD is on immune responses against infection in the skin.

3. Use our understanding of APC in the skin to find new ways to activate these cells to make T cells that kill tumours for cancer immunotherapy.

In order to meet these aims, we need to understand the basic biology of how APC and T cells move into the skin and how they talk to each other in the complicated skin environment. Then we can use this knowledge to alter these interactions to make better cancer immunotherapies without the side effects due to GVHD.

What 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 provide 3 potential benefits: 1. We will get basic information about the biology of immune responses in the skin. The architecture of the skin and the white blood cells within it are very similar in mice and humans. This research is vital for us to understand how we fight infections in the skin, and what is going wrong in people with skin disease like eczema or psoriasis. We also need to know the basic science before we can develop new ideas to manipulate immune cells as treatments for cancer and other diseases. 2. Our work will contribute to the development of new approaches to activating T cells to kill tumours. We expect that our findings will be used to inform clinical trials in the future. 3. Patients who have recovered from GVHD often suffer from re-current skin infections, such as fungal infections. Our work will help clinicians understand why this happens, and will form the basis of finding new ways to manage and treat these diseases in patients.

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

We will use mice for this research. The skin immune cells in mice are very similar to humans and so are an excellent model for the complicated immune cell interactions that we study. There are also a lot of experimental tools available that allow us to test precise hypotheses in mice, for example we have can label T cells with fluorescent markers and track them in the mouse as they enter the skin and tumours. The numbers of animals have been worked our from our previous home office licenses. We use approximately 2000 mice a year for breeding and 2500 a year for experimental procedures 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 overall aim of our work is to find new ways of treating cancer, while preventing the side effects (GVHD) that commonly occur with treatments that activate T cells.

Because of this we need to cause these diseases in mice and this means that our programme has a moderate level of severity. To mimic what happens in patients, we will irradiate male mice to kill their white blood cells and inject bone marrow cells from female mice, with T cells. The transplant recipients will develop a new white blood cells from the donor bone marrow, but the T cells will kill healthy male cells in the skin and in other sites, causing GVHD. The mice will initially show clinical signs of GVHD including weight loss, and loss of activity, and will feel unwell (as patients do after transplants) but most of them will recover. In the lab we have developed a very sensitive scoring system which allows us to monitor the mice closely, and if they are not recovering from the GVHD we will kill them in a humane way. For some experiments we will also either inject tumour cells that grow, or breed mice in which growth of tumours can be activated by painting an inducer on the skin. These experiments are necessary for us to test whether our new strategies to activate T cells to kill tumour cells will work. We make sure that the tumours grow in places that will not get in the way of the day-to-day activity of the mice (e.g. moving, eating, grooming) and the mice are monitored to make sure the tumours do not grow too big. Therefore, we do not expect the tumours to make the mice feel unwell. In the rare cases where tumours suddenly grow very quickly, or break down to cause wounds, the mice will be killed in a humane way. With these models we will use standard techniques such as injection of cells, or substances to alter immune responses, and imaging of cells in the skin of live animals. We do not expect injection of cells or substances to cause any effects other that some discomfort at the time of injection. In order to use microscopy to visualise the immune cells in the ear, we need to restrain the mice in a specially designed "rig" so that they do not move. To do this mice will receive a general anaesthetic to put them to sleep. The imaging itself is not invasive and will not cause discomfort to the mouse. In most experiments, the mouse will be given enough anaesthetic that it does not wake up again and is killed humanely at the end of the experiment. In the vast majority of experiments mice are killed humanely at the end of the experiment so that we can fully characterise the cells in their immune (lymphoid) organs and skin following the different experimental protocols. We are interested in studying immune responses in the skin, and so used models that cause dermatitis, or that model fungal infection. These models involved applying substances or fungus directly to the surface of the skin. This may cause some discomfort to the animals in the specific area where the substance has been applied, but this should be short-lived. No animals are being re-used for this programme. Genetically altered mice that have been bred (protocol 1) will be transferred to one of the experimental protocols as continued use.

Application of the 3Rs

Replacement

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

Replacement

Our work focuses on understanding complicated interactions between lots of different white blood cells within a 3-D tissue environment (the skin or tumours). Part of these interactions need cells to be recruited from other parts of the body via blood vessels. Because of this, these experiments can only be done in live animals. In order to achieve our goals we propose to use the mouse as the model organism to study interactions between cells of the immune system in the skin and tumours in the development and function of the immune system for several reasons. Genetic modification of mice is well-established and their blood and immune system has been intensively studied and bears extensive similarities to that of humans. There are numerous reagents that help the study of the immune system, which is not the in other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this programme to the same extent as the laboratory mouse.{

Mice are the lowest vertebrate group in which well-characterised animal disease models have been developed. Furthermore, the mouse immune system is probably the best characterised amongst vertebrates, and a number of immune models now exist in genetically modified mice that help us to investigate the molecular interactions between immune cells. There has been a lot of work developing human skin equivalents in the lab, but at the moment scientists have not found a way to add white blood cells to these models, which live and function as they would in the body. These models also do not allow us to study the entry of white blood cells from the blood into the skin or tumours. We will continue to follow progress into these models in order to integrate them into our research in the future.

Reduction

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

Reduction

We try to use animals as efficiently as possible by talking with animal technicians, and by careful controlling breeding to meet research needs with respect to numbers of mice needed for experiments. This will be greatly helped by a custom built mouse database that will be established at our establishment, in which every breeding pair and every mouse born is recorded and through which the scientist can readily monitor the numbers of mice they hold. Together these measures ensure that numbers of mice are kept as small as possible.

Our experiments are designed so that, at the end of the experiment, we can make optimal use of several tissues (skin, lymph nodes, spleen) to look at multiple cell types and their activation status in the lab. This approach allows us to get the maximum information from as few mice as possible. A big advantage of working with the skin is that there is a lot on each mouse, so we can use it for multiple readouts (histology for microscopy, characterising cells by flow cytometry, gene expression

studies). We also plan to use new techniques such as single cells gene expression studies. These studies need very few mice to get 100-1000 cells, but we get huge amounts of information about the expression levels of all the genes in those cells. These types of experiments tell us a lot about how different cells are responding to signals in the skin or tumour, and allow us to carefully design new experiments to test our hypotheses.

Robust scientific data requires experiments to be repeated on several independent occasions to make sure that the findings are real biological observations. We have talked to statistics experts in order to work out how many animals we need in our test groups, and in the controls, to make sure that our work is of the gold-standard expected by the scientific community. Our experiments will be planned so that we can publish them in widely-read journals according to the guidance that has been prepared by the NC3Rs. This guidance helps optimise data presented in scientific journals and to plan studies that avoid biases.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 a very carefully worked out scoring system in the laboratory (that has been adopted by many other groups which share our animal unit). This recorded monitoring allows us to identify mice that are looking more unwell then we expect from our experiments. Depending on the score, these mice will either be given soft food to help them eat and drink to aid their recovery, or, if necessary killed by a humane method before they suffer too much.

We have a lot of experience with our GVHD model and have refined it to minimise suffering for the mice. This includes giving the irradiation as 2 separate doses over a period of 2 days. This reduces damage to the intestines caused by higher doses of irradiation and therefore the mice are healthier. We have also tested the number of T cells transferred to cause clinical sub-lethal GVHD, where most of the animals recover.

By working with the skin, we can often paint a substance onto the skin rather than injecting it into the mouse. This reduces suffering both because we avoid the use of needles for injections, and also because we can induce a local response that does not make the animal feel unwell.

For some of our work we may be able to use inducible genetically engineered models which are more refined because they allow us to investigate the consequences of changing a gene or cell-type at defined time-points.

In the event that any of our animals are unexpectedly unwell, then the vet will be called to advise on how we can minimise suffering.

PROJECT 42: STEM CELLS AND NICHE CELLS IN LUNG REGENERATION, REPAIR, AND CANCER

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.

Word limit; 1000 words

Project Title	Stem cells and niche cells in lung regeneration, repair, and cancer
Key Words	Lung; epithelium; stem cells; stromal cells; disease
Expected duration of the project	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):

Our lungs are built and maintained by the actions of tissue-specific stem cells. Their behaviour must be controlled with exquisite precision so that they produce new cells of the correct type at the correct time in the correct place. Incorrect control of stem cell behaviour can be a contributory factor to several degenerative lung diseases and to lung cancer. The objective of the project is to advance our understanding of the regulation of lung stem cell behaviour. Specifically, we will study the regulation of lung stem cells by cell-to-cell signalling pathways and by their neighbouring 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 British Lung Foundation estimates that approximately 12.7 million people in the UK have a history of longstanding respiratory illness including lung cancer and idiopathic pulmonary fibrosis (IPF). Lung cancer is the third most common cancer in the UK (2014), accounting for 13% of all new cases. In 2012, about 32,500 people had IPF in the UK and the incidence has continued to rise. One of contributing factors is that these diseases are multifactorial diseases that likely result from complex interactions between genetic and environmental factors. An improved understanding of cellular interactions and their impacts on stem cells during tissue regeneration and injury repair will advance not only the fundamental stem cell biology of the lung but also the better therapeutic development. The long-term goal in this work will advance our understanding of stem cell contribution to human lung diseases including IPF, Cystic fibrosis, and lung cancer for developing potential therapies.

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

This project will use mice, including genetically modified strains. The maximum number of mice that could be used over a 5 year period would be 14,680.

In the context of what you propose to do to 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 cell behaviour in regeneration, repair, and early tumour formation. To do this we will use techniques such as drug administration to genetically modify animals in order to either label cells or induce specific cell type damage, which will allow us to understand how stem cells generate specific cell types. Sometimes we may replace naturally occurring cells with our modified and traceable cells using transplantation techniques, so that we can precisely study how these cells behave. All animals are carefully checked regularly and if there are any concerns animals are examined and weighed. The majority of the animals will be used in protocols under which they suffer either no, or very mild, adverse effects. For example, transient pain or discomfort following an injection. A smaller number of animals (<5% of the total) will be exposed to protocols of moderate severity to model different aspects of human lung disease. Some weight loss with or without other signs of ill health may be seen. In these cases, 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

Lung stem cells are regulated by their surrounding environment. This includes other lung cell types, nerves, blood vessels, and immune cells from the circulation. This complex environment cannot yet be fully recreated in a dish in the laboratory and so needs to be studied within the context of the whole organism. Nevertheless, some aspects of lung stem cell regulation can be studied in cultured cells and where possible we use this non-animal alternative. REDACTED We will extensively use this powerful tool prior to use of animal models to prove our findings.

Reduction

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

Reduction

We will perform preliminary experiments on lung stem cells which are grown in organoid culture system that we have established. We use age-matched animals of the same background strain to minimise variability within each experiment and therefore reduce the numbers of animals required. We will also use statistical techniques to calculate the minimum number of animals which are required to obtain a conclusive result in each experiment. By labelling cells using fluorescent markers in the confetti mouse model, fewer mice can be used compared to conventional approaches where an increased number of animals were required to understand changes in cell 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

To study lungs in the context of the whole animal, we are restricted to studying an air-breathing vertebrate animal. We choose mice as a species as they have lungs very similar to our own and are also easy to study and make genetic changes to in the lab.

All methods proposed in this project use refined techniques that minimise animal stress. These include refined cell damage approaches with minimal side effects as opposed to the traditional usage of chemical-induced lung injury models. The lowest dose that is sufficient for observing stem cell activation with mild clinical signs will be determined and used in our experiments.

Cell based-studies will be used to plan experiments with animals, reducing number of animals and refining the experimental design.

PROJECT 43: DRUG EVALUATION IN PRE-CLINICAL ONCOLOGY MODELS

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.

Word limit; 1000 words

Project Title	Drug evaluation in pre-clinical oncology models
Key Words	Cancer, pre-clinical, efficacy, models, imaging
Expected duration of the project	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;
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 primary objectives of this project are:
(i) the use of established and validated mouse models of cancer to evaluate candidate anti-cancer agents and combination therapies, to support progression of effective anti-cancer treatments to human trials, ultimately resulting in validated effective final products.
(ii) To support objective (i) through the development of patient relevant pre-clinical models for the evaluation of candidate anti-cancer agents and combination 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)?

According to studies published in the public domain, 77% of 800 cancer drugs entering early clinical trials failed to reach patients and this failure was attributed in the majority of cases to poor response to the anti-cancer agents in the patients tested. This data highlights the growing need by the Pharmaceutical, Biotech Industry and academia for more patient-relevant and predictive cancer modelling before clinical trials begin especially with new generation of targeted cancer drugs, immuno-therapeutics and combination treatments that are presenting new opportunities for patients with cancer. The aims of this project is to provide the scientific community with a high level of centralised 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. In some cases this may result in programme cancellations; whilst this may seem of negative benefit, cancellation of candidate anticancer agents either ineffective or unsuitable for further development can be considered a positive benefit in the longer term as it limits the progression of ineffective therapies brought to early phase clinical trials and allows the direction of resources and patients to other projects. As the understanding around the mechanisms behind cancer progression continues to increase, so does the

requirement to develop and validate relevant models in parallel to test new strategies. Thus the best way to benefit the scientific institutes that we work with, industry and thus patients as a whole, is by the development of pre-clinical cancer models that exhibit greater patient relevance for their application to the development and testing of novel anticancer agents. We are very proactive in attendance at relevant national and international scientific conferences and actively share our research where possible with the global scientific community through abstract submission to national and international conferences. Once validated, all models are added to the proprietary databases; access to which is free to all users, so one of the immediate benefits of the model development process is that model data, including growth and response to standard therapies, histologic and genetic characterisation is freely available to the scientific community which makes these databases extremely powerful tools for research. What types and approximate numbers of animals do you expect to use and over what period of time? Mice will be used for the entirety of the 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+) making this species most amenable to this course of research to investigate different cancer types which include breast, prostate, lung, brain, bladder, leukaemia, lymphoma, multiple myeloma, colorectal, fibrosarcoma, gastric, head & neck, kidney, liver, thyroid, melanoma, oesophageal carcinoma, ovarian and pancreatic. Over the course of this project we'd expect to use 115,700 animals to model these cancer types, the different stages of cancer and novel anti-cancer agents and combinations.

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

Mice will be used for the entirety of the 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+) making this species most amenable to this course of research to investigate different cancer types which include breast, prostate, lung, brain, bladder, leukaemia, lymphoma, multiple myeloma, colorectal, fibrosarcoma, gastric, head & neck, kidney, liver, thyroid, melanoma, oesophageal carcinoma, ovarian and pancreatic. Over the course of this project we'd expect to use 115,700 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 mice used in this project will be used to support candidate anticancer agent development through the following stages/types of projects and model development: I. Pharmacokinetic testing: Mice will be dosed with candidate anticancer agents to

determine the fate of a chemical from the moment that it is administered up to the point at which it is completely eliminated from the body. This information is used to guide dosing regimens to ensure sufficient agent is delivered for a sufficient period of time to achieve effective target efficacy (achieve mechanism of action) in later project stages. II. Pharmacodynamic (PD) testing: Mice will be dosed with candidate anticancer agents to generate information about the efficacy of the candidate anticancer agent against its tumour target. Taken in consideration with PK analysis they can be used to assess suitability for progression to efficacy testing. The majority of mice will undergo subcutaneous tumour implantation which are visible and measured by callipers (length and width); less commonly surgical tumour implantation into the brain or organs such as liver/lung under anaesthesia which are then measured once/twice weekly throughout the study by imaging under anaesthesia to determine internal size. Once small tumours have established single doses of candidate anticancer agents by standard routes (oral, intravenous, subcutaneous etc.) will be administered followed by scheduled in life and terminal sampling. Tumour and tissue samples will be used to determine impact of agent/dose on modulation of tumour target. The short study/dosing duration and small tumour burden means that the prevalence of treatment-related adverse effects is uncommon in these studies, and any adverse clinical signs are expected to be transient. All mice will be killed at the end of the studies. III. Tolerability testing: The key aim is to ensure that candidate anti-cancer agents are tolerated at the proposed dose levels/regimens prior to entering into larger efficacy testing protocols. Mice will undergo minimally invasive procedures: short dosing phases (up to 2 weeks) at regimens reflective of follow-on efficacy studies by standard routes (oral, intravenous, subcutaneous etc.); occasionally in-life blood (tail or saphenous vein) sampling or terminal sampling is carried out. Care is made to select a dose regimen to minimise toxicity and informed by PK/PD studies; however, body weight loss (BWL) and/or adverse clinical signs may be evidenced as a result of acute or cumulative dosing. Body weight will be monitored daily and will be used to guide to intervention. Persistent adverse clinical signs e.g. subdued behaviour patterns even when provoked etc. will result in humane killing regardless of body weight measures. If the initial dosing regimen produces evident toxicity, doses will be reduced by a stepped approach (~30-50%) prior to testing in further tolerability studies. All mice will be killed at the end of the studies. IV. Subcutaneous (s.c.) efficacy testing: For s.c. efficacy testing, the key aim is to assess the efficacy of candidate anti-cancer agents, either as monotherapy or in combination with other candidate anti-cancer agents on the growth of mouse or human tumours. Mice will undergo subcutaneous tumour implantation by cancer cell injection or tissue implantation under anaesthesia. Dosing of candidate anticancer agents (refined through earlier work) by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response or humane endpoints for tumour size, mean diameter ≤15mm, are achieved. Provision of supporting tolerability data or acute phase tolerability studies (section E) means that the frequency of treatment-

related adverse effects are 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 e.g. subdued behaviour patterns even when provoked, will result in human killing regardless of body weight measures. All mice will be humanely killed at the end of the studies. V. Efficacy studies with genetically modified mice which carry the same mutation to that in human colon cancer resulting in similar tumour formation and progression will be dosed with candidate anticancer agents, refined through earlier work, by standard routes until scientific endpoints are achieved i.e. development of adenomas in the small and large intestines by 18 weeks; Alternatively, a surrogate survival format may be employed using a humane endpoint i.e. the onset of anaemia; in this setting, the study can be terminated at that point at which a statistically significant effect on surrogate survival can be determined. All mice will be humanely killed at the end of the studies. VI. Translational studies: experimental metastasis: Experimental metastasis models mimic latter stages of disease progression that may be difficult to model utilising spontaneous metastasis models where primary tumour size may drive the model endpoint. Mice will undergo tumour implantation by cancer cell injection (intraperitoneal, intracardiac, or intravenous). 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 optical imaging) or humane endpoints for tumour progression e.g. abdominal distension (peritoneal ascites), changes to gait (bone metastasis), or respiratory changes (lung metastasis.) Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects are 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. All mice will be humanely killed at the end of the studies. VII. Translational studies: Models implanted in relevant organ sites are known to better model cancer in patients with respect to various criteria as they form a single focal disease area as in the patient situation, facilitate metastatic spread via lymph nodes and show a reduced response to chemotherapy. Mice will undergo tumour implantation by cancer cell injection and surgical tumour implantation into the brain, lung or liver under anaesthesia. 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 optical imaging) or humane endpoints for tumour progression e.g. abdominal distension (peritoneal ascites), lack of coordination, head-tilt (brain tumour), or respiratory changes (lung tumour) Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects are 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. All mice will be 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

In vitro methodologies have 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, and can certainly 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 in vivo, such as blood vessel formation, 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 PK, PD and pilot toxicity studies. The use of complex 3D in vitro assays can be applied to pre-screen studies and compound selection prior to advancement into animal testing (thus reducing animal use). Careful use of pilot studies and statistically powering the study design can be used to optimise animal model use and reduce overall use of animals. The use of optical imaging technologies can reduce the number of animals required to generate study outcomes.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 a large panel of mouse cancer models to enable the immune system interplay with the tumour to be investigated. Genetically engineered mice with mutations representing those seen in humans, for example in colon

cancer, will also be used to assess the importance of potential oncogenes and mice are the lowest species in which this technology can be applied as require an appropriate mammalian architecture. There will be 2 main approaches to tumour implantation. The majority of mice will have tumours implanted subcutaneously as this enables immediate and accessible measurement of tumour growth for a wide range of cancer models. The second approach is to implant tumour 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.

Although subcutaneous cell line models lack direct translation to human studies, they are extremely well characterised within the scientific community (peer-reviewed scientific literature). As such they can be a useful tool if used with acknowledgment of their limitations i.e. as a tool to help 'dissect' a specific molecular pathway, gene fusion or driver mutation. In this context they allow a flow of work from early in vitro studies, through to PK/PD and efficacy assessments, thus assessing proof-of-concept in a minimally invasive scenario. Furthermore, using optical imaging technology they can be translated to more complex organ-specific and metastatic modelling to offer a more translational context.

PDX models offer significant translational power, they preserve both the genomic integrity and heterogeneity of the original disease and allow the generation of data that closely resemble clinical data. The translational power of PDX models is increased with their application to pre-clinical Phase II-like mouse clinical trials (MCTs) that closely reflect the human trial design, studies can be used to inform on patient selection or dosing strategies in human trials.

Organ-specific models are known to better model cancer in patients as tumour grows in the correct environment which facilitates spread to other organs via the lymph nodes as seen in the clinic and also show a reduced response to chemotherapy. The use of optical imaging will be used to refine the methods used as well as minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled termination, thus potentially reducing the duration of regulated procedures.

Experimental metastasis models mimic latter stages of disease progression e.g. escape from primary site, establishment at the metastatic site, compartmental separation etc. that may be difficult to model utilising spontaneous metastasis models where primary tumour size may drive the model endpoint. Experimental metastasis models are therefore useful for assessing candidate anti-cancer agents directly targeting the development of metastasis, or metastatic treatment strategies which often differ to those used for primary disease in the clinic. In the case of intracardiac administration of cells (i.e. experimental bone metastasis), this results in a much more refined model than direct injections into the bone as the circulating cells encounter the target organ e.g. the capillary beds of the bones, in the same way as circulating metastatic tumour cells arising from a primary tumour. In the capillary beds they are compelled to invade into the tissue, thus only the clone of the cell population having the required capabilities e.g. tropism conferred by possession

of the bone metastasis gene expression signature will survive and grow into a tumour. Direct injection into the bone introduces the cells directly into the site and does not model the escape of cells into the bone site. Furthermore, direct injection may result in the mechanical disruption of the bone itself, which is not only aversive to the animal, but could also compromise the development of lytic lesions that are characteristic of many breast and prostate bone metastases.
The development of relevant pre-clinical models of oncology is a key stage for the evaluation candidate anti-cancer agents and proposals for model development will undergo a review by the company's internal research and development (R&D) committee. Following completion of the model development phase, a report will be generated and the outcomes of the model development process will be carefully reviewed by the R&D committee before the model is considered suitable for use in client studies. As part of the ongoing commitment to the highest levels of scientific output and welfare, a regular review period will be set up for each model following completion of the model development process. This will look to follow-up on the current use and applications of the model to ensure that the most refined science and animal welfare is being utilised. Where areas of potential refinement are identified, these will be assessed through further pilot and validation ?studies.
in summary the methods that minimise animal suffering include the following:

- 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 NVS/NACWO 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.
- Presentation of adverse clinical signs, behaviour patterns or BWL relating to treatment or model progression should be de-risked by supporting work, and managed as detailed in the relevant project plan and protocol sections.
- Sampling will be in line with established welfare guidelines (see general project plan comments), 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.
- The use of supplemented diet or drinking water may be used for both candidate anti-cancer agents as well hormone supplementation, but in such circumstances, care should be taken to carefully monitor intake, to ensure that that the change in composition doesn't affect normal feeding/drinking behaviour.
- For hormone dependent models (some oestrogen-dependent breast/ovarian models, and some androgen-dependent prostate models) hormone supplementation using the most refined method that results in consistent tumour growth.
- For test agents whose efficacy may be impaired by the blood brain barrier, small proof-of-concept pilot studies may be carried out whereby dosing is achieved by

administration directly into the brain or tumour site. Where multiple doses are required use of an intracerebral/intraventricular cannula will be used to reduce the number of invasive procedures.

- Use of pilot tolerability studies to ensure there are no unexpected adverse effects associated with new models or unexpected toxicity as a result of tumour:drug interactions and to ensure the drug levels used are not associated with any cumulative effects.
- Mouse tumours implanted in mice with a fully functional immune system display higher levels of ulceration, therefore appropriate scoring system with defined endpoints and escalated actions has been put in place as a refinement to these models, and ensures that the welfare of the animals isn't compromised and the risk of harm is minimised throughout the model but scientific endpoints are still achieved.
- Non-invasive imaging will be used to refine the methods for all orthotopic and metastatic cell line models as well as minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled termination, thus potentially reducing the duration and of regulated procedures as described in Section D general comments.
- During surgery where there is a need to go through the muscle wall local anaesthesia will be used as additional pain relief. Pain scoring will also be carried for 3 days post op. A standard approach to post-operative pain management is to provide analgesia for 3 days post op, by giving a NVS recommended analgesia in flavoured jelly reducing the need for further procedures, animals are given untreated jelly 5 days prior to surgery to acclimatise. If evidence of persistent pain beyond this time is observed then the animal will be humanely killed. Following surgery the mice will be weighed daily and monitored at least once daily for changes to normal behaviour/clinical signs (typically more frequently) as well as assessment of the surgery site for bleeding.
- All procedures will be carried out in accordance with established welfare guidelines and published scientific guidelines.

Through continual professional development, new techniques for current/new models are developed and refined through the NVS, the research community and animal technology institutions or relevant veterinary expertise.

PROJECT 44: UNDERSTANDING COMMON HUMAN NEURODEGENERATIVE DISEASES

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.

Word limit; 1000 words

Project Title	UNDERSTANDING COMMON HUMAN NEURODEGENERATIVE DISEASES
Key Words	Mechanisms of neurodegeneration,, propagation of protein aggegrates
Expected duration of the project	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):

Neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, are among the most common age-related human diseases. Alzheimer's disease is characterized by dementia, whereas Parkinson's disease is primarily a movement disorder. Existing treatments only treat disease symptoms without modifying the underlying disease process. Our aim is to understand more about the mechanisms leading to neurodegeneration, with the view to developing mechanism-based therapies.

The presence of abnormal protein clumps inside nerve cells defines most human neurodegenerative diseases. Besides Alzheimer's disease and Parkinson's disease, they include dementia with Lewy bodies, progressive supranuclear palsy, corticobasal degeneration, Pick's disease and other frontotemporal dementias, multiple system atrophy, Huntington's disease and prion diseases. A small number of proteins make up the abnormal inclusions. Two of these proteins, tau and alpha-synuclein, are central to the present project.

In normal brain, tau and alpha-synuclein are soluble proteins. In diseased brain, they assemble into abnormal thread-like structures called filaments, indicating the existence of a pathological pathway leading from normal, soluble, to abnormal, insoluble tau and alpha-synuclein. This pathway is central to the origin and cause of these human diseases. It follows that a toxic property of the proteins that make up the abnormal inclusions underlies the inherited forms of these diseases. The precise molecular nature of these toxic species remains to be identified.

The discovery that specific mutations in tau and alpha-synuclein cause disease has

made it possible to produce animal models that exhibit the essential molecular and cellular features of the human diseases. These models are already leading to a better understanding of the mechanisms causing neurodegeneration. Studies on human tissues have greatly contributed to this field. However, one is almost always looking at end-stage disease. Animal models make it possible to identify the early changes; this is important, since future therapies will almost certainly have to target the early stages of inclusion formation. In human brain, by the time the first clinical symptoms appear, inclusions are already widespread. At present, there are no cell culture systems that would allow one to perform similar studies. To study the effects of this process on nerve cells and the ability of nerve cells to handle the abnormal filaments, one needs animal models. Of the existing models in vertebrate and invertebrate species, mouse models recapitulate the human diseases the closest.

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

Improved mouse models of human neurodegenerative diseases will lead to the development of new strategies and the validation of new methods for assessing the therapeutic compounds.

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

We will use a maximum of 45,000 mice over 5 years, most of which will be 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?

Mice will be housed in a social environment whenever possible and provided with environmental enrichment such as chew sticks, fun tubes, nesting material and platforms. Some mice will be left to age to allow development of the neurodegeneration which causes weight loss and progressive hind limb impairment. Some mice will undergo behavioural tests to check for neurodegeneration such as motor function or memory tests. These tests are non invasive and there are no expected adverse effects. We may administer substances by a variety of routes, choosing the most suitable and least invasive route possible. Some of the routes will be non-invasive such as food or water. Other routes such as injection (under the skin, into the peritoneal cavity, into a vein, into a muscle), gavage, inhalation, intranasal will cause mice transient pain and/or transient stress. Some mice may receive surgical injections into defined areas of the brain: mice will be anesthetised and a small hole will be drilled into the skull at the site of injection. Surgery mice will be given pain relief prior to recovery from anaesthesia and whenever necessary to alleviate pain as advised by the veterinarian. Following the administration of substances mice will be aged to observe the possible development of neurodegeneration in brain and spinal cord, in which case these mice will experience

weight loss and pain due to progressive hind limb impairment. At the end of experiments, mice will be humanely killed and samples collected for analysis. The mice may be killed under terminal anaesthesia by exsanguination to collect sufficient blood for protein analysis or by perfusion fixation to allow the subsequent analysis of whole tissues. Alternatively the mice may be killed under terminal anaesthesia for collection of cerebro-spinal fluid for protein analysis.

Application of the 3Rs

Replacement

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

Replacement

The identification and evaluation of genetic and pharmacological modifiers of neurodegeneration requires the use of experimental animal models. Prior to being used in humans, there is a legal requirement for virtually all potential disease modifiers to be tested in animal models for the disease(s) in question.

The brain structure of all mammals is similar and so the mouse is best suited for this work since, of all existing models, mouse models are the closest to the human diseases

When possible, we also carry out experiments in cell lines in our laboratory but whilst this will provide some information, cultured cells do not provide physiological conditions nor the complex interactions amongst different cell types. Tissues will also be used but only provide an isolated response which is not completely representative of in vivo response because drug action often involves metabolism and interplay among different tissues.
We have established cell assays to study mechanisms of uptake and of cell to cell propagation of tau and alpha-synuclein misfolded and insoluble protein clumps in vitro. These data are taken into account when designing animal experiments.

Reduction

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

Reduction

Mouse breeding will be carefully monitored to ensure that surplus animals are not generated.
We will use the minimum number of animals needed to give a statistically significant result. A statistical expert will be consulted regarding experimental design and the most appropriate statistical analysis. Prior experiments and expertise with the animal model used will be taken into account when deciding on the number of animals

needed for an experiment. Mice will be randomly allocated to the various treatment groups using a computer-generated sequence and the histological analysis of the samples will be blinded in order to reduce sources of variability. Cryopreservation is being used routinely to preserve important mouse lines and to remove the need to breed mice only 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

Existing animal models of common neurodegenerative diseases exhibit the essential features of the human diseases, a necessary prerequisite for the identification and evaluation of disease modifiers. As such, they are true models of at least some of the molecular and cellular features of the human diseases. At present, no valid alternative model exists.
Only mice used in experimental studies will be allowed to develop neurodegeneration. Otherwise, only young mice will be kept.

Genotyping will undertaken from ear biopsies except in those rare cases where more DNA is required when a tail biopsy will used.

When new lines are generated and bred for the first time, animal technicians will be specifically informed and the first litters carefully monitored. Any untoward phenotype will be discussed with the NACWO, veterinarian and if appropriate, the Home Office inspector.

The surgery will be carried out to minimum standards for asepsis and we will aim to follow "Guiding principles for preparing for and undertaking aseptic surgery" (2010) as closely as possible. Mice will be given analgesia 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.

PROJECT 45: ROLE OF INNATE LYMPHOID CELLS IN CANCER.

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.

Word limit; 1000 words

Project Title	Role of innate lymphoid cells in cancer.
Key Words	Immunology, cancer development, metastasis, inflammation
Expected duration of the project	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;
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):

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. 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 5,000 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 'Severe' (1-2% of mice in this study may experience temporary severe symptoms). 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 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.

PROJECT 46: THERAPIES & BIOMARKERS IN TRAUMATIC BRAIN INJURY

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.

Word limit; 1000 words

Project Title	Therapies & Biomarkers in Traumatic Brain Injury
Key Words	Traumatic Brain Injury, Acquired Brain Injury, Treatments, Neuroprotection
Expected duration of the project	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;
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.

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):

Acquired brain injury (ABI) (brain injury due to e.g. trauma, ischemia or hypoxia) is a major cause of death and disability throughout the world. In developed countries TBI is the main cause of death and disability in those aged under 45, with falls and traffic accidents being the leading causes. In the UK approximately 1 million patients present in hospitals with head injury, of which 10% have moderate or severe injury. The average cost per patient of treating TBI in the UK is £15,000. In spite of its widespread occurrence there are no effective treatments specifically aimed at preventing brain-cell loss following head injury. Blast-induced brain injury is becoming recognised as a distinct class of traumatic brain injury. The increased use of improvised explosive devices in both military conflicts and civilian terrorist attacks has seen a large increase in the incidence of blast-TBI. Exposure to an explosive blast-wave may not result in obvious externally visible lesions. Nevertheless significant behavioural and cognitive deficits may result after injury. The high economic and social costs of dealing with head injury combined with its high incidence mean that there is an increasing need to develop new treatments for TBI. Other types of ABI include ischemic brain injury (*eg* stroke) and hypoxic brain injury (*eg* neonatal asphyxia, or exposure to toxic compounds such as carbon monoxide). Although the primary insults are different in each type of ABI, there are similarities in many of the mechanisms thought to play a role in delayed brain-cell death (*eg* excitotoxicity and oxidative stress). The aim of this program of work is to develop novel neuroprotectants as treatments for ABI.

What 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 present there are no effective drug treatments for Traumatic Brain Injury, subarachnoid haemorrhage, blast-induced TBI or carbon monoxide exposure. Treatments for other forms of ABI are equally limited; therapeutic cooling is used in the treatment of neonatal asphyxia; carbon monoxide toxicity is treated with oxygen therapy either at atmospheric pressure or elevated (hyperbaric) pressures. The aim of this program of work is to determine the effectiveness of novel treatments to prevent or minimise brain damage resulting from these injuries. We will also investigate whether novel biomarkers (EEG and blood or CSF samples) can be used to predict outcome and recovery after injury. These biomarkers could be of great use in clinical diagnosis of brain injured patients. EEG will be measured before and after injury and blood and CSF samples analysed to determine whether these can be used to predict outcome after injury.

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

We will use rodent models of acquired brain injury, controlled cortical impact, blast-traumatic brain injury subarachnoid haemorrhage and CO-exposure. Rats and mice are the least sentient species in which we can determine the effects of novel treatments on clinically relevant behavioural outcomes such as memory and locomotor function (eg walking pattern). We will use both rats and mice in this program because each species has particular advantages: Mouse models offer advantages in modelling certain aspects (eg locomotor function & gait) and are more convenient for studies on outcomes in aged animals, while rats offer the advantage that the effects on physiological parameters (eg blood pressure, heart rate, intracranial pressure) are more easily measured. Rats and mice will be subject to brain injury under controlled conditions and functional behavioural outcomes will be used to assess the protective effect of our novel treatments. We will use the minimum number of animals necessary to obtain statistically significant data. We estimate that over a 5 year period we will use a total of 3500 rats & 4250 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 rodent models of ABI that we use are widely used in pre-clinical studies to determine the efficacy of neuroprotectant drugs. We expect that animals will have some degree of locomotor and cognitive impairment after injury. The animals will be closely monitored after injury and any that show signs of pain or distress will be given pain relief as required in consultation with a veterinary surgeon. Any animals in extreme distress will be humanely killed in order to minimise suffering.

Application of the 3Rs

Replacement

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

Replacement

Using a simple *in vitro* models of ABI we have identified novel neuroprotectant substances. The events that follow TBI *in vivo* are complex. Although some of these processes can be studied *in vitro* (and we will pursue such studies where possible) there are no alternatives that can effectively model these processes that do not use animals. In order to allow progression to clinical use, the use of animals is unavoidable.

Reduction

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

Reduction

The proposed models, experimental design and statistical analysis have been discussed with a Statistician. For the quantitative experiments we may use power analyses to decide the sample sizes. If the desired power has been achieved we will not use further animals. For the qualitative measures we will use the minimum amount of material required to provide an adequate description.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 program of work will use rats and mice. Rodent models have been widely used in pre-clinical ABI studies and are the most well characterized and reproducible models. In order to increase the relevance of the results, the study designs will where appropriate incorporate the principles of the STAIR (Stroke Treatment Academic Industry Roundtable), CAMRADES (Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Stroke) and NC3Rs ARRIVE guidelines All animals will be closely monitored after injury and any that show signs of pain or distress will be given pain relief as required in consultation with a veterinary surgeon. Any animals in distress will be humanely killed in order to minimise suffering.

PROJECT 47: DEVELOPMENT OF NOVEL NEUROTHERAPEUTICS FOR MULTIPLE SCLEROSIS

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.

Word limit; 1000 words

Project Title	Development of novel neurotherapeutics for multiple sclerosis
Key Words	Multiple sclerosis, Experimental allergic encephalomyelitis
Expected duration of the project	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;
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.

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):

Multiple Sclerosis (MS) is characterised by brain injury caused by inflammation and nerve cell damage and cell death. A limited number of therapies are currently available and many have modest benefits and some have serious side-effects.

The objective of our research is to test the potential of 4 novel therapy approaches in a mouse model of MS called experimental allergic encephalomyelitis (EAE). At the end of this body of work (5 years), if the approaches we have proposed show efficacy and safety, we would hope to start further development with the aim of eventual human testing of at least one of these approaches.

These approaches are not interdependent and could independently lead to success.

We are proposing the following four novel approaches:

1. The development of drugs that protect the nerve cells and that have multiple mechanisms of action. These types of drugs have not been previously developed and tested in MS.

2. The development of a therapeutic vaccine for MS that would allow the treatment of large numbers of patients who have MS.

3. To better understand the body’s own natural protection mechanisms against MS and to understand how these messages maybe transmitted to subsequent offspring. This knowledge could be used to develop new therapies.

4. To study the effect of combining nerve cell protection with stem cell 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)?

If we are successful in developing a new therapy for MS, this could have huge benefits for humankind. MS is a major cause of adult disability worldwide. A new therapy for MS that could benefit large numbers of patients is desperately needed.

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

We plan to use only mice for this body of work. We plan to use 1600 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 model we will use is the EAE model which models human MS. Some of the animals are expected to reach severe level of disability. However, these animals will be closely monitored and suffering will be minimised using husbandry techniques developed through the study of other neurological conditions. Any animals exhibiting significant levels of suffering will be humanely terminated. All animals will be humanely terminated at 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

Our previous work has used *in vitro* approaches to select candidate drugs and therapies which underpin the three approaches that we have proposed in this body of work. Our previous work has allowed us to select candidates with the highest likelihood of success. However, going forward, *in vitro* work cannot replace animal experiments which are needed to confirm efficacy and safety.

The outcome measures that need to be tested include functional and behavioural testing which cannot be tested in cells. In addition, MS injury involves a complex interplay of many different cells in the brain and many organs systems. This cannot be tested using *in vitro* approaches.

Reduction

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

Reduction

The number of animals will be kept to minimum by ensuring that the experimental designs to be used are rigorous and that all personal licensees (and dedicated animal care staff) working on this project are appropriately trained and suitably competent. This will enable a high success rate to be achieved with minimum number of animals being used.

Statistical approaches such as Anova will be used so that the number of animals in each study is minimized by using multiple comparisons within a set of data, thus eliminating the need to carryout multiple group 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

An ongoing aim is to constantly refine the use of experimental animal models and reduce the impact on the animal. We are working on refining our experimental protocols that are used to induce EAE which can affect the results and also reduce the impact/severity on animals. This will be done by ensuring that operators use video recording of mouse functional outcomes to ensure accuracy and reproducibility. We will work with other investigators in the field to explore the use of non-invasive imaging of the eye to monitor nerve damage, so the effect of disease could be detected earlier so that experiments of shorter duration could take place. These techniques include optical coherence topography and confocal corneal microscopy.

Stress and discomfort or pain due to priming injections

Injection of Freund's adjuvant under loose skin in the flank is very well tolerated and no major problems associated with disease induction. As a consequence of Freund's adjuvant, granulomas may be formed at the site of adjuvant injection.

Refinement: Injection in the flank reduces these adverse reactions and the animals show no untoward side effects as a result of this protocol. Although unexpected (incidence <1%), should the injection sites develop full thickness ulcerations the animal will be killed. We will not inject into footpad or tail base. Analgesia will be provided as needed. We will use sharp needles of the narrowest possible gauge.

Paralysis, which may cause distress or anxiety: loss of tail tone, hind limb weakness, hypo-motility, limb paralysis

Experimental autoimmune encephalomyelitis causes neurological disease that interferes substantially with the normal responses of animals and results in immobility and considerable weight loss. Animals exhibit complete hindlimb paralysis (>90%) usually for less than 4 days but unusually lasts for 7 days, prior to recovery. Animals may develop quadriplegia (incidence <1%), prolapsed of the penis in males (<2%) that does not retract with 2 days (incidence <1%), or incontinence/loss of bladder control (<5%). The disease may be so severe that mortality (~5%) may unexpectedly occur despite efforts to implement endpoints prior to death of the animal. Almost complete remission (>90%) occurs by about 25 days post-injection. Paralytic relapse (~100%) develops with various levels of remission in the intervening period and an accumulation of neurological deficit (paresis) develops. Relapse animals exhibit complete hindlimb paralysis usually from 4 to 10 days, prior to recovery. Mortality due to the severity of clinical relapse is not anticipated (incidence <0.5%).

Refinement: Measures will be put in place to get the animals through this crisis period. We will monitor urinary function, use manual expression of bladder if necessary (monitoring carefully for signs of pain or distress following bladder emptying). Animals with incontinence and soiling will be groomed to clean the anal region and to remove wet hair and cleaned to limit urine-induced irritation. We will ensure adequate refuges and nesting material are provided. We will provide constant access to water and food placed in containers on the cage floor. We will provide soaked food, fluid blocks, liquid nutrition or subcutaneous supplementation as needed. In some cases, we will place on paper rather than bedding to allow ease of movement, nesting material to allow them to generate heat as they will not be moving as much.

Weight loss

Following disease induction, animals show an initial weight loss after 7-10 days (<10% overnight compared to pre-disease), then limp tail, ataxia and usually reversible hindlimb paralysis for a period of 2-5 days as weight loss (up to 35% depending, on the strain used) progresses.

Refinement: Weight loss is a feature of the disease process and occurs even when animals are given food and fluid orally by gavage). Although weight changes are not a good discriminator of which animals recover, animals developing more than a 35% body weight loss will be humanely terminated.

Humane Endpoints: **Hypothermia is** a prognostic marker of disability. Animals developing significant hypothermia (<32˚C) will be killed. (Most animals will exhibit some degree of hypothermia). Heat pads may be used during relapses. *If animals develop complete hindlimb paralysis (EAE score 4) for more than 5 days without evidence of weight-gain, or any animal with hindlimb paralysis for 10 days during the relapse, will be humanely killed.* Animal developing quadriplegia, prolapsed of the

penis in males that does not retract within 2 days, or incontinence/loss of bladder control, for more than 4 days, seen by wetness and soiling of the fur, to which the animal cannot groom itself to remain healthy, will be killed immediately.

Inspection of EAE animals

As part of our routine disease monitoring animals will be independently checked by both researchers and animal care staff trained appropriately to observe any adverse effects at both poles of the working day. “Animals at risk” will be identified by means of a label, and researchers will be contactable. As is normal, the animal care staff have the right to decide if an animal needs to be killed humanely. The frequency of monitoring is commensurate with the severity of anticipated severity of disease, which has shown some yearly variations in other labs. This may include institution of scheduled out of hours monitoring so that animals may be killed if their condition deteriorates. Standard operating procedures are monitoring of animals and the adverse effects of the disease will be displayed to assist animal care staff determine the duration of the side effect to help ensure are maintained within defined (above) endpoints.

The NVS, NACWO and animal technicians will be consulted to ensure that all agree the clinical signs and the associated score assigned.

[FSL 20/6/17- Please provide additional details included in body of application which outline refinement for individuals within the project especially steps taken to reduce suffering in end stage mice]

PROJECT 48: PRECLINICAL DEVELOPMENT OF INTERVENTIONS AGAINST EMERGING PATHOGENS

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.

Word limit; 1000 words

Project Title	Preclinical development of interventions against emerging pathogens
Key Words	Preclinical, Emerging, Viruses, Interventions, Pathogens
Expected duration of the project	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;
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.

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):

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 influenzalisted 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.

PROJECT 49: RETRIEVAL-RELEARNING TO STRENGTHEN MEMORIES

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.

Word limit; 1000 words

Project Title	Retrieval-relearning to strengthen memories
Key Words	Memory, Behaviour, Reconsolidation, Retrieval
Expected duration of the project	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):

The retrieval of a memory can lead to the opportunity for it to be updated. One form of updating is the modulation of memory strength. In this project, we aim to explore whether retrieval allows for subsequent relearning to strengthen an existing memory more effectively than other behavioural approaches.

What 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 for retrieval coupled with relearning to greatly strengthen memories has implications in two areas: First, this may be a mechanism, by which fear memories become stronger and more persistent, thereby perpetuating conditions such as post traumatic stress disorder. If we confirm that it does strengthen fear memories to a point that they resemble posttraumatic stress disorder, then this may become a target for therapeutic intervention. Second, retrieval and relearning has the potential to maximise memory gains in educational and aging settings. Therefore, if we confirm that memory is improved across different ages, then it may become a useful memory strategy.

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

We plan to use both rats and mice. Our preliminary experiments have been conducted in rats and this will be continued for the fear element of the project. The study of conscious memory can be conducted in both rats and mice. However, colonies of aged mice are more readily available, and so this element of the project will be conducted in mice (to allow for cross-age comparisons). We anticipate using 1150 rats and 1500 mice over the course of 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?

The majority of the experiments will be purely behavioural in nature. These will involve initial memory training, followed by retrieval and retraining. The behavioural study will culminate in a single test, or a series of tests. The fear memory element of the project will involve exposure to mild electric footshocks, which cause transient pain, whereas the conscious memory experiments have no expected adverse effects. For some experiments, we will target the brain mechanisms of the process, which will involve drug administration, either as an injection or by infusions directly into the brain via a cannula previously implanted under anaesthesia. For the experiments in aged mice, there is expected age-related health decline. The expected level of severity is mild for the conscious memory behavioural experiments, and moderate for the footshock, surgical and aging experiments. At the end of the experiments, the rats and mice will normally be killed by a schedule 1 method. Should we need to analyse brain tissue post-mortem, this may require preparation of the brain 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 key measurement in the project is behavioural memory performance, which cannot be modelled or replicated in non-animal alternatives. Moreover, given that the outcomes of the project are anticipated to have translational relevance to human memory, non-protected animal alternatives are not of sufficient relevance to human neuroanatomy, neurophysiology and behaviour.

Current computational models of the brain are not sufficiently sophisticated to model true behaviour, although there is potential for progress to be made to a point when some objectives could be achieved, at least in part, computationally.

Reduction

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

Reduction

We will design experiments carefully, using appropriate group sizes and control conditions in order to draw valid conclusions from our data. In order to minimise variability in behavioural performance, we will control environmental and experimenter conditions carefully (e.g. testing at the same time of day).

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 sufficiently similar to humans in their biology and behaviour that the outcomes of the project will have relevance to the understanding of human memory processes.

Declarative memory testing causes no lasting harm or suffering, and we will use the minimum intensity of footshock to motivate robust behavioural outcomes. We will continue to use best practice in surgical procedures and will use the earliest age at which memory decline is robustly observable in order to minimise age-related health welfare costs.

PROJECT 50: THE PHARMACOLOGY OF THE PULMONARY CIRCULATION: NEW TREATMENTS FOR PULMONARY ARTERIAL HYPERTENSION

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.

Word limit; 1000 words

Project Title	The pharmacology of the pulmonary circulation: new treatments for pulmonary arterial hypertension
Key Words	pulmonary hypertension, drugs, obesity, sex
Expected duration of the project	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):

Pulmonary hypertension (PH) occurs when the blood vessels in the lungs close up and die causing the right side of the heart to fail. There is a very poor survival with around half of the patients dying within three years of being diagnosed. Modern drug treatments do not improve survival. Women get PH up to 4-fold more often than men but men die quicker. Obesity is common in PH patients and can facilitate the disease process. We do not know if sex or obesity affects the development of PAH or the effectiveness of treatments. Here we will look at the effects that sex and obesity has on the development of PAH and the response to drugs by using the best animal models. We will also examine the effectiveness of novel drugs in these 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)?

Our work previously has led to new drugs going through clinical trials but we still need drugs that better reverse or treat the changes in the arteries and heart. Here we wish to find out why women get PH more frequently than men but why men die quicker. We also wish to find out why obesity contributes to the disease. We wish to discover new drugs that better treat the disease and improve survival.

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

We will use the minimum number of rats and mice to find out why women get PH more than men but men die quicker. Legally all new drugs need to be tested in animals before entering clinical trials so we will study very good rat and mouse models which have led to previous drugs successfully entering clinical trials and going on to treat the disease. In order that we ensure robust results we need to study

enough to make results statistically significant. Over 5 years we could use up to 1500 rats and 5000 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?

Some animals (we compare males with females) will be placed into hypoxic conditions (by putting them at simulated altitude) for up to 6 weeks which causes PH like that seen in patients in that blood vessels in the lungs close up slightly and put strain on the right side of the heart. This is like putting them up a mountain and doesn't cause them any distress. Some animals may be given a drug called sugen that makes the disease moderately more severe in that very small blood vessels in the lungs completely close as seen in patients. This does not normally cause adverse events. Some animals are given a drug called monocrotaline that also causes blood vessels in the lungs to close up and put moderate strain on the right side with associated inflammation of the lungs to model what happens in some patients. This does not normally cause adverse events. These animals may be treated with novel drugs, hormones, steroids, genes or gene modifiers to test their ability to ether prevent the onset of PH or to reverse it. When we administer drugs these are given by the most appropriate route such as orally (via drinking water or a small tube into the mouth), intra-venously (usually via a vein in the tail), directly into the lungs through the airways via a modified syringe), via small pumps that are implanted under the skin or drug releasing pellets implanted under the skin. The doses, length of dosing and routes are carefully designed such that therapeutic effects are optimal but adverse effects are not expected. Drugs given are given at known non-toxic doses. Some genes are given via special viruses and some via special fluids that enable the genes to take effect quicker. Some animals are made obese by feeding them a high fat diet for up to 30 weeks. Their teeth are checked regularly for signs of over-growth which is the only expected adverse event. As we are interested in how sex affects the development of PH, some female animals may have both ovaries removed and males have their testicles removed prior to any of the above. This is done under general anaesthesia after which the animals are allowed to recover. The very small wound is closed with clips or sutures and they are given thorough pre- and postoperative care by the vets and monitored for any signs of ill-health. This may include bleeding or wound breakdown. If so the vet will recommend treatment or repair or that the animal is put down immediately. All animals are monitored daily for any signs of ill health. If signs occur a vet is called and either the animals will be treated or put down in a human fashion. Sometimes we wish to examine how the heart is affected by the closure of the lung artery directly. To do this rats are put under general anaesthesia a ligature placed around a large lung artery. After this the wound is closed and the animal allowed to recover for up to 20 weeks. The wound is closed with clips or sutures and animals given thorough pre- and postoperative care by the vets and monitored regularly for any signs of ill-health. The surgery may have adverse effects just as in humans including

bleeding, infection, weight loss and pain (this is indicated if the animal hunches up and has fur standing up). If so the vet will recommend treatment or repair or that the animal is put down immediately. Following any of the above procedures, animals are put under general anaesthesia and catheters placed in their hearts and blood vessels to measure heart and blood vessels pressures and function. Some are placed in special scanners that image the heart to see how it is working. This will tell us if the potential new life saving drug has actually worked by reversing or preventing this terrible disease. The animals will not be allowed to recover from anaesthesia in these experiments.

Application of the 3Rs

Replacement

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

Replacement

In PH patients the changes in the lungs causes the right side of the heart to work harder and eventually the patients die of right heart failure. We can only study this complex interaction between heart and lungs in a living animal. Patients are already very ill when they are diagnosed and so experiments cannot be carried out on them. Therefore animals are essential if we are to find new drugs to save the lives of these patients.

Reduction

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

Reduction

For every experiment we use the minimum number of animals that we need to give statistical significance. Only this data would be considered by clinicians when looking at new therapies to put through a clinical trial on patients. The number is calculated by the variability in experimental design as well as the magnitude of any change we wish to measure. Wherever possible we would never repeat experiments. For example if we can, in one study, we will look at males and females with and without drug, fat and lean in one go. Some of these animals may also have had ovaries or testicles removed or had their lung artery occluded to simulate strain on the right heart.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 selected mimic aspects of the human disease we wish to study. In PH patients the blood becomes hypoxic (low in oxygen) as thickening and loss of lung arteries means oxygen is not effectively transferred, from the air we breath into the airways into the arteries. The rat/mouse hypoxic model mimics these effects. The addition of an injection of the VEGF antagonist sugen prior to hypoxic exposure refines the model such that vascular occlusive lesions appear similar to those seen in patients and this infers a pulmonary pressure which is usually higher than with hypoxia alone. The monocrotaline model exhibits additional changes in lung blood vessel function related to inflammation as this is commonly seen in some patients. Transgenic mice allow the study of one gene and its influence on the development of PH. These are selected when a mutation in a gene has been reported amongst patients with PH or changes in that gene have been shown to play a role in PH. Putting a tie around a lung artery causes a strain on the right heart as seen in patients. This allows examination of events that cause the right heart to fail and the patient to pass away. Certain diet pills caused many women to die of PH in the past and so we may also examine the effects of new drugs or interventions on drug-induced PH. All animals are studied by fully trained researchers and regularly checked by vets for any signs of ill health, especially if they have had a surgical procedure. Analgesia is always given by experienced vets where needed. Any animals showing signs of distress or ill-health will either be treated accordingly but would be put down immediately at the advise of the vet. Where drugs need to be given every day, if possible we will administer these through a drug-eluting pellet as this will mean the animals are not repeatedly dosed by mouth.

PROJECT 51: DEVELOPMENT OF NOVEL THERAPEUTIC AGENTS TO TREAT CNS DISORDERS

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.

Word limit; 1000 words

Project Title	Development of novel therapeutic agents to treat CNS disorders
Key Words	CNS, Drug development, Neurological disorders, Pharmacology, Efficacy studies
Expected duration of the project	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:
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):

The experiments we are planning to perform in this project will support the development of novel medicines for the treatment of mental diseases, such as dementia or depression. The World Health Organisation has already highlighted that mental diseases are one of the main causes of disability in the world. Mental illnesses have an impact not only on the well-being of patients, but also on those taking care of them. With global increases in life-expectancy, age-related mental diseases such as Alzheimer are becoming leading causes of mortality in UK and other western countries.

There are currently no cures for the vast majority of mental diseases. Available medicines only work for some symptoms and usually produce undesired side-effects, such a weight gain, changes in mood or uncontrolled movements. Over the last two decades researchers have made significant progress trying to understand what originates these diseases, and which changes in the brain are responsible for the observed symptoms. With the help of this information, a great number of novel ways to treat mental diseases have been proposed and are currently being tested.
The objectives of this project are to predict whether a novel drug has potential to become a medicine, to calculate the required dosage to be given to a patient and to predict if it will help to treat the symptoms or to cure 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)?

Current medicines used to treat mental disorders are still not good enough to adequately manage the growing problem of mental diseases. The ultimate potential beneficiaries of this work are among the millions of patients suffering from mental

conditions. This project will provide the pharmaceutical industry and academic researchers technical expertise and state of the art instrumentation to test if their experimental drugs have the potential to become a medicine. We will do that at specific stages of the complex process of developing a medicine. Completion of each of these stages will constitute the milestones and will help us to measure the short-term benefits of each particular project. Overall, we will provide decision making information that will help the pharmaceutical or academic laboratory to decide if the drug they are working with has the potential to become a medicine or if, on the contrary, they need to re-think their strategy or improve the properties of the drug.

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

We predict to use approximately 2200 mice and 2450 rats over the 5 years of the project licence.

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

For ethical reasons, it is not possible to test new medicines in humans without knowing if they are safe or without a clear idea if they have the potential to cure their disease or treat their symptoms. Testing potential medicines on rats and mice is a well validated approach accepted by the scientific community as a preliminary and necessary step before testing novel medicaments in humans. Sometimes medicines produce undesired effects which are usually associated with how much of the drug is given. Since this project relates to how much of the drug needs to be given to be effective against a disease, we will carefully determine the minimal dose required. Doses that produce side effects will be discarded on further experiments. For this reason we can predict that the majority of the animals are not expected to show signs of adverse effects that impact materially on their general well-being. No more than 5% of animals are expected to show clinical signs of a mild or moderate severity as a result of unpredicted side-effects of administered compounds. These may include diarrhoea, sedation, transient discomfort or irritation. In addition, drugs will have to be injected or administered, which may cause transient pain or discomfort. Occasionally, blood may sampled from the tail vein, causing transitory discomfort. In order to measure changes in the brain physiology, some animals will be surgically prepared. This usually involves the placement of a recording device or cannula within the brain. Slight post-operative pain is inevitable and we will give a analgesics before and after the operation. However, no long lasting pain is predicted as there are no pain receptors within the brain. Previous experience shows that animals recover rapidly and are mobile and active soon after cessation of anaesthetic. Local inflammation can be seen in rare cases (<2%). For the nature of the therapeutic area investigated, we expect animals to be as close as possible to their normal state since alterations in the animal well-being may produce undesired

interferences with the scientific measurements. All animals will be humanely killed at the cessation 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

Cell based work is not good enough to predict if the drug will reach the human brain once it has been administered. Animal work is also needed to confirm that the drug will produce the desired effects that have been previously observed in cell assays. This is because cell based assays are still unable to replicate the complexity of the vertebrate brain or the interaction of the neurons with the vascular and immune systems. Similarly, non-protected species also lack the ability to replicate the complexity of the human brain. Hence, in the last stages of research, rodent work is still a necessary step to predict if the drug will produce the desired therapeutic effects in humans. Approximately, only 1% of the compounds that showed good potential in cell based assays can be eventually tested in humans. This is because there are no current reliable alternatives which would give us a precise idea of how the drug will behave in a living animal.

However, for some scientific questions, we may not need to replicate the whole complexity of a living brain. In these particular occasions we will contemplate alternative strategies. For example, in studies investigating a specific cellular response to target manipulation, those studies can be carried out on cultured or primary cells. We will also contemplate the use of animal tissue instead of using a living animal where possible.

Reduction

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

Reduction

We will use the minimum number of animals needed to give meaningful results. This will be mainly based on the extensive previous experience in conducting animal experiments to discover new medicines for mental diseases. Statistical analysis of previous data will help us to calculate the minimum number of animals needed in an experiment. Where possible, we will track the changes in variability over time and consider further reductions in the number of animals used. For completely new assays, where variability and magnitude of responses is unknown, the optimal number of animals may be determined by pilot studies using a limited number of animals. In addition, testing will be only carried out with compounds that have been

shown to work on cell based assays and that we know they have the ability to reach the brain. Experiments will be carefully designed to allow the maximum amount of information without compromising animals' welfare or the quality of 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

Species of choice will be agreed with the client, either rat or mouse, depending on the properties of the potential medicament, the scientific test and the knowledge of the target. The overall premise of the license is that the most refined, most relevant and less invasive method will be used at each stage. We will expect the greatest number of compounds to be tested in models with minimum burden on the animals (duration of study, stimulus required for experimental window and end-point). When the experimental question justifies additional burden to the animal (i.e. surgery, single housing or repeated drug administration) we will aim to keep such burden to minimum levels. If invasive techniques are needed, such as implantation of recording devices or surgical techniques, post-op recovery standards will be continuously monitored and revised, including the use of combinations of analgesics to keep operative pain to a minimum. On some occasions it may be necessary to single house the animals to be able to perform the experiment, but we will try to minimise these instances and compensate them with additional environmental enrichment or human handling. Most of the medicines for mental diseases only work after repeated administration and this may need to be replicated in animal studies. In these cases, the dose route will be carefully discussed with the client and alternatives to repeated injections, such us implantation of mini pumps or subcutaneous pellets, will be contemplated.

PROJECT 52: ANALYSIS OF GENE EXPRESSION CONTROL IN VIVO USING GENETICALLY MODIFIED MICE

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.

Word limit; 1000 words

Project Title	Analysis of gene expression control in vivo using genetically modified mice
Key Words	Genetic alteration, mouse, breeding
Expected duration of the project	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):

The scientific unknowns being addressed are how genes function in animals. Genes are made of DNA, yet to function they have to be copied into another molecule called RNA. A number of proteins bind to RNA and control how it works during development and in adults as well. These RNA binding proteins are very conserved in different animals, but what they actually do is largely unknown. It is only possible to find out exactly what the physiological role of a gene or protein is by using a genetic approach to knock out its function within an animal, and monitoring the effect of this.

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

Science will be advanced by showing how animals normally develop, and how this might go wrong in disease. More than 90% of human and mouse genes are controlled at the RNA level, but the importance of this kind of control, or the proteins that regulate it, are very poorly understood. The research undertaken using genetically modified animals will relate to what might be going wrong in some forms of male infertility, and these studies investigating the mouse will help us understand what is happening in humans.

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

Mice will be used, and it is expected that up to 5000 mice will be used over 5 years.

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

A few animals (<100) will be used to establish new breeding colonies at our facility. These animals will undergo surgical implantation of embryos that have been genetically altered, and these animals will give birth to transgenic offspring that are used to start a breeding colony. In order to prepare the mice for implantation of the eggs, they will be mated with male mice who have been made sterile by vasectomising them (a procedure also undertaken as part of this project, to approximately 100 mice). Alternatively, adult mice will also be imported from elsewhere, and then bred to produce more. To check their genetic make-up, small tissue samples may be taken, for example a small piece of ear. Pain-killing drugs or anaesthetics will be given whenever these are likely to be needed, so that the minimum of pain and distress is caused. We have found so far that all mice bred with genetic alterations to RNA binding proteins show very minor or no adverse effects. The genetic modifications will also be maintained as heterozygotes: this means that the mice carry only one copy of the altered gene which is sufficient to provide function, and would only be abnormal if they have two copies of the altered gene. When we expect that there might be harmful effects when two copies of genes are removed, we will remove these gene copies only in specific cell types to minimize these effects, and also monitor gene function within a non-essential tissue (the testis) to reduce any negative effects on animal welfare. To further reduce the number of animals that are produced with adverse effects (such as abnormal development of particular tissues or organs), we will carry out many studies on tissues obtained from animals humanely killed at an early stage of development.

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 research questions that we will start in mice to identify endogenous targets and functions for our proteins will be followed up using experiments using cells grown in culture rather than animals. We will only use mice when it is absolutely necessary for the particular research question that we are undertaking - this will be largely identification of regulated targets and their importance within whole animals.

This work involves studying the development of complex organ systems and so requires some work in whole organisms. This is because the complex interactions between different organ systems and cell types cannot yet be studied fully in isolated cells and tissues grown in a dish in the laboratory.

Reduction

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

Reduction

We will minimise animal use by only performing work in whole animals when alternatives are unsuitable. We will minimise the numbers of mice bred by careful colony management. We will aim to make tissues available from any animals bred to other research groups either directly, or by participating in national and international schemes for sharing such resources. Where specific types of mice are readily available from academic or commercial sources, mice will be acquired for each study, to avoid maintaining a breeding colony. Once we have identified physiological targets and processes regulated in whole animals, we will use non-animal alternatives such as tissue culture to reduce animal numbers as much 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

Mice will be used for these experiments because the genetic make-up of the mouse has been successfully decoded, and all of its genes identified. This includes the RNA binding proteins we will be investigating in our project, which are shared between mice and humans. The welfare costs of the work are minimized in two main ways. When animals undergo surgery (for vasectomy or embryo transfer) pain will be prevented by use of analgesics, and distress will be minimized by high standards of perioperative care. When genetically altered mice are bred, they will be monitored for any adverse effects of the genetic alteration. If there is any harmful effect, then carefully defined criteria are established to limit these effects. This usually involves humanely killing the animals before their health is compromised. We expect most of the animals bred on this project will remain clinically normal throughout their life-span.

When a new type of genetically altered mouse is to be imported, details of the anticipated effects of the genetic modification will be obtained from the supplier and this will inform the initial decisions in relation to breeding and care of the mice. When possible, “mouse passport” data that contains more specific husbandry advice will be sought. During establishment of the initial breeding colony, litter size, number successfully weaned, and any specific adverse effects will be documented by regular (daily) examination of the animals. Husbandry modifications (eg use of soft diet, later

weaning dates for smaller juveniles, additional bedding etc) will be adopted as required. If the genetic alteration could lead to a reduced resistance to infections, we would change the way we house the mice to reduce the risk of them being exposed to disease agents.

PROJECT 53: IMPROVING THE CONTROL OF FASCIOLA HEPATICA IN SHEEP AND CATTLE

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.

Word limit; 1000 words

Project Title	Improving the control of Fasciola hepatica in sheep and cattle
Key Words	Fasciola hepatica, cattle and sheep, triclabendazole resistance, immunomodulation, vaccination
Expected duration of the project	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;
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 aim of this project is to improve the control of liver fluke infection in sheep and cattle. Liver fluke is a common and highly pathogenic parasite infection which kills sheep and causes chronic disease and reduced productivity in cattle. Control is based almost exclusively on the use of medicines to kill the parasite in the animal, but the parasite has evolved resistance to these medicines, meaning control is becoming increasingly difficult. The objectives of this project are to develop targeted control programmes to reduce transmission and thereby reduce levels of infection in sheep and cattle; to improve diagnosis of infection and to develop more precise methods of detecting resistance to the wormers used to treat infected animals and to develop vaccines. Liver fluke affects the immune system of infected livestock, making them more susceptible to other infections and affecting the diagnostic test for bovine tuberculosis. This project also aims to understand the immune perturbation associated with liver fluke 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)?

Through this research we will deliver better methods for diagnosing liver fluke infection so farmers can target treatment specifically at those animals that are infected; we will deliver better advice on how to minimise transmission, so reducing levels of infection and we will develop better methods for identifying resistant parasites. We will also increase our understanding of how liver fluke infections affect their host's immune system. This will help us better understand why diagnostic tests for other pathogens fail and why fluke infected animals may be more susceptible to other infections. This information will also inform how vaccines against fluke, as they become available, are best delivered to maximise their effect.

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

We will use up to 72 sheep to maintain the parasite life cycle and in particular to maintain our well defined resistant and susceptible isolates of liver fluke. These are required to define the regions of the fluke genome and ultimately the genes, which encode resistance. By sampling up to 3000 sheep and cattle on farms, we will be able to identify risk factors for infection and so design control programmes to reduce transmission. Finally by testing up to 400 cattle for fluke on farms during their TB tests, we can ascertain if fluke affects TB diagnosis. To refine the assays needed for this work we will infect up to six cows with fluke.

In the context of what you propose to do to 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 infect sheep and cattle with fluke, but at a level that will not cause those animals to show any sign of disease. At the end of the experiment the animals will be killed using a Schedule 1 method. We will collect blood, faeces, milk and saliva from animals on farms and test the samples for fluke and also immunological correlates. These tests are no more invasive than those used for normal diagnostic techniques. When animals have been sampled, they will return to their normal farm groups.

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 the success of the project. We can only to maintain the life cycle of the parasite and in particular maintain our genetically and phenotypically characterised clones within the host.

The life cycle of *F. hepatica* involves infection of a snail intermediate host. Eggs are shed from the mammalian host and a miracidium develops in the egg. This miracidium is released from the egg and goes on to infect the snail intermediate host. After several rounds of multiplication, the fluke leaves the snail and encyst, normally on grass or other vegetation. These metacercariae are infective for grazing animals, such as sheep and cattle. The parasite migrates from the gut to the liver where it develops and matures. It is not possible to maintain this part of the life cycle outside the host. We maintain the life cycle of the parasite in sheep, which are a natural host for *F. hepatica* and tolerate low numbers of fluke (~100 parasites) without showing clinical signs.

We have developed mathematical models for *F. hepatica* that can be interrogated to provide information on a range of parameters, reducing the need for in vivo studies. For example, the model has been used to define the properties required from a vaccine if it is to be commercially viable. Further development of this model is allowing us to test the impact of vaccines on egg output, pasture contamination and transmission; the impact of novel drug combinations and programmes on control in the face of growing resistance; and finally to evaluate the concept of refugia on spread of resistance genes. These in silico models will refine future experimental design and lead to further reductions in animal usage.

Reduction

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

Reduction

All our work relies on maintaining the life cycle of the parasite, and importantly maintaining six clones of defined resistance to triclabendazole, which are a unique and valuable resource. For both in vivo and in vitro assays we require substantial numbers of metacercariae (~60,000). To produce this number, it is essential to have a constant source of eggs to infect snails and derive metacercariae and juvenile fluke.

For each step of our experimental design we have drawn on the extensive experience from our previous research to ensure we use the minimum number of sheep to maintain the parasite. We have reduced the need for animals by developing *in vitro* assays to test resistance within fluke populations, however this can only be done using the newly excysted juvenile stages. It is not possible at the moment to maintain the parasite long enough in vitro to test efficacy of drugs against adult parasites although we are supporting work with colleagues to extend the life span of the parasite in vitro by providing metacercariae from our snail colony.**We ask statisticians to work with us to calculate the minimum number of animals required in each study to give meaningful outputs whilst cognisant of sources of variation. We use a range of experimental designs, depending on the type of analysis required and the nature of the experiment. For example for maintaining the fluke life cycle, we will use one animal per clone/isolate, because we have maximised the efficiency of our infection systems. We have reduced the number of animals that need to be blood sampled on farm by developing diagnostic assays that use milk and we have shown we can use faecal samples collected from the floor rather than per rectum.**

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 appropriate host species (sheep and cattle) rather than rats and mice, to maintain the life cycle. Rats and mice do not tolerate fluke infection well whereas sheep and cattle tolerate low levels of infection well. Animals are kept under normal farm conditions, so their welfare is not compromised by hosting the parasite. When sampling animals on farm, we use the same diagnostic procedures as those used during normal farm practice, normally at the same time as a veterinary visit. This reduces unnecessary stress for the animals.

PROJECT 54: IMMUNITY TO PARASITIC PATHOGENS OF RUMINANTS

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.

Word limit; 1000 words

Project Title	Immunity to Parasitic Pathogens of Ruminants
Key Words	Ruminant, Vaccine, Tropical, Immunology, Parasite
Expected duration of the project	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;
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):

Theileria and trypanosome parasites are major causes of livestock disease and death in many low-middle income countries, having severe animal health and welfare effects and placing large economic burdens on farmers in the affected countries. The overall aim of this project is to provide a better understanding of the parasite and host parameters that determine the outcome of *Theileria* and *Trypanosoma* infection of ruminants that will contribute to the development of improved strategies to control these important livestock diseases. This will be achieved through the following objectives:

1. Define and characterise how *Theileria* and *Trypanosoma* spp. interact with host immunity to either delay or prevent parasite clearance.

2. Analyse immune responses induced by different candidate vaccines and compare these to protective immunity to identify immunological parameters that confer protection.

3. Examine the genetic diversity of ruminant loci that have direct influence on immune responses.

4. Generate infective *Theileria* materials (to facilitate the studies relevant to objectives 1 and 2).

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

Generation of effective vaccines for many diseases remains challenging. The work in this study will provide a better understanding of the immunological mechanisms that determine vaccine success and failure and also the strategies used by parasites to evade host immunity. The studies will also result in the generation of new reagents/technologies that can be used to refine our understanding of ruminant immune responses. This information and novel tools will contribute to advances in

the development of vaccines (and/or other interventions) to tackle these important veterinary diseases.

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 estimate to use ~2500 cattle and ~600 sheep respectively. The majority of these animals (2000 cattle and 500 sheep) will be used for genetic studies and will be subjected to a single blood sample in order to obtain RNA/DNA. This number of animals is required to provide a high-level description of the highly diverse genes that are being analysed. The remainder of the animals will be used in infection and immunity studies (and the generation of infective Theileria material needed to conduct these studies). The numbers of animals allocated for these purposes is based on the number of anticipated trials and previous experience of the group sizes required to obtain robust and reliable scientific data from such trials.

In the context of what you propose to do to 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 the majority of animals the adverse effects are expected to be minimal (single blood sample) and the animals will be immediately re-homed following sampling. For the animals that are used for infection and immunity studies studies the experience of individual animals will vary dependent on the scientific purpose of the trial being conducted. Most of these animals will have multiple blood samples taken, will be vaccinated (by administration of candidate vaccines either under the skin, into the muscle, into the lymph node or into the vein) and also will be infected with virulent pathogens. The latter of these may lead to symptoms of disease such as lethargy, recumbency and complete loss of appetite which if marked will only be permitted for a period of 12 hours prior to intervention. In addition some animals may have needle biopsies of lymph nodes and skin biopsies taken (the latter under local anaesthetic). If recovery from infection is required for scientific objectives to be achieved anti-parasitic and palliative medication will be administered. In a limited number of animals lymph nodes may be removed under general anaesthetic and a catheter to collect lymph inserted. To prevent dis-lodgement of the catheter these animals will have to be ‘single-housed’ for a period of upto a month (but still in visual contact with other animals of the same species) and have restricted capacity to groom. All animals used for infection and immunity studies will be euthanased at the end of the study or if humane end points are reached. Animals used for maintenance of tick colonies will have cloth bags attached to their ears and ticks applied within the cloth bags. The numbers of ticks used are known not to have direct detrimental effects on the animals. Cattle/sheep-infective Theileria material is generated from ticks that have acquired infection from an infected animal. Animals used to generate infective Theileria material will be infected with Theileria parasites leading to the development of clinical symptoms as described above; however due to the need to permit the

parasite to mature within the cattle/sheep these marked symptoms may be present for upto 3 days. Symptoms will be mitigated by the administration of NSAIDs and anti-parasitic medications as necessary. To monitor the progress of the infection (key to apply the ticks at the correct time for ‘pick-up’ of the parasite as well as to clinically evaluate the animals) multiple blood samples and lymph node needle biopsies will be taken. To prevent dis-lodgement of the cloth bags these animals will have to be ‘single-housed’ for a period of upto two weeks (but still in visual contact with other animals of the same species) and have restricted capacity to groom. All animals used for tick colony maintenance and generation of Theileria material will be euthanased at the end of the study or when humane end points are reached.

Application of the 3Rs

Replacement

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

Replacement

The animal work will be supported and complemented by a large amount of *in vitro* and computer-based analysis. However, due to the absence of *in vitro* systems that replicate the complexities of the mammalian immune system and host/pathogen interactions it is necessary to use *in vivo* animal experiments to achieve the aims of this project.

Reduction

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

Reduction

We are actively exploring new experimental models that will reduce variability in the results and therefore reduce the number of animals required to reach statistical significance. For example we are adopting a model in which non-successful and successful vaccines can be trialled in the same individual to avoid inter-animal variability (a major source of variation in vaccine/infection trials). We will also consult with colleagues who are trialling non-animal based technologies for maintaining tick colonies and are also looking at modifying techniques that will allow archived material to be used for surveys assessing immunogenetic diversity of British cattle and sheep populations. A statistician is being consulted in the design of experiments to ensure that group sizes are sufficient to obtain valid results using the minimal 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

Due to the lack of transferable animal models the use of the target species is essential for achieving the aims of the project and therefore offer the most refined experimental model. We will continue to implement procedures to reduce the severity of the protocols used in this licence (e.g. using accumulated data to modify the clinical scoring index to permit earlier endpoints in vaccine/infection studies where possible) and to ensure use of palliative treatment to mitigate clinical symptoms wherever possible. We have, and will continue to, actively seek enhanced methods in which to monitor experimental models to reduce the duration and impact of experimental protocols and number of samples required. By incorporating comprehensive *in vitro*, bioinformatic and molecular analyses we will seek to ensure that the maximum data can be derived from non-regulated activities that will complement the work conducted using regulated procedures.

PROJECT 55: REACTIVE OXYGEN SPECIES IN THE HEART

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.

Word limit; 1000 words

Project Title	Reactive oxygen species in the heart
Key Words	infarction, reperfusion injury, heart
Expected duration of the project	1 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):

The study is to test the concept that a novel type of medicine might be effective in reducing the damage caused to the heart in the immediate aftermath of a myocardial infarction (heart attack). The agents to be tested have already shown promise in preventing “oxidative stress” in other physiological systems. This study aims to characterise their effectiveness in the heart.

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

Existing treatments to reduce “reperfusion injury” (the damage caused when blood flows back into the heart after an heart attack) are not always effective, so new treatments could be beneficial in helping patients recover better or more quickly.

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

Mice, up to 200 over a one-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 potentially therapeutic agents will be given to the mice for a short period of time (usually a week). The mice are not expected to show any adverse effects as a result of the medicines given. The mice will be anaesthetised and given drugs that influence cardiac function and heparin which prevents blood clots (used in people), before being killed under anaesthesia and the heart being removed for further scientific studies.

Application of the 3Rs

Replacement

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

Replacement

Heart attacks occur when the blood supply to the arteries that supply the heart muscle with oxygenated blood is interrupted. The reperfusion injury occurs as this supply is re-established. Neither event can be modelled satisfactorily in anything other than an intact heart

Reduction

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

Reduction

We have calculated that we shall need 8-10 hearts to determine precisely the effects of a single dose of a single drug.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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, like other mammals, has a four-chambered heart which works physiologically exactly like the human one. We will avoid inducing myocardial infarction and/or reperfusion injury in the animals by removing the heart from animals under non-recovery anaesthetic and studying it in isolation, in an apparatus designed to mimic the normal blood flows. The animals will therefore only be exposed to the potentially therapeutic agents (at doses known to be safe) and to the treatment with standard anti-clotting medicines.

PROJECT 56: IMMUNO-VIROTHERAPY FOR HAEMATOLOGICAL MALIGNANCIES AND METASTATIC DISEASE

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.

Word limit; 1000 words

Project Title	Immuno-virotherapy for haematological malignancies and metastatic disease.
Key Words	Cancer, Virus, Immune system
Expected duration of the project	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;
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):

We aim to develop novel treatments for haematological cancers, such as leukaemia, and cancers that have spread to distant sites (metastatic disease). More specifically, the efficacy of oncolytic viruses, viruses which can kill cancer cells directly but also activate the immune system to eradicate cancer cells, will be tested either alone, or in combination with complementary therapies.

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

The development of new treatments for haematological malignancies and metastatic disease that can be transferred into the clinic will lead to improved outcomes for patients. Viruses that target cancer cells and the immune system have already been trialled in patients and we aim to facilitate the translation of these agents into different disease settings.

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

We anticipate using 1500-2850 mice over the 5 years of the project. Most of these will be immune competent animals but a small number will be immune-deficient as this will allow us to identify the mechanisms involved in successful treatments and facilitate the design of complementary therapies with enhanced efficacy.

In the context of what you propose to do to 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 inoculated with tumour cells and treated with therapeutic agents. These injections, and the resulting cancer masses, may cause discomfort. Viruses

and drugs may be administered by different routes which may cause some discomfort; where necessary, treatments will be delivered under anaesthetic to reduce discomfort. Tumour burden will be monitored in order to assess the effects of therapy. All animals will be killed humanely at the end of the experiment and are expected to suffer no more than a moderate degree of adverse effects during the entire experiment.

Application of the 3Rs

Replacement

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

Replacement

Although we conduct many *in vitro* experiments to investigate the immune responses induced by our viruses, these models are not fully representative of what may happen in patients. Immune responses involve complex interactions between different immune cell types and these take place at different locations in the body. In addition, cancer cells exist along with other cell populations which support cancer growth and development. Therefore *in vitro* experimentation provides a limited amount of information regarding potentially useful treatments. For this reason we need to test our therapies in animals that have a fully functioning immune system and complex cancer environment.

Reduction

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

Reduction

The number of animals used will be minimised by conducting *in vitro* analyses before proceeding to test our data in *in vivo* studies. We will estimate the size of the likely therapeutic effect, using pilot studies, in order to perform statistical power calculations and design experiments with appropriate group 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 models planned are the lowest form of mammal recognised as relevant for human cancer.

Mice do not develop spontaneous cancer at a rate compatible with experimentation, therefore tumour cells will be implanted into the mice. Studies will be performed on animals with HM and metastatic disease and mice will be monitored daily for symptoms of disease or adverse effects of treatment. Our work is based on humane treatment of animals at all times and all work will be carried out by fully trained staff to ensure the highest standards are maintained in our work. Discomfort and distress experienced by the animals will be limited to unavoidable procedures required to conduct valid research. If at any time an animal is found to be showing signs of ill health it will be killed humanely.

PROJECT 57: UNDERSTANDING MECHANISMS OF ATRIAL FIBRILLATION, VENTRICULAR ARRHYTHMIAS, AND HEART FAILURE

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.

Word limit; 1000 words

Project Title	Understanding mechanisms of atrial fibrillation, ventricular arrhythmias, and heart failure
Key Words	Arrhythmias, heart disease, breeding
Expected duration of the project	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;
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):

Cardiovascular disease affects a large proportion of the population, with around 30% of deaths caused by cardiovascular disease. The aim of this project is to enable identification of novel molecular pathways driving cardiovascular disease (including atrial fibrillation, cardiac hypertrophy and contractile dysfunction leading to heart failure and ventricular arrhythmias)) and development and discovery of novel therapies to treat patients with cardiovascular disease. Further aim is to assess the effectiveness of novel therapies in mouse models of cardiovascular disease.

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

Improve our understanding of cardiovascular disease development, the mechanisms that contribute to cardiovascular disease, and how the interaction with aging and obesity can modify and benefit these processes. -Shared results and resources to better understand cardiovascular disease development. -Translate findings to human use to prevent or delay cardiovascular disease development.

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

Over 5 years, we anticipate no more than 16,000 mice in total to breed the genetically altered strains. We estimate a further 1100 mice to be provided from other sources like collaborating universities or purchased from commercial providers. We expect no more than 600 mice to be used for ageing studies (protocol 3), and further 9,000 are expected to be used in protocol 4 for assessment of functional electrophysiological and structural properties of murine hearts. Most of the animals will be used to investigate mechanism leading to atrial fibrillation and ventricular

arrhythmias, whereas a small number of animals (less than 5%) will be used to investigate mechanisms leading to heart failure.

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

Breeding of GA animals – Most of the mouse lines we propose to use are only expected to experience adverse effects of mild severity. None of the mice that we breed show a pronounced decline in physiological function. Small number of mice used for breeding may experience adverse effects of moderate severity. If any of the mice develop severe phenotypic changes, the animals will be humanely culled. Aging- Only a small number of mice may be used for aging studies. We expect adverse effects to be of moderate severity with approximately 85% survivorship at 24 months. A number of reasons exist for the 15% non-survival rate at 24 months including rare natural causes, tumour burden or acute neurological disease such as stroke. Adverse events include: -deterioration of body condition such as skin sores for which may be resolved with analgesic creams. - Increased barbering or repetitive behaviours for which further enrichment actives and conditions will be used. - Overgrown teeth will be clipped if possible. If these conditions are not resolved the animal will be humanely culled if palpable tumours manifest, there is weight loss of up to 20%, or visible sings of suffering are detected, including continued piloerection, obvious pain or discomfort, rapid loss of fur condition and general malaise beyond that expected for an aged mouse, intermittent hunched posture or reduced activity. Obesity - supplementation diets- Only mild adverse effects are anticipated. Bodyweight and food intake will be closely monitored for signs of ill health and behavioural abnormality, though this is not anticipated. In some cases excessive grooming may occur resulting in skin irritation. If skin becomes broken and inflamed, and is not resolved with topical analgesic cream and monitoring the animal will be humanely culled. Drug treatment - Pharmacological agents to be used are: antiarrhythmic drugs, pre-load reducing substances and diuretics, anti-inflammatories, antifibrotics, calcium/sodium handling modifiers, anticoagulants, steroids/hormones, steroidal enzyme inhibitors, metabolic modulators, new compounds with therapeutic potential shown in other models like zebrafish, or interventions to counter regulatory pathways involved in the genesis of atrial fibrillation, hypertrophy, heart failure, stroke or ventricular arrhythmias, circulating factors, synthetic analogues, antagonists. These have previously demonstrated not to have any major adverse effects from administration and any adverse effects will be mild/moderate. However if animals display signs of toxicity such as reduced mobility and grooming and up to 20% weight loss compared to the controls, the terminal experiment will be performed or mice will be humanely culled. Certain drugs, such as preload reducing therapy (reduces fluid retention) or some anti-obesity drugs may lead to weight loss as a wanted effect; laboratory staff and animal unit staff will be informed about these expected weight loss effects prior to treatment.

Application of the 3Rs

Replacement

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

Replacement

No in vitro or alternate system exists to accurately model the complexity of a whole heart. While individual cardiac cells can be cultured and examined to answer specific questions, they cannot represent whole heart responses that may be age-dependent.

Hypertrophy, cardiac dysfunction, atrial fibrillation and ventricular arrhythmias are complex diseases involving the whole body. Hormones, the central nervous system, the kidneys, etc all actively influence the disease progression. It is impossible to simulate these complex scenarios in cells in culture or in computer models

Reduction

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

Reduction

Statistical analysis and pilot studies will ensure that we use the minimum number of mice per group while maximising data quality. We will use strain and age-matching, using multiple outcomes in the same animal when possible.

To maximise the information gained from a single animal we aim to perform multiple *in vivo* and *in vitro* analyses. Where possible, cell line work and *in vitro* manipulations have been designed to yield the maximum possible information and reduce 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

The mouse has become the preferred animal of choice for many studies as it is cost effective to house in large numbers, it has a short gestation period and, in captivity, breeds readily and repeatedly just as it does in the wild. This makes it the species of choice for manipulating its genome (that is adding, deleting or mutating its genes) to test the role of specific genes or proteins. The environment is enriched for the animals. All surgical experiments are done using anaesthesia and analgesia (pain

killers) as would be provided to humans and post-operative care is designed to minimise stress and suffering. Any animal found to be suffering outside the expected limits of this application will be humanely killed. All new genetically altered strains of mice to be examined will be closely assessed for undetermined phenotypes that may present through the course of experiments.

PROJECT 58: COMPANION ANIMAL VACCINE DEVELOPMENT

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.

Word limit; 1000 words

Project Title	COMPANION ANIMAL VACCINE DEVELOPMENT
Key Words	Companion Animal Vaccine Development
Expected duration of the project	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:
Yes	(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.
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);

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):

This project has two aims:

1.To develop new vaccines for cats and dogs

2.To update and improve existing vaccines for cats and 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)?

The key benefit of this project will be the development of up-to-date companion animal vaccines that are safe and work well. Vaccination to prevent disease is preferable to relying on treatment once the animal becomes sick. Without vaccination, contracting the disease can be debilitating or, at worst, fatal. This project will make sure that existing and newly developed companion animal vaccines protect the animal from currently circulating strains of disease without negatively affecting its overall wellbeing.

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

Dogs: 1100 over a five year period Cats: 900 over a five year period Chickens: 100 over a five year period Ferrets: 50 over a five year period Rats: 100 over a five year period Mice: 100 over a five year period

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

The majority of techniques required by this licence are expected to be mild in severity and limited to vaccination, blood sampling, swabbing and monitoring of temperature. These techniques are similar to those that a cat or dog would experience during an annual vaccination and/or veterinary check-up. Since these techniques are used in routine veterinary inspection, only minor and short lived discomfort is expected. For example: some tenderness and/or minor swelling at the

site of vaccination which may last a few days. In order to demonstrate to the regulatory authorities that a vaccine works, a number of vaccinated and non-vaccinated cats and dogs need to be given the relevant disease. Although vaccinated animals should be protected from disease and would only experience mild adverse effects, the/these non-vaccinated animals will succumb to the illness. Depending on the disease, they may develop clinical signs such as a high temperature, lack of appetite, depression, a runny nose, diarrhoea or vomiting. If an animal gets sick, we will nurse and look after it carefully. Once the scientific objective has been obtained the animal will be humanely euthanized to prevent unnecessary suffering. The number of animals required for these studies are laid out by the licensing authorities and in all cases, the number of animals used will be the minimum necessary. When non-vaccinated animals are used, veterinary intervention will be used to ease any suffering experienced. Whenever possible, the cats and dogs will be re-homed at the end.

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 licence is to develop vaccines for cats and dogs. During vaccine development a lot of work is done in the laboratory prior to testing the vaccine in the animal for which it is intended. We need to test the vaccine in the animal in order to ensure that it works and is safe.

Vaccines work by mimicking a microbe and teaching the immune system to recognise and destroy it. This protects the animal from disease if it encounters the genuine microbe in the future. The way in which a cat and dog's immune system reacts to a vaccine or an invading microbe can only be confirmed by using the whole animal.

Reduction

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

Reduction

The minimum numbers of animals required in safety and efficacy studies are governed by specific European Pharmacopoeia and European Medicines Agency guidelines. The number of animals used in each study will be guided by the legal minimum number 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

As studies in the animal are the only sure way of showing that a vaccine is safe and works well, it is not possible to use an alternative animal (e.g. mice, rats) or computer model. The use of cats and dogs is therefore the most refined choice to fulfil the objective of this licence.

The severity of the regulated procedures required is expected to be mild in the majority of animals used, with a very low degree of pain, distress or suffering anticipated. Since adverse reactions to vaccination are highly undesirable (from both the animals' and owners' point of view), studies are largely conducted using vaccine formulations that have already been shown to be acceptable.

When the level of antibody present can tell us whether the animal will be protected from disease, this will be used in preference to challenge tests. However, in a small number of non-vaccinated animals, disease will be experienced. In these cases, humane end points will be monitored frequently under the care of the attending veterinarian in order to minimise any adverse effects. In addition, whenever possible pain killers and anti-inflammatories will be used to alleviate pain.

PROJECT 59: GENETIC AND ENVIRONMENTAL REGULATION OF ADIPOSITY

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.

Word limit; 1000 words

Project Title	GENETIC AND ENVIRONMENTAL REGULATION OF ADIPOSITY
Key Words	Diet, adipose tissue, genetics, zebrafish
Expected duration of the project	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):

Body fat accumulates in diverse locations throughout the human body. Patterns of body fat distribution can both increase or decrease susceptibility to a range of cardiometabolic diseases including cardiovascular disease, diabetes and cancer. For example, increased fat accumulation at the abdomen increases cardiometabolic disease risk; whereas, fat accumulation in the legs and thighs protects against cardiometabolic disease. Body fat distribution is inherited, suggesting a strong genetic influence. However, the genetic basis underlying body fat distribution is almost entirely unknown. The objective of this project is to understand how genetics influences body fat distribution.

What 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 utilize genetic data from human populations to identify important genetic mechanisms that influence body fat distribution and susceptibility to cardiometabolic disease. As such, our results will be directly relevant for humans, and provide new therapeutic targets to modulate cardiometabolic disease risk. Furthermore, with the dawn of personalized medicine, identification of important genetic elements in this project will enable prediction of cardiometabolic disease risk in patients prior to actual disease onset.

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

Genetic data from humans will be tested in cell culture and zebrafish (Danio rerio). Zebrafish will be used as they provide a simple and tractable system for studying body fat distribution that is highly homologous to humans. 7,500 zebrafish will be

used for experiments over 5 years (500 under a potentially severe protocol). Along with 16,000 used to produce new lines and maintain existing 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?

In general, this project proposes experiments with mild severity, and utilizes non-invasive in vivo imaging to minimize suffering to the animals and maximize physiological relevance. However, we also perform some diet manipulation experiments with a high level of severity. As obesity occurs due to an interaction between genes and diet, it is essential to conduct these experiments. To minimize suffering we will conduct pilot experiments to refine the experimental technique, conduct extensive monitoring of animals that are potentially suffering, and humanely euthanize any animal exhibiting adverse effects. At all times the general health and well-being of the animals will be the primary concern. After the experiments, zebrafish will be humanely euthanized. Any zebrafish showing signs of ill health or distress during the experiment will be humanely euthanized

Application of the 3Rs

Replacement

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

Replacement

We will utilize culture of human cell lines where possible. However, to properly understand body fat distribution, laboratory animals must be used that recapitulate the interactions that occur in the physiological context of a highly complex living system.

Reduction

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

Reduction

We will utilize statistical methods to determine the smallest adequate sample sizes that are able to detect the effects of genetic manipulation. Where possible, culture of human cells will 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 will use a less sentient vertebrate, zebrafish, to minimize the potential for suffering. Furthermore, we will use imaging techniques that do not harm the animals and provide more accurate readouts of body fat biology. Finally, statistical methods will be employed to ensure maximal information is gleaned from each experiment, and to increase experimental reproducibility.

PROJECT 60: IMMUNOLOGICAL MECHANISMS IN PREGNANCY-RELATED DISORDERS

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.

Word limit; 1000 words

Project Title	Immunological mechanisms in pregnancy-related disorders
Key Words	Pre-eclampsia, Pregnancy, Genetic susceptibility, Immunology
Expected duration of the project	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;
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):

This project aims to understand why some women develop pregnancy related disorders such as pre-eclampsia during pregnancy. The condition, which affects about 3-5% of all pregnant women, is characterized by high blood pressure, protein in the urine and symptoms such as severe headaches. Pre-eclampsia increases the chances of preterm delivery, smaller baby size and even death. It can cause long-term health problems for the mother and child, and remains a major cause of maternal death worldwide if untreated. The disease is caused by the placenta (afterbirth) and currently the only treatment for the disease is to deliver the placenta (& the baby) at whatever stage of pregnancy the disease strikes. There is, therefore, a large unmet need to predict, prevent and satisfactorily treat pre-eclampsia before it causes such problems. Unfortunately, however, our understanding of pre-eclampsia has progressed very little over the last 50 years, in part because of the lack of appropriate models. Our proposal directly addresses these needs through the study of disease-causing factors and potential targets for early detection and treatment of the condition.

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

This project holds high clinical relevance and offers several benefits by aiming to understand the reasons that influence development of pregnancy related disorders such as pre-eclampsia, smaller baby sizes and recurrent miscarriage through understanding the genetic risk factors which may contribute to the development of these conditions. This data will be used to inform clinical practice by providing better screening strategies of expectant mothers as well as having the potential to target specific pathways that are involved in the development of these pregnancy related

disorders. Ultimately, it is hoped that this could result in fewer mothers and babies dying across the world due to these complications of pregnancy.

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

We plan to use both genetically altered (GA) and normal mice for our experiments. Up to 1000 GA mice will be bred and maintained over a 5 year period. Approximately 500 mice will be used over a 5-year period for studies involving administration of substances and monitoring of mice during pregnancy.

In the context of what you propose to do to 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 that are pregnant are not expected to experience any adverse effects due to their genetic background alone, as these animals have been bred for several years without developing any symptoms detrimental to their health. In some cases, substances or cells will be administered to pregnant mice by injection or orally. The administration of the substance or cells is likely to cause momentary discomfort but there are no expected adverse effects from the substances or cells administered themselves. Blood samples may be drawn from mice at intervals over the course of the experiment, which again is unexpected to cause any long terms problems following the procedure itself. To check for development of blood pressure during pregnancy, blood pressure will be measured in conscious restrained animals. As restraining of animals is typically stressful and is also likely to cause slight discomfort where the cuff is clamped to the tail, the blood pressure of the animals is likely to increase by virtue of the procedure itself. Therefore, all animals will be acclimatised to blood pressure monitoring gradually over the course of 5 days before taking any scientific readings. This is likely to reach a moderate severity for the animals under this procedure. In some instances, where we need to monitor the presence of protein in the urine of pregnant mice, mice will be moved from their normal cages into small metabolic cages so that the urine can be collected over a period of 24 hours and assessed for presence of protein. This is a small chamber without bedding and is considered a stressful experience for the animal being away from its normal environment. Therefore, mice will be monitored closely during this time to make sure that they do not exhibit any signs of distress or abnormal behaviour. This is a moderate severity procedure for the animals. We suspect that handling and treatment of pregnant animals may result in additional stress than that observed by non-pregnant animals, which may result in higher preterm delivery or miscarriage. However, our handling and restraining techniques will keep this to a minimum. All animals will by killed by a standard, or specified non-schedule 1 method, so that we can obtain tissues and cells for post mortem analysis.

Application of the 3Rs

Replacement

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

Replacement

We continually strive to find alternative approaches to restrict the requirement of animal models. We use several experiments using cultured human cells and human samples, whenever possible, to test the role of risk genes. However, complexity and variation in the genes that contribute to development of pre-eclampsia make it impossible to assess their role in human patients alone and hence the animal model will play a crucial role in understanding the pathways and genes that control development of disease in pregnancy. Due to the similarities between the human and mouse placenta, mice are a good model to study features of placenta development and human pregnancy. In contrast, lower animal models are not an appropriate choice for this study due to significant differences in their reproductive biology as well as the absence of a placenta.

Reduction

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

Reduction

We have a statistician working as part of our group who will be consulted on experimental design. We will use the minimum and suitable number of animals that are required to detect a significant treatment. Preliminary studies using other non-animal techniques will also ensure that any experiments requiring animals are dedicated and specific to cell types or pathways within the disease and therefore will require fewer animals overall to answer the scientific questions 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

There are various similarities between mouse and human pregnancy, which make mice and especially humanised mouse models suitable to study disease pathways influencing development of pregnancy-related disorders. We have several years of experience working with mice and within this time have developed several refinements to minimise animal stress during handling of mice.

To minimise stress during handling, pregnant mice will be transferred between cages, for example, from a normal cage to a metabolic cage, by moving them in their

normal housing tunnel, instead of lifting them by the tail. There will be no additional procedures performed on the mice while they are housed in metabolic cages, so that the stress that they experience is limited as much as possible.

As pregnant mice are going to be typically larger, we will use appropriate sized restrainers when administering treatment or taking blood from the tail vein. We will also use red/black plastic or covered restainers, to minimise the stress associated with the use of bright lighting administering substances or taking blood samples. When measuring blood pressure in conscious animals, the mice will be acclimatised to have blood pressure readings over a course of 5 days prior to the actual readings, where the number of recordings taken for one blood pressure measurement will be gradually increased per day, reaching a limit of 20 recordings for 1 measurement.

PROJECT 61

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.

Word limit; 1000 words

Project Title	Neural basis of tactile behaviour
Key Words	Neuroscience, electrophysiology, neuroimaging
Expected duration of the project	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):

The general aim of the project is basic research into the mechanisms of how the nervous system represents tactile information and makes decisions based on touch. The project addresses two important gaps in our knowledge. First, most past experiments on this topic have been undertaken in anaesthetised animals. It is unclear how tactile perception operates in the conscious brain. Second, despite the fact that the sensory regions of the brain contain millions of brain cells, most past experiments measured the activity of only one or a handful of neurons at a time. Coordinated activity amongst large numbers of brain cells is likely to be crucial to perception, but is poorly understood. The specific objectives of the project are: (1) to determine how touch-related parts of the brain respond to touch; (2) to develop a method for studying the activity of brain cells by measuring signals related to cell Calcium; (3) to determine the coordinated response of many brain cells to touch.

What 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 short-term and long-term benefits: (1) The project will advance our understanding of one of the great mysteries of science – how the activity of brain cells allows us to perceive the nature of the outside world. (2) Basic science such as this project is fundamental for brain health, since it will provide the clinicians of the future with a richer and more useful scientific base, from which to develop improved therapies for neurological disease. (3) The powerful new methods that we develop during the course of the project can be applied to animal disease models and thereby get more insight into disease mechanisms than was previously possible.

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

We expect to use not more than 4600 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?

We will measure the activity of cells from the brains either of behaving animals, trained to perform a task for fluid reward, or of anaesthetised animals in response to touch. Recovery surgery may cause post-operative pain and/or infection: these will be prevented by delivery of analgesics/antibiotics and by use of aseptic techniques. Restraint may cause stress: this will be minimised by habituation and training. No more than moderate severity is expected. Animals will be killed at the end.

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 how sensory pathways process sensory information is incomplete. Hence a pure computer modelling approach cannot be used. In order to determine how neurons respond to sensory stimuli, the full circuitry from sensory receptors, including the sensory organ (here the whiskers), must be intact. This precludes in vitro approaches.

Reduction

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

Reduction

By using advanced techniques for measuring neuronal activity (multimicroelectrode arrays and imaging), we will maximise the number of observations measured per animal. This will reduce the required number of animals. The minimum number of animals will be determined by a combination of statistical analysis and pilot 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

Rodent species are of low neurophysiological sensitivity. We will use the most refined techniques possible that minimise adverse effects or discomfort to the animals. To minimise harms, the project includes the development of a refined, non-invasive imaging technique for measuring the activity of brain cells.

PROJECT 62

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.

Word limit; 1000 words

Project Title	Gene regulation during cell fate specification
Key Words	Pig, Germ Cells, Chimeras, Stem Cells
Expected duration of the project	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;
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):

This project will study the role of key genes participating in how cells determine their identity (whether a cell becomes muscle, skin or sperm) in pig embryos. The pig is the chosen species for this work because they grow similarly to humans. Thus, investigations using pig embryos are very likely to reflect the mechanisms of human development.

Objective 1: We don't have good understanding of how the precursors of sperm and egg (or primordial germ cells (PGCs)) are formed during early human development. Recent studies showed that genes involved in primordial germ cell formation in humans and mice are different. We recently demonstrated that germ cell development in pig embryos resembles that of humans, making the pig a valuable model for understanding early human development. Our first objective in this project is to determine what genes are critical for the formation of PGCs in the pig. Identification of these critical regulators will be used to gain basic understanding of germ cell formation in humans. This new knowledge will help us better understand the underlying causes of infertility and the origin of germ cell cancers.

Objective 2: Another aim of this project is to determine whether recently derived pig embryonic stem cells (pESC) can give rise to all cell types within a fetus. We have developed novel protocols for the propagation of these cells in vitro and the properties of these stem cells can be assessed in the laboratory. However, in vivo determination of their differentiation potential by testing the contribution to a chimeric animal is a critical test to evaluate the properties of these cells. A chimera is an animal made from cells from two different organisms. For example a chimeric embryo is generated when embryonic stem cells derived from an embryo are introduced into a host embryo. In this project chimeric pig foetuses will be generated

by introducing pig stem cells into pig pre-implantation embryos. Chimeric foetuses will be studied using laboratory tests to determine whether pESC contributed to different cell in the body of these fetuses (for example skin, liver, muscle, etc).

What 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 determine the role of key genes required for the formation of pig primordial germ cells (the precursors of sperm and eggs). This information will be relevant to understanding human development, since we have recently shown that pig and human embryos develop similarly. Understanding how germ cell precursors form will improve our understanding of how cells decide what cell type to become during early development. An abnormal program of germ cell formation underlies the causes of certain types of infertility and the origins of human germ cell tumours. These have only been partially studied in humans, due to lack of accessibility to early human embryos. Our work will provide evidence for the first time of how PGCs originate in vivo in pigs, which has similar development to humans. Our second objective will have important biotechnological impact, since pESC can be used for the generation of genetically modified pigs (GM). GM pigs can be generated to model human diseases and can help us understand the onset and progression of a disorder (for example, metabolic syndrome), but also be useful tool for developing new treatments for debilitating diseases such as age related macular degeneration.

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

We will use females (n=146) over a 5 year program. These animals will be used either as embryo donors and/or as embryo recipients to carry embryos between 12-35 days old.

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

Embryos obtained from young pig females (killed via schedule 1 technique) will be transferred, following gene editing, into the womb of recipients females using midline laparotomy under general anaesthesia. The oviduct will be briefly exteriorized to insert a catheter into the oviduct and deposit the embryos. This is a rapid procedure (about 30 minutes per animal). After surgery animals will be given analgesics and antibiotic treatment as directed by the named Veterinary Surgeon, and will be housed in our facilities. The animals are expected to make a rapid recovery after the anaesthetic. No adverse effects are anticipated, although there is a small probability of systemic infection. Daily observations will alert us of this possibility. After 12 or 35 days the pigs will be humanely killed using schedule 1 technique to allow collection of embryonic tissue.

Application of the 3Rs

Replacement

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

Replacement

The study is based on using pigs because it is the most suitable animal for modelling early human development. The program of germ cell (sperm and eggs precursors) formation is different in mice, therefore it cannot be used as model for humans. Non-protected animals (invertebrates) have very different modes of germ cell development, and are not relevant to humans.

The period of embryo development (Day 12 and Day 35) of the study cannot be replicated in vitro, therefore it is essential to perform embryo transfers.

Reduction

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

Reduction

Embryo recovery and embryo transfer are well established methods that yield large number of embryos in pigs, which will minimize the need for many sows.

We used Power calculation to estimate our requirements for the project. We assume a probability of success of 1% (1 foetus/100 showing chimeric contribution) and Power= 80%. Based on these assumptions we need to generate 185 chimeric embryos to determine the ability of a cell line to contribute to a chimera. Day 5 embryos will be retrieved from 14 donor sows/cell line (56 for 4 cell lines). We will transfer ~20-25 embryos/recipient and we expect >90% pregnancy. We need 10 recipients/cell line and we will test 4 cell lines. A total of 48 recipients will be needed to test 4 cell lines (we will oestrus synchronize an additional 20% to account for those animals not responding to oestrus synchronization).

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 shares a developmental program that closely resembles that of humans, making it the most relevant animal for gaining insight into human development. The pig genome sequence is also available, which facilitates genetic modification, and pig embryology is very well characterized. In addition, pigs are litter bearing species, which means that multiple embryos can be retrieved from a single female, which

makes the system cost effective and reduces the number of animals needed to obtain sufficient embryo material.

The potential harms to the animals are related to the surgical (midline laparotomy) intervention. The operation will be performed using aseptic techniques to prevent potential infections. Administration of post-operative analgesics and antibiotic according to veterinary advice will minimize the probability of infection.

PROJECT 63

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.

Word limit; 1000 words

Project Title	Refinement of anaesthetic protocols for research animals
Key Words	Anaesthesia, Refinement
Expected duration of the project	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;
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):

Anaesthesia of laboratory animals should represent a refinement of research methods, as it should prevent pain and distress caused by research procedures. However it is important that the best anaesthetic methods are used. This project aims to develop improved methods of anaesthesia for a range of different species, and suitable for a variety of different research projects.

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

Improved methods of anaesthesia would benefit animals used in research, as it would reduce the incidence of side-effects such as prolonged recovery periods, reduced food and water consumption after recovery. Improved methods of anaesthesia would also reduce complications such as slow recovery from the anaesthetic, which can increase the risk of death during or after an anaesthetic. These complications can also affect the quality of scientific data obtained from the animals, so the potential benefits are better science, as well as better welfare for the animals used.

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

The numbers of animals used, as well as the species, will be determined by the need for development of particular anaesthetic methods, but will require no more than 300 rats, 300 mice, 44 rabbits and 44 guinea pigs 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?

Most animals will undergo an anaesthetic, and some will be allowed to recover. The anaesthesia may be repeated. Some anaesthetics can cause slight pain on injection, or can be unpleasant to inhale, but these effects should only cause mild distress and the animals would rapidly become anaesthetized. These procedures would be classified as mild. A very few animals may need surgery to implant monitoring devices to measure the effects of anaesthesia, or to allow infusion of materials or withdrawal of blood. This could cause pain or infection, but we expect to be able to prevent these adverse effects by administering pain relief, and antibiotics, and by carefully monitoring that these are being effective. These procedures would be classified as moderate. Some animals would receive anaesthetics and would not be allowed to recover. These procedures would be classified as non-recovery. Other animals would recover, so that the longer term effects of anaesthesia could be assessed, and these animals would usually be humanely killed once the study was completed. These procedures would be classified as either mild, or moderate. However, when possible, animals that have not undergone any surgery may be rehomed.

Application of the 3Rs

Replacement

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

Replacement

Since the project aims to develop improved methods of anaesthesia, this needs to be undertaken in living animals, although some aspects of the work (eg developing new apparatus) can be done without the use of animals.

Reduction

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

Reduction

At each stage, the number of animals used would be minimised by:

a) Conducting pilot studies using very few animals (typically 1 or 2). This is often sufficient to determine whether a new anaesthetic regimen represents an improvement over existing techniques.

b) Using statistical calculations to determine the minimum numbers of animals needed to show whether a new method is an improvement on older methods of anaesthesia.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 age and species of animals selected will be those that match the animals in which the new information obtained on the project will be applied.

When possible, animals will not be permitted to recover from anaesthesia. When recovery is needed, animals would not normally have undergone any surgical procedure. Surgery (to implant telemetry devices) would only be carried out when no alternative approach could be used, and these animals would receive post-operative analgesia to alleviate pain. All of the animals would receive high standards of care during anaesthesia, for example provision of warmth, and monitoring of body temperature to ensure these measures are effective. Animals would also be carefully monitored to ensure no unintended complications occurred during anaesthesia, for example respiratory distress because of inadvertent obstruction of the airway

PROJECT 64

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.

Word limit; 1000 words

Project Title	New approaches for prevention of tuberculosis and other infectious diseases
Key Words	Tuberculosis, vaccine, immunotherapy, dengue, Buruli
Expected duration of the project	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;
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):

Tuberculosis (TB) is a major human disease, killing millions of people worldwide. Current control measures are seriously compromised by poor efficacy of the current TB vaccine (BCG), the increasing incidence of HIV/TB co-infection and the emergence and spreading of multi-drug resistant disease. This project endeavours to explore novel treatment and prevention approaches against TB. This will include generation and testing of novel TB vaccine approaches and novel immunotherapies that could be applied in HIV/TB patients or those suffering from multi-drug resistant disease.

REDACTED

Immunotherapy of multi-drug resistant tuberculosis (MDR-TB) would be highly beneficial to patients, since the current treatments are very protracted, toxic and poorly effective. We propose to test the potential of immunotherapy with antibodies and cytokines, both natural components of our immune system, to shorten MDR-TB treatment and improve treatment efficacy in mice before this approach could be considered for human application.

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

Primary benefit (i.e. control of disease) concern the potential application of new vaccine candidates for prevention of infectious diseases (i.e. TB, dengue, Buruli ulcer and possible others) in man. This could have a substantial health impact on millions of people globally. In addition, our objective is to determine if immunotherapy for TB has the potential for human application for treatment of multi-drug resistant TB (MDR-TB). Secondary benefit of this study concerns scientific community. The

scientific impacts may be multiple and will include: 1) advancement of the vaccine immunology field; 2) better understanding of the immune responses to infection, and 3) novel research tools and technologies (i.e. nanoparticle and spore vaccine platforms, molecular engineering, novel animal models of infection). As the results generated from this project will have multiple applications, we anticipate that the findings will be of interest to both academia and Small/Medium Enterprises.

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

The project will require up to 6000 mice, over the 5 year period. These will include wild type mice and also genetically modified mice. A typical experiment will have 6-8 experimental groups of 6 mice/group, including controls and test groups. Experiments will last 1-6 months, typically 3-5 months. To ensure reproducibility, each experiment will be repeated at least once, either on its own, or as a component of a subsequent experiment, where possible.

In the context of what you propose to do to 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 animal suffering in this project will be mild or moderate for the vast majority of animals. Immunisation procedures (which account for > 80 % of animal experimentation in this project) will generally cause only minor and often transient adverse effects. For example, local haematoma may occur during blood sampling, brief respiratory discomfort may occur during intranasal inoculation, localised dermatitis (skin lesion) may occur in some animals immunised with the BCG vaccine, oral gavage may cause a temporary discomfort etc. None of these adverse effects is expected to cause any lasting harm to the animals. Very occasionally, vaccination via respiratory route may cause an excessive inflammatory response in the lungs and if that happens and animals show any signs of distrees or suffering (hunched posture, poor eating and grooming habbits) they will be humanely killed. Infection of mice with Mycobacterium tuberculosis bacteria (that cause TB) is a well described experimental model. Though animals may harbour significant numbers of bacteria in their lungs, they show very little sign of clinical disease, as they are naturally more resistant to this infection than other species (eg. guinea pigs or humans). It takes a protracted course of infection (typically more than 3 months) for mice to begin to exert clinical symptoms of TB such as weight loss, irregular breeding, reduced mobility etc.). This project will not include such prolonged infection studies as all experimental readouts can be obtained before the onset of clinical symptoms. The footpad model of Mycobacterium ulcerans infection (causing a neglected tropical disease called Buruli ulcer) is also well described and does not cause any major suffering to animals. The infection causes painless (due to attacking nerve endings) swelling of the footpad which can be objectively categorised and experiments concluded before it becomes excessive as to significantly impair animal mobility or cause visible ulcerations of the tissue. In one of our experimental protocols

(aerosolised infection of mice with Mycobacterium tuberculosis), a very small proportion of animals (in our experience, typically <2 % animals from this particular protocol, and < 0.5 % for the overall project) may unfortunately be lost to sudden death due to equipment shortcoming or failure and this is being continually addressed with the manufacturers and improvements have been made and will continue to be made. Animal welfare will be always a primary consideration and all measures and precautions will be taken to minimise animal suffering and distress. Where necessary, anaesthesia will be used prior to initiation of a procedure and appropriate care given to the animals after the completion of the procedure. At the end of the experimental protocol, animal 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

Use of animals is justified, as this is the most appropriate experimental model for testing of immunotherapy and vaccine immunogenicity and protective potential, proposed in this study. Mouse models of tuberculosis is well described and it serves as a good initial screen of the vaccine immunogenicity and efficacy. Extensive *in vitro* evaluation of the proposed vaccine candidates using dendritic cells and tonsil tissue culture will be performed prior to deciding whether to proceed with immunisation of mice. Similarly, effects of immunotherapy will be tested in vitro, using human cell lines or blood samples, to select the most active/therapeutic antibodies and determine the best combinations.

Reduction

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

Reduction

The experimental design in this project took into account previous experience and the published information from other research groups. Importantly, the statistical aspects (including power calculations) and data analysis have been given full consideration. Furthermore, the replacement protocols described above (i.e. tonsil tissue culture, human blood, dendritic cells) will identify non-performing candidates, thus reducing the need to use animals for their testing. Wherever possible, multiple tests will be performed using the same control groups, thus avoiding repetitive use of control 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

Experimental tuberculosis in mice shares many immunological features with the infection in man, and therefore, the mouse model is the most appropriate for the proposed work. None of the objectives listed above could be achieved satisfactorily without the use of animals. However, extensive *in vitro* evaluation will be conducted on all new vaccine candidates and only those that ‘pass’ the *in vitro* test will be considered for evaluation in mice. REDACTED The proposed protocols and experimental designs have been continually refined to improve animal welfare and reduce stress and suffering during experimentation.

PROJECT 65

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.

Word limit; 1000 words

Project Title	Assessment of Subcutaneous Biomaterial Implants
Key Words	Biodegradation, Polymers, Inflammatory response
Expected duration of the project	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;
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 project is focussed on understanding the *in vivo* biological degradation behaviour of novel biodegradable polymer materials in order to enable them to be advanced towards clinical applications ranging from resorbable implants to advanced drug delivery devices.

While a large number of materials can be developed from a synthetic stand point, only those with appropriate *in vivo* degradation profiles and that cause a minimum of an inflammatory or foreign body response are appropriate to be applied. As such, the primary objective of this project is to evaluate and identify leading candidates.

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

While several polymer materials are applied for in vivo application, many are limited from application in a broader range of medical devices by either their lack of in vivo degradation behaviour or their poor mechanical properties. Despite this, the desired advances in personalised medicines require the further development of biomedical materials in order to achieve the large step forward that are desired in the areas of tissue engineering, regenerative medicine and controlled release/delivery. An essential step to delivering such high performance biomedical materials is to study their in vivo degradation/release and the inflammatory response that results from their implantation. There are several examples of applications in which the advancement of biodegradable polymer materials will greatly benefit humans and advance science. Those of most relevance to this project are: 1) Controlled release of active pharmaceutical ingredients from biodegradable subcutaneous implants enable active pharmaceutical ingredients to be delivered to a site of action and

released over an extended period of time. For example, for pharmaceutical use when administered into the body the biodegradable polymer slowly breaks down, releasing the drug over an extended time period. One of the key advantages of such formulations is the reduction in the frequency of dosing, which greatly improves patient convenience and compliance. Additionally, healthcare costs are reduced, as treatments are more effective and fewer visits to healthcare providers are required. 2) Bioengineered tissue scaffolds use biodegradable materials as scaffolds. Seeding and culturing with patient's own cells enables the realization of bioengineered tissue replacements. The application of degradable polymers in these scaffolds is highly desirable to enable gradual erosion of the polymer matrix as the cells proliferate to ultimately provide a bioengineered living implant derived from the patient's own cells, closely resembling the natural tissue and capable of self-maintenance. Advances in materials design could deliver bioengineered tissue replacements for a large range of tissues. In both of these areas new advanced biodegradable materials are required and an essential aspect of the materials development is understanding the degradation/release profiles and inflammatory response in vivo.

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

All experiments will be conducted using rats. It is anticipated that a maximum of 1000 rats will be used throughout the duration of the project (5 years 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?

All animals will be checked visually following recovery from anaesthesia and will be monitored daily after surgery for a minimum of 7 days and weekly thereafter. No adverse effects of anaesthesia are anticipated. Mild to moderate adverse effects are expected to arise the days following surgery. Moderate responses are possible in response to the implanted materials, but their incidence is unlikely to be higher than 10%. All animals will be killed using a schedule 1 method if the severity limit is overcome or at the end of each experiment.

Application of the 3Rs

Replacement

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

Replacement

In vitro tests on murine and human cell lines will be performed prior to any animal studies with a material to demonstrate it's non-toxicity. Beyond these studies, no suitable models are available to accurately reproduce *in vivo* degradation and

inflammation response. Only by studying the material in an animal will the full complex biological picture be available.

No, non-animal-based alternatives are currently available (backed up by recent literature searches). Alternate *in vivo* models are available using mice, but would require a l arger number of animals for the study since only one test and/or control polymer can be implanted per animal. Sprague Dawley rats are preferred since they will permit the implantation of test and control polymers within the same animal, thereby also allowing within and between animal comparisons. Literature will be continued to be reviewed periodically to ensure no new alternatives are developed.

Reduction

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

Reduction

The project will focus on sub cutaneous implantation of biodegradable polymer materials in rats. This model is widely used, in part as 4 samples can be implanted into each rat thus reducing the numbers of animals required in each study.

The minimum number of repeats necessary to provide statistically robust data will be used thus minimising the number of animals required. Furthermore, studies will be conducted at appropriate points when sufficient materials variables are established to eliminate the need for repetition of control materials experiments, each of which would require the use of control animals.

Power calculations have been used to identify the minimum number of animals per group required to provide statistically robust datasets. These will be used to inform experimental design.

Outputs from the studies in this project will be published according to the ARRIVE guidelines to further minimise animal usage in other 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

Rats provide an accessible small animal model system for subcutaneous implantation of polymer materials with their size allowing multiple materials to be implanted per animal thus reducing the number of animals required and providing more robust data. A considerable body of literature has been generated using subcutaneous implantation of polymer materials into rat models and this will provide

substantial dataset with which to compare the results of the project. Literature searches did not reveal any further refinements in techniques, further replacement models'' or methods for reducing animal numbers.

Surgical procedures for subcutaneous implantation of materials have been refined to minimize dis//comfort and advice/supervision/training from NVS will ensure selection of the best anaesthetic and good surgical practice.

All experimental animals will be monitored daily immediately after surgery and weekly thereafter. An observational scoring sheet (developed in conjunction with the NACWO) will be used to identify signs of distress and assess the severity of the pro edure scored using a standard protocol. Inflammation/infection markers will be used to help refine the end point. Rats showing the most severe symptoms will be culled using a schedule 1 method.

PROJECT 66

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.

Word limit; 1000 words

Project Title	Inflammatory changes and fibrosis in renal transplantation
Key Words	kidney, transplant, scarring, early function
Expected duration of the project	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;
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):

Kidney transplantation is an excellent treatment for many patients with kidney failure, but there are insufficient organs to meet the demand, and so patients die waiting for a transplant. Our work focuses on undertaking experimental work that is clinically relevant and has the capacity to translated into clinical practice. Two specific areas will be examined: Early post-transplant injury: about 50% kidney transplants do not work immediately, due to inevitable damage as the kidney is removed, transported and transplanted. Work in my previous licence led to a clinical trial of a drug aimed to improved this rate of function, and further work is proposed to examine the mechanisms of injury and the effect such reduction in injury has on long term outcomes of kidney transplants. Long term damage is the second area of interest: 5% kidney transplants fail every year due to scarring and there is no treatment for this. In this work, we will examine possible treatments aimed at reducing fibrosis which might be translated into clinical trials.

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

The work will improve our understanding of early post-transplant injury, and late failure due to scarring. The benefits will be improved mechanistic understanding and potential translation into clinical trials. Improving the lifetime of transplants benefits the individual patient and other patients waiting for a transplant, as fewer patients need to return to the waiting list.

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

Approximately 3000 mice over the total period of the licence

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

We propose two models: one of early injury is a native model i.e. does not involve a transplant, but we will stop the blood supply to the kidney for between 20 and 45 minutes, causing reversible injury This will allow early investigation of possible treatments, and will minimise the number of transplants needed to be performed. The transplant operation is classified as severe, as it is technically demanding. We have developed stringent measures to assess the mice at the end of the procedure and to cull them if survival is unlikely. In the majority of cases, it is apparent at the end of the operation whether the kidney is likely to function or not, and if not, the animal is culled at this stage. In some circumstances this is less clear, and the animals may become unwell over the following 48hours, they are sluggish and don't feed. Animals are kept under close observation and are culled if they do not recover well.

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 necessary in the complex area of transplantation, and this will be accompanied by relevant cellular work. In addition, as some of the work will be undertaken in parallel with a clinical trial, we have the opportunity to study the clinical relevance of blood and urine samples of transplanted patients.

We continue to review relevant literature and attend scientific meetings of relevance to determine whether there are alternatives to animal experiments in this context and wherever possible seek *in-vitro* alternatives to animal use

Reduction

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

Reduction

The models described are well-established and we have published work based on them. We therefore know the approximate minimum numbers required for each experiment and will plan experiments accordingly. We have significant experience in calculating sample sizes to minimise number of animals. We also use the native IRI model, which is moderate, to undertake preliminary relevant experiments in order to further reduce the number of animals exposed to the substantial transplant procedure.

As a general principle we publish in journals that support the use of the ARRIVE guidelines and make full use of the NC3R's research design tool to ensure all work is well thought through and uses the minimum numbers of animals to obtain meaningful scientific 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 procedures involved are surgical procedures, requiring general anaesthesia, and animals will be monitored closely in the post-operative period. They will be maintained in a warm box for the first 24 hours, and will be given fluids and analgesia as advised. We have a clear protocol that is maintained for each animal, documenting their recovery, with a scoring system with indicators for culling if their recovery is delayed. This has been in practice successfully within our group for several years.

After every piece of work 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.

PROJECT 67

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.

Word limit; 1000 words

Project Title	Investigations into Lumpy Skin Disease virus
Key Words	Poxvirus, Lumpy skin disease, Capripoxvirus
Expected duration of the project	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;
No	(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):

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 again 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 they 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.

PROJECT 68

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secreta=ry 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.

Word limit; 1000 words

Project Title	Tight junction signalling in health and disease
Key Words	cornea, retina, vasculature, inflammation, diabetes
Expected duration of the project	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;
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):

Cells make different types of physical connections with each other and those connections enable tissue formation and normal function of entire organs. One type of such “cell to cell” connection is called tight junction (TJ). TJ connections are essential for cells to form barriers like the skin or the surface of our eyes that separate and protect our bodies and tissues from the outside world. Common disease complications lead to TJ dysfunction. Defects in specific TJ components can lead to diseases affecting different organs including the eye. TJ dysfunction commonly leads to loss of normal organ function with several pathological consequences. For example in the eye, loss of normal TJ connections can lead to blindness through degeneration of ocular cells. This happens in many blinding diseases. Additionally, mutation of TJ proteins has been linked to different human conditions, such as diabetes, deafness, and infertility. To develop new therapies for these conditions, it is imperative to understand how cell-to-cell connections are formed and how they develop and function when they are healthy or diseased.

In this project we aim to characterise novel drugs to manipulate TJ defects in disease and to better understand the role of TJ proteins that we have identified and analysed extensively already in the laboratory so that our knowledge is improved about how their malfunction may contribute to human disease development and progression. Disruption and malfunction of TJ proteins are being linked to an increasing variety of different diseases; hence, there is a clinical need to understand the role of cell-to-cell connections during disease to develop therapeutic tools to repair dysfunctional cell-cell connections and thereby rescue the loss of organ function 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)?

Different diseases in the young and the old as well as the working age population affect the eye, thereby causing a severe burden on society. As human beings so heavily depend on visual function, any visual impairment has major consequences for social and economic costs as well as the employment capacity and quality of life of an individual Additionally, cell-to-cell connection defects can arise from diabetes and inflammatory conditions affecting many other organs in our body apart of the eye such as the kidney, lung, and intestines. Therefore, understanding how cells make connections and how they function in different types of tissues (whether healthy or diseased) is critical for the development of novel therapies. Thus, this project is of a major importance, not only for the individual, but also for society as a whole.

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

We expect to use around 1200 mice per year for the duration of the project licence of five years, a total of 6000 mice and 200 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?

The majority of mice bred under this licence will not experience any adverse effects as they will either have harmless or non-symptomatic genetic mutations. A proportion (around 20-30%) of animals may undergo procedures including general anaesthesia to facilitate imaging of the eyes so that we can analyse how their eyes are affected by specific treatments. Nevertheless, these animals are not expected to suffer any pain. An even smaller proportion (around 2-5%) are expected to undergo procedures with a moderate severity limit such as minor surgery, exposure to increased oxygen concentrations, or to light or chemically induced diabetes. Every effort will be made to minimise discomfort and distress and all animals will be humanely killed at the end.

Application of the 3Rs

Replacement

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

Replacement

Cell-to-cell connections enable tissues to form barriers between different areas. For example, the intestinal lining separates the intestinal duct from the body. We have studied TJ functions in laboratory models of disease but many TJ functions cannot be recreated in a test tube and the consequences of TJ dysfunction affects not only the cells that form TJs but also neighbouring cells and tissues. Therefore, by only

using cells in a laboratory, it is not possible to study all aspects of TJ functions, including what happens to them when diseased, and how they can be manipulated for therapeutic purposes. We hence need to start to use animal models to complement our experiments with cultured cells and to test and further develop therapeutic tools that we have generated and explored using cells in a laboratory. Nevertheless, experiments with so-called “cultured” cells will continue to develop and refine our experimental approaches so that we keep the number of animals required and the discomfort caused as low as possible.

Reduction

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

Reduction

All animal experiments will be based on prior detailed analysis of tests in a laboratory to ensure experiments with animals can be designed as precisely as possible and using the fewest required animals with experimental tools that are refined as far as possible. For all our studies we carry out power calculations in advance to establish the minimum number of mice needed to obtain statistically sound data. Furthermore, we have developed in vivo imaging methods that allow us to follow animals over time, thereby reducing the total number of animals needed to study different time points. We will also do pilot experiments to assess which approaches will yield the greatest amount of useful information. Additionally, we will plan our experiments ahead to breed and use the minimum number of genetically modified animals and we will use both males and females for our 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 have chosen rodents as they replicate most features of human disease. We are not aware of any alternative to animal models that provide the necessary complexity to enable us to achieve our objectives. Cell-to-cell junctions have the same functions and similar structure in mice and human beings, and it is relatively easily be observe and manipulate them in rodents. Rodents are therefore one of the most useful model systems in cell-to-cell junction biology. Furthermore, recent developments in genetic engineering allow the manipulation of specific genes in a time-dependent manner in specific cells. This means the effects on the animals of such manipulations are minimal.

To minimise pain and distress, we will carefully evaluate what is already known about the specific substances we plan to use. We shall also seek veterinary advice to ensure that the least invasive route of administration with the fewest adverse effects is used. Moreover, we plan to use animal models that are well established along with well-established assessment techniques. Potential side effects are consequently well understood and the required measures can be taken to minimise pain and distress. Undertaking careful monitoring of all animals that undergo procedures to evaluate welfare will be a principal objective.

PROJECT 69

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.

Word limit; 1000 words

Project Title	Molecular mechanisms underpinning behavioural stress responses in the rodent
Key Words	Stress, adaptation, genes, coping, rats
Expected duration of the project	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):

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 out 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 amoungst 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 of 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.

PROJECT 70

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.

Word limit; 1000 words

Project Title	Mechanisms of speciation in cichlid fish
Key Words	hybridization, biodiversity, cichlid
Expected duration of the project	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):

Hybridizing individuals from different populations of a cichlid fish generates phenotypic change in the F2 generation. We will quantify this, and use genomic approaches to understand the mechanisms involved.

Test that male cichlid bower shape is a heritable trait by comparing variance in bower shape within and between individuals.

Test that female preference for bower shape exists by a) allowing free choice between bowers of known shape, and b) offering alternative shapes, and DNA paternity testing offspring.

Develop technical tools to help understanding the underlying molecular mechanism of adaptation and speciation, by altering the functionality of candidate genes from genomic analyses.

What 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 has been REDACTED demonstrated that hybridization can lead to morphological variation in the F2 generation of lineages in cichlid fish, and we now seek to use genomic techniques to understand the precise mechanism of this. This will have advanced our understanding of the role hybridisation may play in the generation of biodiversity, thus significantly advancing the fields of evolutionary biology and conservation. Additionally, understanding the genomic mechanisms in this system will have wider importance for other systems- we will be able to look for similar signatures in other groups of species, but also in species important as food sources (plants and animals) where producing hybrids can lead to a change in important

traits for cultivation, and understanding precisely how will be important for future development. Our work examining the heritability of an extended phenotype, male bower shape is crucial in assessing whether bower shape is a trait that could be under selection, and therefore play a role in speciation. In the longer term, we anticipate that the results from these studies will provide insights into the mechanisms responsible for speciation, furthering our fundamental understanding of evolution in general, and assisting conservation and management of the cichlids of Lake Malawi. The amendments we propose allow us to take this work further. The discovery of novel gene functions and the understanding of the related gene networks may lead to better understanding of human genes important for our adaptation to the environment. REDACTED. By collaborating with other research groups and providing mutant lines, we sought to consolidate scientific findings and to minimise duplication of effort leading to fewer animals being used overall. These mouth-brooding cichlid fish are ideal since the fertilised eggs are held in the females mouth and can be easily removed without damage to the female or eggs. Using the traditional observational methods, large numbers of replicates are required to become statistically significant to draw conclusions. The generation of customised mutations for phenotypic study is the most powerful way to confirm the causative relation between gene functions and morphological appearance. As a result, to have the same level of certitude, fewer animals will be used.

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

We will use a maximum of 1200 fish over the duration of the project. We use cichlids broadly identified as Haplochromis sp.,including Nyassachromis cf. microcephalus and Astatotilapia calliptera, Rhamphochromis "chilingali", Trematocranus placodon.

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

These are mild procedures, and we anticipate no adverse effects. We will target regions of the genome involved in morphological and colour change, but it is possible that some of the mutants we produce may not be healthy, in which case they will be euthanised as outlined in the protocol above. Most animals will continue to live in our aquarium facilities until they reach the end of their natural lives.

Application of the 3Rs

Replacement

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

Replacement

Our experiments focus on the empirical outcome of specific hybrid crosses and mate behaviour, so there is no way of achieving the benefits of this research without the use of animals. These species (*Astatotilapia calliptera* and *Nyassachromis* cf. *microcephalus*) address the specific questions we investigate. REDACTED Other closely related species kept in the aquarium facility may also be used.

Random mutations in the genome may lead to morphological novelties, which under natural selection results in the present biodiversity. What we need to understand is precisely the relation between genetic variance in the complex gene network of the whole animal and the resulting morphological/behavioural difference. As little is known about the genes responsible for each morphological/behavioural trait and their function, we cannot avoid using intact animals for the purpose of this project.

Additionally, we know they breed and thrive in our aquarium facility, and they are robust enough to withstand the mild procedures we propose with the minimum of stress.

Reduction

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

Reduction

1. Quantify that hybridizing individuals from different populations of a cichlid fish generates adaptive change in the F2 generation.

This study has been carried out successfully under the previous license, and we know that the numbers we propose allow statistically meaningful interpretation of the data REDACTED. We now seek to continue work to allow genomic analysis from fin clips (such technology was not previously available or cost-effective).

2 & 3. Test that male cichlid bower shape is a heritable trait by comparing variance in bower shape within and between individuals.

In order to minimise the number of males undergoing the protocol, we will estimate upper bound heritability using repeatability, which describes the proportion of variance in a character that occurs among rather than within individuals, calculated using the equation: $r = s^2_A/(s^2 + s^2_A)$, where s^2_A is the among groups variance component and s^2 is the within groups variance component. Estimates of variance increase in accuracy with sample size. We will use 12 males in two pools, and allow them to build bowers. Based on field observations of bower density, we predict 8 males will be able to build bowers, but we need the additional 4 males to promote a low level of competition to instigate bower building. We will allow these males to build bowers as many times as possible but we estimate that three times within the time limits of the project is realistic. The total number of males that will undergo the protocol is therefore **24.**

3. Test that female preference for bower shape exists by a) allowing free choice between bowers of known shape, and b) offering alternative shapes, and DNA paternity testing offspring. We will allow females to mate with males occupying the bowers in objective 3. Our null hypothesis predicts that spawning decisions conform to a discrete uniform distribution and we will test the goodness fit of our data with that distribution using Pearson's chi-squared test. *A priori* power analysis given 7 degrees of freedom (assuming 8 bowers) alpha 0.05, power 0.8 and large effect size 0.5 indicates a sample size of 58 spawnings is required. Therefore, **58** females will undergo the protocol. The experiment will be replicated three times using the same females to examine consistency of choice within females and minimize the total number of animals that need to be tagged.

A more rigorous procedure will be used to disentangle the effect of bower and male on female choice. Six compartments will each hold a male on an artificial bower in one of three categories (high bower, low bower, no bower with two replicates of each). *A priori* power analysis given 5 degrees of freedom, alpha 0.05, power 0.8 and large effect size 0.5 indicates a sample size of 52 females is required. In this case, each female needs to be offered a different combination of male/bowers so after each spawning, one male will be removed and replaced. A total of 47 males will therefore be needed, bringing the total number of fish undergoing the protocol to **99** for objective 4b. These experiments (a and b) will run simultaneously.

4. Establish an efficient genome editing toolkit for East African cichlid fish with which to link genetic variance to morphological difference

To reduce the number of animals kept under regulated procedures we will strive to maintain the smallest number necessary to carry out a particular experiment, e.g. for each mutation line, only F0 animals with confirmed germ line transmission will be mated (or alternatively, sperms of which will be used to fertilise eggs from WT females) to breed into adult F1 fish. We will also endeavour to preserve lines that are not currently being used by sperm freezing thus only maintaining actively used animals in the facility. In addition, the use of homozygous mutant animals allows us to reduce the total number of animals to characterise a gene. Under protocol 1, we would raise such homozygous mutant animals to adulthood to be used in the characterisation of mutant gene function.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 the procedures we propose are mild. The species we propose are evolutionarily important, and are large enough that small fin clips and the insertion of microchip PIT tags are relatively uninvasive. We know they breed and thrive in our aquarium facility, and they are robust enough to withstand the mild procedures we propose with the minimum of stress.

Where possible we will use the most up to date technology (i.e. smallest PIT tags) available. Unfortunately tag systems are not compatible so a swap to different technology would mean re-tagging some of the fish that are already tagged and in use. Instead, we will try to bring one part of the work to a close, using current fish, and schedule the most appropriate time to swap systems maximising data collection from those animals already tagged.

Objective 1 and 4 requires that the animals are tagged for identification purposes. This enables them to be returned to community stock tanks and interact freely with other individuals, instead of having to be kept in isolation. *Rhamphochromis sp.* and *Trematocranus placodon* have the most distinct morphology and behaviour REDACTED, therefore the traits are easily distinguishable and a smaller number of fish are needed to demonstrate functionality.

PROJECT 71

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.

Word limit; 1000 words

Project Title	Exposure of wild birds to neonicotinoids
Key Words	Birds, wild, farmland, neonicotinoids, exposure
Expected duration of the project	2 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);
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):

Neonicotinoid insecticides are used worldwide as a seed dressing to control pests of economically important crops. We aim to establish circulating levels of neonicotinoids in wild birds in the UK. Alongside establishing likely exposure routes, we also aim to determine whether there are any subtle or sub-lethal effects, which may cause adverse health effects in wild birds. Currently, very little is known about the sub-lethal effects of neonicotinoid pesticides on birds, especially outside of aviary-based protocols. Our research programme will fill this knowledge gap, to better inform current policy and legislation concerning the use of pesticides. Currently neonicotinoids are subject to a temporary and partial moratorium while their impacts on pollinator species are assessed.

Birds have not been included in this moratorium, thus neonicotinoids are still used as a seed coating for non-flowering crops such as sugar beet and winter wheat. Farmland birds have been seen to feed on seeds and seedlings of these crops, but the effects on their health and fitness have yet to be fully assessed.

There are few data showing true exposure of wild birds to neonicotinoids (by measurement of residue in biological samples) and to our knowledge no studies concerned with potential sub-lethal effects of neonicotinoids in relation to wild birds *in situ*. Our work will therefore have policy, environmental management and regulatory implications.

What 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 help us to understand the complex links between exposure to neonicotinoid pesticides and the effects on birds including health and parasite load. This will have broad implications for both our understanding of avian physiology and how animals are likely to cope with environmental change. Thus findings from our study may provide data useful for the conservation and management of wild birds. In addition, this research will help to improve the environmental risk assessments for neonicotinoids. Currently, there is an EU-wide moratorium on the use of neonicotinoids on flowering crops while further data are gathered. If a full ban is implemented starting in 2018 or 2019, then there is even more urgency to complete this study.

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

600 wild birds (maximum) over two field seasons. The project focuses on farmland birds. Each bird will have a maximum handling time of 30 minutes during a capture event and immediately released back into the wild.

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

After capture, identification and taking non-invasive measurements using standard methods, we will simply be taking a small blood sample and returning birds to the wild. Capture and handling stress will be minimised because only experienced researchers will be involved and best practice will be followed. The adverse effects are minimal and the severity rating for this work is MILD.

Application of the 3Rs

Replacement

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

Replacement

The protocol is based on studying the physiology of wild birds in relation to an exposure that takes place in a natural setting, so there is no alternative to the use of live sentient animals. We are selected species based on their diets, how easy and robust they are to capture, as well as their propensity to live on farmland.

Reduction

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

Reduction

Statistical analysis has been used to determine the optimal sample size. The project licence holder is experienced in running statistical analyses, but will seek specialist advice where required to maximise the results acquired from the minimum number of animals.

In the wild, environmental and genetic variability mean that relatively large sample sizes are needed when investigating exposure and effects. The sampling effort will be adjusted to ensure that we achieve the sample size that we calculate is required. In line with best practice, we will maximise the data acquired from each individual by ensuring that we take multiple non-invasive samples and measures, as well as biological sample(s).

Refinement

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

Refinement

Birds are the ideal target species for this project because:[

a) Their foraging behaviour and physiology are well characterised;

b) The research team have worked on our planned focal species before and know how to safely capture and handle them;

c) Birds are a sentinel of environmental contaminants.

Having such detailed knowledge of a particular system is rare and vital to the success of this project.

Minimising welfare costs:

All researchers participating in this experiment have prior experience in handling birds. Where training is required for staff and students it will be done by experienced staff with guidance from a veterinarian. We will use the most sensitive analytic techniques in order to minimise the volumes of blood required (a maximum of 1% of body mass which equates to a maximum volume of 10% of the circulating blood volume). also we will validate less invasive methods of assessing residue concentrations using faecal rather than blood samples.

PROJECT 72

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.

Word limit; 1000 words

Project Title	Using Genetically Altered (GA) mice to model cancer
Key Words	Cancer, Oncogene, genetic
Expected duration of the project	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):

Oncogenes are genes that when mutated or switched on inappropriately can contribute to the cancer development. Some cancer cells absolutely depend on the action of these genes to continue to survive and growth. We wish to understand which genes are essential to specific cancer types and if switching these genes off can result in complete regression of the cancer. We are particularly interested in those genes that act as master switches capable of turning whole sets of genes on and off. The aim is therefore try and identify new weak points in specific forms of cancer, and we will be testing mainly blood cancers and breast 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)?

The potential benefits are to find and exploit key vulnerabilities in cancer cells. Such an approach could aid the development of specific drugs and therapies in particular forms of cancer. Our experiments will determine which cancer types may be suitable for testing with these new drugs.

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

Experimentation with mice is required because tissue culture based systems provide only a limited insight into gene driven changes in cell behaviour. However, tissue culture work is used as an alternative to animal experiments whenever possible. It is estimated that the project will use approximately 7000-10000 mice over the next five years. Given the very large numbers of animals used it is worth noting that the majority of genetically modified mice do not succumb to disease and remain healthy throughout the study period. In those animals that do develop cancer suffering is

minimised by humanely euthanizing mice at defined end points and generally before the disease causes distress.

In the context of what you propose to do to 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 be predisposed to cancer. Approximately 80% 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 20%) will develop cancer because of their genetic makeup or because tumour cells have been implanted and allowed to grow. This may require administration of an inducing 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. A proportion of animals may be treated with substances to inhibit cancer or modulate oncogenic signals and the response to these treatments monitored. 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. Any animal that displays signs of illness such as weight loss of 15%, 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.

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 in the lab, it is not easy 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 (a process called metastasis) is very difficult to do other than in a mammalian 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.

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 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 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 study group.

PROJECT 73

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.

Word limit; 1000 words

Project Title	Heart failure & ventricular arrhythmogenesis
Key Words	Heart Failure, ion channel, myocyte, ventricular arrhythmia
Expected duration of the project	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;
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):

Sudden cardiac death is a major cause of death in patients suffering from heart failure. It is well recognised that the changes that occur within heart cells during heart failure predispose patients to the development of cardiac arrhythmias and this explains sudden cardiac death, however the precise mechanisms by which these changes occur remain unclear. Consequently, effective treatments to prevent sudden cardiac death have not as yet been identified.

What 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 overarching aim of the work is to advance understanding of the precise changes that occur in calcium and electrical signalling in failing heart cells. The knowledge gained by these studies will then be used to identify novel treatments that may be used to prevent arrhythmia and reduce the risk of sudden cardiac death. In the longer term, the work is expected to contribute to the development of safer and more effective therapies for the treatment of patients suffering from heart failure.

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

The project will be conducted using cardiac preparations from rabbits (up to 275 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 covered in this licence include killing using a humane method in order to obtain viable tissue and the induction of heart failure by coronary artery

ligation or tachypacing by surgery under full anaesthesia. The procedures used are refined to minimize suffering and post-operative analgesia will be given in line with best veterinary practice. The severity is defined as severe, because the procedures carry a risk of sudden death. However, with the exception of the immediate postoperative period, during which the animals will be given analgesics, the animals should not experience any suffering as they develop heart failure. Humans suffering from the early effects of heart failure, which correlates to that experienced by the animals, report only minor discomfort. The animals will be humanely killed once heart failure has developed to a point comparable to that used for initial clinical diagnosis in humans.

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 alternative to the use of animals for these studies as the experiments require viable heart muscle cells which have developed the pathological changes associated with heart failure. Such diseased cells cannot be reproduced using cell culture systems as cultured heart cells do not perform the contractile action essential to the functioning of the heart and do not undergo the changes found in failing heart cells. Consequently, these studies cannot be undertaken without the use of living animal.

Reduction

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

Reduction

The group size for the study has been estimated on the basis of power calculations from published data and data provided by colleagues using the models used in these studies. This approach ensures that we achieve statistically significant data using the minimum number of animals. The ex-vivo approach used enables large amounts of data from numerous heart cells to be obtained from a single animal, thereby maximising data output whilst minimising animal usage. In addition, as part of the programme of work the data generated from animals and cell cultures will be used to develop a computer based model to help predict drug dose-responses without the need to use animals to optimise drug levels. This development will enable us to significantly reduce the overall number of animals needed to meet our objective.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 represents a well-characterised model for studying abnormalities in repolarisation of the ventricles. The rabbit is the most relevant laboratory species for these studies as the conduction and contractile characteristics of its heart cells more closely replicate those of human heart cells than either the rats, mouse or lower species. Consequently, the rabbit heart has been widely used in the study of cardiac function and there is extensive background information available on its physiology and anatomy. Reliable, reproducible and well refined models of heart failure in rabbits are well-established. The procedures used in this study have been refined REDACTED and are designed to minimise suffering through appropriate analgesia and anaesthesia during surgery and standard post-operative veterinary analgesia. In addition, the endpoint for the study are set at a level that ensures that animals are killed before they experience any actual suffering as a consequence of heart failure.

PROJECT 74

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.

Word limit; 1000 words

Project Title	Pre-clinical cancer models for identifying and screening novel drug and imaging agents for cancer diagnosis and treatment
Key Words	cancer, treatment, imaging, refined models
Expected duration of the project	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;
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 purpose of this licence is to develop more accurate cancer models which can then be used to validate new tumour target and develop novel therapies, drug delivery systems and imaging agents.

This will be accomplished via the following 5 objectives

The specific objectives are:

1. To develop and improve animal models of human cancer (including breast, ovarian, colorectal, gastric, kidney, pancreas, oesophagus, brain, melanoma, lung, bladder, prostate, leukaemia)

a) incorporating relevant aspects of the tumour microenvironment (other human cell-types and/or molecules present in patient tumours that influence the behaviour of cancer cells, including their growth rates, ability to spread throughout the body and their response to treatment)

b) growing tumour cells in relevant organs, e.g. (breast) including secondary sites of growth e.g. bone

2. The identification and validation of potential drug targets for clinical use in the treatment of the above cancer types.

3. To evaluate how potential drugs and imaging compounds behave in the body including how long they remain in circulation, which organs they localise to and safe doses that can be used.

4. To identify and test the effectiveness of new drugs, drug delivery systems and imaging compounds prior to use in patients

5. To develop and improve imaging (scanning / visualisation) technologies for use in animal models of human cancer

a) To apply imaging systems already being used in the clinic e.g. MRI, CT scans in order to improve our understanding of how results obtained in the animal models can be transferred into use in patients.

b) to provide readouts of the biology of the cancer cells and their microenvironment

c) to test novel imaging compounds, which may also be associated with drug treatments to provide a combined theranostic,(therapy / diagnostic) approach with both imaging and treatment in one delivery 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)?

The main benefit will come from the application of superior cancer models which are more representative of the patient's tumour in terms of biology and response to treatment. These will provide more appropriate models that are more predictive of patient response to new drugs, and allow assessment of new imaging (diagnostic) agents (for e.g. MRI and PET scans) and drug delivery systems, thereby shortening drug development times. This will reduce unnecessary use of animal and the costs associated with drug development by identifying drugs/imaging agents unlikely to be effective in a patient and eliminating them at an early stage in the drug discovery process. These benefits are already being realised in ongoing studies based on the models we have already developed and intend to continue to use under this new licence. In addition, as the research will contribute to a better understanding of the biology underlying cancer development, spread and response to drugs, in particular, the way in which other components of tumours influence the behaviour of cancer cells, it may allow identification of new drug targets and development of drugs which target tumour-related molecules that cannot be assessed in current models. Such benefits are likely to be realised 10 - 15-years following development of drug molecules directed against these targets. Alongside using the models to assess new drugs, we will use tissues collected from the animals to carry out laboratory tests to identify molecules (biomarkers) that would allow treatment to be targeted both by 'personalising' a treatment to an individual's cancer, and allowing treatment to be targeted to a specific growth/response phase when the cancer is most likely to respond to treatment. This idea of a targeted 'personalised' treatment regime is an area that is currently of great interest and a predicted way forward in the clinic. These benefits are likely to be realised within a 5 -10-year timeframe. Improved models will also facilitate the development of novel drug delivery systems such as those which can overcome the problems associated with drugs crossing the blood/brain barrier or other delivery obstacles, improving success rates of early

stage clinical trials. Again, these are likely to result in patient benefit in an approximately 10-year timeframe. In addition to testing imaging systems with potential application in the clinic, we will continue to assess and develop new imaging systems for use in the cancer models. The development and application of biologically-based visible systems for monitoring of tumour establishment, spread and biology will allow us to identify relevant and potentially earlier scientific endpoints, reduce the numbers of animals required for pre-clinical drug assessment, as well as increasing our understanding about the influence of the environment surrounding and interacting with the tumour and how this can affect cancer progression, growth, spread and drug responses, and how cancer cells interact with imaging techniques and drug delivery systems. These benefits are already being realised within our laboratory and the further work we will do, will continue to have immediate benefit in our own research. Further benefits will be derived from publication of findings in peer-reviewed academic journals which may enable other groups to use our models or data to develop new treatments or diagnostic assays. Also, our improvements in techniques will be reported at appropriate scientific meetings to disseminate best practice in the 3Rs. This will further enhance the impact of our work, increasing its extent and the probability of the benefits being realised. We will also provide a service for external collaborators who wish to benefit from our expertise and experience in the field of in vivo (in animal) cancer modelling, where it falls within the remit of this licence.

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

Mice-10,500 Rats-500 over 5 years Some of these animals will have a reduced immune system in order to implant human tissue without the problem of rejection by the animal’s own immune system.

In the context of what you propose to do to 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 or implanted with various tumour cells types in a number of organs and then drugs, molecules designed to improve drug delivery or imaging molecules will be given to the animals via a variety of routes such as via a vein or under the skin. Adverse effects could include tumour ulceration, unexpected effects of the treatments under test or surgical complications, but good techniques combined with careful monitoring by well-trained staff will keep these to a minimum to give an overall severity level of “moderate”. In addition to standard methods of monitoring tumour growth, we will apply and further develop imaging systems which allow monitoring in a more detailed way. These will be particularly helpful in monitoring models in which cancer cells are grown in organs deep within the body, allowing us to identify relevant timepoints for ending experiments which allow scientific information to be obtained prior to excessive growth of tumours that would otherwise potentially cause adverse effects. At the end of the experiments, all

animals will be humanely killed, with organs collected for further analysis in the laboratory and data generation.

Application of the 3Rs

Replacement

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

Replacement

Through development of “close to human” laboratory-based cell culture models we are pioneering in our research unit, we are working towards replacing the use of animal models for some purposes. For example, 3D models that incorporate relevant aspects of the tumour microenvironment, are being used in the lab to examine drug sensitivity, or potential drug toxicity in normal tissues, which will partly assist in achieving the overall objectives of the programme of work. Thus, such laboratory methods will be used where possible to identify candidate drugs suitable for animal testing. These methods will include cell growth, viability, and death as a result of drug treatments, possibly in new combinations, when compared to current available treatments. The use of tissue generated in our animal models is important for validating such laboratory-based models.

However, while in some cases we have managed to develop laboratory-based models which are superior to the standard models used in cancer drug development, it is still not possible to recreate some aspects of *in vivo* biology in the cell culture setting e.g. the development of blood vessels and thereby relevant drug access or the many physiological mechanisms in play in the body that allows relevant tumour biology drug/imaging compound assessment. Furthermore, research and development of anti-cancer agents involves a multi-step process in which animal studies still form an intrinsic, required part of the regulatory process linked to the international approval of new drugs, therefore, some use of animal models is still required.

Reduction

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

Reduction

Pilot studies will be performed on any new tumour model to identify experimental variation in tumour growth and this will be used directly in calculating group sizes to provide statistical significance.

In addition, laboratory studies will be used to evaluate dose ranges confirming effectiveness which will be translated into clinically relevant doses for use in the pilot

tolerability studies, which will ensure the drug has no side effects in a tumour bearing animal.

We will obtain advice from clinical colleagues regarding clinical treatment protocols (cycles, dosing and routes of administration) and pharmacy colleagues will be consulted when considering how new compounds will be carried around the body.

Study design for all objectives of this licence will use both negative and positive controls; the negative control for example may be an untreated group, while a positive control may be a drug already used in the clinic.

The use of short-term tolerability studies may be avoided if supporting data is already available in a relevant model.

Conventional approaches to monitoring disease and the effects of treatments in these models frequently rely on analysis of tissue obtained post-mortem, to look at the progression of the disease. Using this approach would require substantial numbers of animals to look at the development of disease over time and the effectiveness of any treatment. By the use of the established whole animal imaging technology, we are able to look at the progression of disease and treatment within an individual animal. This approach removes variation between animals in the disease model, therefore refining the experimental approach. Definition of scientific end points, before death occurs, is also a key aspect for refinement of existing models; we are able to gain sufficient information of effectiveness of disease and treatment earlier in the disease progression, so where relevant and available, the use of different types of imaging (scanning) technologies, e.g. MRI, CT/PET, CT/SPECT, will be used to reduce the numbers of animals used: for example, for an tumour model whose internal tumour dimensions cannot be measured, tumour growth would normally have to be assessed by timed terminations of multiple study groups; using scanning, this could be assessed in a single group of animals by multiple image points. Likewise, for a treatment study looking at tumour uptake of a particular compound, the compound's ability to target the tumour, treatment and how quickly it is removed from the body can be done in the same animal without the need for multiple termination groups. However, while allowing an overall reduction in numbers, it should be borne in mind that the cost to an individual animal in terms of the number and potentially the severity of the procedures it may be subject to will increase. Therefore, each protocol has a maximum number of optional procedures any one animal will undergo, and the potential increase in side effects will also be carefully monitored.

Group size will be determined by statistical power calculations which will be based on the known variation within the model, as determined by pilot studies which will use the minimum number required to achieve significance and the anticipated effectiveness of the agent in relation to blood levels as determined by laboratory

evaluation. The determination of group size will be aided by pilot growth studies to establish take rates for the different tumour models

The number of groups and, therefore animals required for a specific experiment, is dependent on the particular study rationale, which will be detailed in its individual study protocol. For example, for a simple growth study, there may be only 2 groups of 8 mice, each group being dosed with differing tumour cell concentrations, but for a new treatment study, there may be 5 groups of 8-10 mice, namely

1) vehicle control (untreated control not required as growth rate already established through growth studies)

2) drug A

3) drug B

4) drugs A+B combined

5) current recognised treatment drug

We have access to statistical support through in-house and external colleagues and from websites which we utilise to provide additional information on statistics and experimental design. Use of analysis by dry blood spot (DBS), (where available), or microsampling (taking only a very small sample of blood), would reduce the number of animals required for these types of studies as serial sampling could be used on the same animal, rather than a set of animals per time point.

For each study, as part of good laboratory practice, we will write an experimental protocol which includes:

- a statement of the objective(s)
- a description of the experiment, covering such matters as surgical protocols, experimental treatments, imaging, the size of the experiment (number of groups, number of animals/group), duration, and the experimental material
- tissues to be collected and method of processing at termination
- details of potential adverse effects and humane end points
- an outline of the method of analysis of the 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

Tumour models grown in the organ of source (“orthotopic”) are known to better model cancer in patients with respect to various criteria as they form a single focal

disease area as in the patient situation, facilitate spread to other parts of the body and show a reduced response to treatment. The choice of model used is dependent upon the target cancer; brain tumours for example, present a very different set of problems to that of other target organs as any treatment must cross the blood:brain barrier to be effective; furthermore, the underlying biological mechanisms of cancer growth and progression are often influenced by the tissue of origin. Orthotopic models better represent the human situation and, therefore, treatments developed in these models can more easily and accurately be translated into the clinical setting. However, as part of this project, we hope to further improve the subcutaneous (under the skin) models by incorporating elements of the tumour microenvironment so they better represent the orthotopic model system but is less invasive and, therefore, less harmful to the animal.

Mice and rats are the lowest species in which a genetic mutation has produced a weakened immune system which allows the growth of human tumours. All such animals will be housed in enclosed, self-contained systems with appropriate “super clean” husbandry procedures, in order to prevent possible infections due to their lower immunity level. Mice will the animal of choice in most instances, although rats will be used in the brain tumour model due to their larger size and, therefore, practicality of use. The body structure of mammals is required to accurately replicate tumour development and spread to relevant organs. However, where it is possible to use normal mice, for example, when assessing initial scanning agent properties, we will use these instead.

While some groups may study cancer in the zebra fish, it is currently not an appropriate model for our work as the body system does not accurately reflect mammalian physiology. For example, they have no mammary fat pad essential for the implantation of breast tumours. Therefore, drug distribution studies, certain models (e.g. prostate, mammary fat pad), dosing routes etc. cannot be carried out with clinical relevance, if at all. Also, the zebra fish xenograft model only lasts a few days which limits the length of time dosing can be performed, again compromising clinical relevance.

The models we use have been carefully selected to be the most patient relevant while being the least invasive in terms of surgery and procedures. Where surgery is necessary, all procedures will be conducted under sterile conditions using suitable anaesthetics. Where necessary, and as advised by the vet, analgesia will be administered to minimise pain, due to both surgery and to specific painful models. During surgery, a High Definition camera system will be employed to assist with visualisation and introduction of cells into the appropriate organs. This camera allows the surgical area to be observed under magnification on a screen allowing introduction of cells into the correct organ or region with greater accuracy and consistency. Pilot studies for the establishment of novel surgical techniques will be

carried out and advice from the Named Persons and other colleagues in the field will be sought in this respect.

Other model refinements that minimise animal suffering include the following:

- Using a catheter via the urethra for bladder initiation will be the method of choice, as it is less invasive than surgical implantation.

- Delivering hormone supplementation (essential for the growth of some tumour types) in food rather than having to surgically implant a hormone pellet.

- A jacket, to which the animal will be acclimatised, will be employed to hold non-invasive drug delivery devices such as magnets, in preference to gluing or surgically implanting such devices in position.

- Use of pilot tolerability studies will ensure there are no unexpected side effects associated with new models or novel imaging compounds, or unexpected toxicity as a result of tumour:drug interactions, and to ensure the drug levels used are not associated with any long term cumulative effects.

Animals will be monitored by trained technical staff daily; they will be weighed and tumours will be measured physically or by imaging at least weekly. The maximum allowable tumour size will be as detailed in the current Best Practice guidelines. Real-time imaging facilitates the early termination of models in which the primary tumour is at an internal location or if there is spread to other organs.

We select our end points on a scientific rather than an adverse effect basis, with our use of imaging technologies and careful observation and monitoring by our experienced staff allowing us to select and anticipate end points as soon as we have scientifically relevant data and prior to the onset of adverse effects.

We have developed our experimental techniques over many years and will continue to refine these as technology and knowledge gained throughout the course of this licence allows. This is particularly relevant in our surgical procedures where we constantly re-assess existing techniques in order to develop refinements which will improve animal welfare or lead to a reduction in numbers of animals required.

PROJECT 75

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.

Word limit; 1000 words

Project Title	Fish telemetry investigations to inform effective management actions – monitoring tagged fish already at liberty
Key Words	Fish, telemetry, migration, movement, behaviour
Expected duration of the project	0 year(s) 6 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.

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):

Fish populations in the UK, including salmonids, coarse fish and conservation species (e.g. lamprey, eel and shad), face many pressures in the freshwater environment, including exploitation, fish stocking, pollution, abstraction, navigation, power generation, flow regulation and habitat modification. Understanding these pressure, which will be locally specific, is crucial to the implementation of effective management actions and targeted rehabilitation projects driven by legislation. Fish will be implanted with marks and tags, using well established methods and techniques, to monitor their movements, behaviour, and habitat use in order to target and assess improvements to the aquatic environment aimed at enhancing fish, eel and lamprey populations in the UK.

What 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 beneficiaries of knowledge arising from this research is anticipated to be governing bodies (e.g. the EA in England and their equivalents across Europe), who will be able to use outputs to inform and revise policy, regulation or operational best practice. Implementation of policy, regulatory or operational advancements will ensure freshwater environments worldwide are sustainably managed within legislative frameworks to deliver positive environmental outcomes.

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

Brown trout. <1,000

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?

Tagged brown trout have already been detected and appear to be behaving normally so no further adverse effects are expected

Application of the 3Rs

Replacement

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

Replacement

This study is focused on wild fish in a locally specific situation; therefore a non-animal alternative approach cannot be used and all non-tagging alternatives are technically inadequate.

Reduction

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

Reduction

Non-animal techniques will be used were appropriate to minimise the numbers of animals, including using modern techniques like underwater video monitoring and hydro-acoustics.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 tagging protocol was carried out under anaesthetic to ensure no pain is experienced. In some instances the procedure involved a small incision on the belly of the fish, an inert and sterile transmitter inserted and the incision closed with a suture to ensure full recovery and minimal suffering.

PROJECT 76

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.

Word limit; 1000 words

Project Title	Immunity and Transmission of Influenza Viruses in Pigs
Key Words	Swine Influenza, pigs, transmission, influenza vaccines, local pulmonary immunity
Expected duration of the project	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.

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):

Swine influenza (Flu) viruses cause disease resulting in economic loss to global pig production. In addition, swine Flu can spread to humans and may cause influenza pandemics. Unfortunately the vaccines currently available to control Flu infections in pigs and people are not very effective, and new immunisation strategies are required.

We will study how swine Flu viruses are transmitted between pigs and how they change during transmission. Understanding how swine viruses transmit between pigs and humans will help design effective control policies like quarantine, vaccination or culling.

In addition Flu viruses change constantly which is the reason why Flu vaccines must be updated annually to match the current strain. There is therefore a need for better vaccines and a vaccine which could protect against many Flu strains, “a universal vaccine”, is highly desirable.

Recently it has become clear that giving a vaccine directly into the lungs is much more effective in inducing universal protection against Flu, because it induces white cells, called tissue resident memory T cells, which remain in the lung and rapidly fight lung infection. We shall develop methods to give vaccines to the lung of pigs, analyse whether they induce tissue resident memory cells and measure whether they protect against different Flu strains.

Finally it has been shown that the harmless bacteria which live in the gut can alter immune responses to vaccines. We will work out how the harmless bacteria alter the immune response to vaccines and design vaccines that can overcome this effect.

What 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 effective Flu vaccines would help to prevent the spread of dangerous pandemic Flu strains. Understanding how Flu viruses transmit between pigs and whether vaccines can stop this transmission will help us design better control strategies in the face of Flu epidemics and pandemics. Flu vaccines that worked equally well in different environments would be highly desirable. Since swine Flu is a considerable problem in the UK, costing the farming industry and government millions of pounds annually, better vaccines will be of great benefit. Vaccines that are effective against swine Flu in pigs are very likely to work against Flu in humans.

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

We shall use no more than 436 adult 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?

Most pigs will be vaccinated by injection or intranasally, which requires light and short sedation. Immunisation has few side effects. Very rarely injection can cause local inflammation. Some animals will be exposed to Flu and become infected. Animals infected with Flu will develop mild to moderate clinical signs for a few days. All animals will be monitored post-infection. All animals are euthanised at the end of the experiments by an appropriate method. The 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

We can only measure transmission of Flu viruses and whether a vaccine protects against swine Flu in the living animal. Given the nature and localisation of the Tissue resident memory cells it is not possible to use an *in vitro* system to study how these cells are induced following vaccination, nor it is possible to measure immune responses to or protective efficacy of a vaccine without the use of animals. However after establishing how best to induce these cells, we shall perform a series of *in vitro* experiments to determine what factors influence their maintenance and survival.

Reduction

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

Reduction

Before carrying out experiments we calculate the smallest number of animals needed to obtain a statistically significant difference between experimental and control groups 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

We will use pigs because they are natural host of swine Flu and also because their immune system is very similar to that of humans. Animals infected with Flu or other live organisms will be very carefully monitored for clinical signs of disease by staff who are trained in daily animal handling, husbandry and the recognition of signs of pain, distress, disease and the ethics on the use of animals in research. The pigs will be housed in groups or pairs to allow for normal social interaction. They are given straw beds and supplied with a variety of enrichment.

PROJECT 77

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.

Word limit; 1000 words

Project Title	Establishment and Regulation of Collective T cell immune responses
Key Words	
Expected duration of the project	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):

This project investigates how immune responses to fight infections and tumours are generated.

Very few immune cells are recruited to a response and it is unknown how so few cells organize relative to each other in order to efficiently respond while sparing healthy tissues. Specifically, our studies aim to understand how those rare T cells use their environment to interact and regulate the overall response, and reciprocally, how different environments will shape T cell cellular responses. Our goal is to define the molecular events that allow these systems to communicate with each other and to understand how such inter-relationship shapes short and long-term immune responses to vaccination and infection. We aim to understand the principles of these processes in order to understand how protective immunity is obtained in response to pathogens, but inefficient to fight 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 studies aim to improve our basic understanding of how immune responses are regulated. This work will have direct implication on our knowledge of immune responses to infection, more specifically the relationship between T cells and their environment during infection. It will also give insights into the mechanism used by tumour cells to escape T cell responses. Insights gained from these studies may have long-term implications for our ability to develop novel vaccines that will induce immune response with long-term memory towards multiple virus variants. Finally, by providing a better understanding of how dysregulated response against cancer

develops, our work may help to identify new strategies to treat cancer in the long-term.

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 mice. In addition to wild type animals, we will also crossbreed different types of genetically modified animals, in order to generate animals with defined immune system molecular defects. Crossing different strains and breeding them will require 18,000 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', which means that some animals may experience pain and suffering. The majority of animals will be used for breeding and maintenance of genetically modified lines, and to act as donors for cells for use in vitro or in vivo. These animals are expected to experience no adverse effects and at most a mild level of severity. The majority of animals (~70%) will experience procedures such as injections which will lead to transient pain during the procedure, but will not induce long-lasting harm and suffering. During Influenza or Listeria infection, mice might experience moderate symptoms such as weight loss and reduced motility, but they are expected to recover within 7 to 10 days. During induction of cancer, some animals may experience some adverse effects, described thereafter. Mice will develop either cancer under their skin, or breast cancer, or lung cancer. During the early phases of cancer induction, mice are generally in good health and no sign of cachexia or terminal disease are observed. We aim to kill the mice while they are in this phase. For cancer under the skin or breast cancer, mice might develop ulceration at the site of the tumour. Mice will be killed before the primary tumour metastases. Mice with lung cancer might develop difficulty breathing, but we aim to kill the mice before they reach this point of suffering. To assess the role of the particular immune system genetic changes in disease, a small proportion of mice (<5%) will receive irradiation treatment to remove their immune system and replace it with a new one, a procedure of which will induce transient weakness and fatigue. All animals will be 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 aim of our proposal is to understand the molecular mechanisms controlling immune responses in the context of infection and cancer. This response drastically varies depending on the injury, through factors that we do not yet understand. It is therefore difficult to recreate the correct in vitro conditions that will mimic each injury. Furthermore, the nature of studying immune cell communication requires consideration of the local environment in which the interactions take place (microenvironment). This complex mixture of factors supplied by various cell types within a tissue shape immune responses, making it essential to evaluate such hypotheses in whole body/tissue systems.

These studies directly investigate the complex interactions between immune cells and their physiological environment which cannot be replicated in tissue culture, in vitro, systems or lower organisms.

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 required:

We will use appropriate statistics and careful experimental design. We will combine our extensive experience with literature to adequately determine the minimal number of animals needed to obtain a significant result for each experiment.

This will perform early ‘pilot’ experiments to understand intra and inter-group variation and optimization of experimental design e.g. including age and sex matching mice used to reduce variation in data. Reduced variation will allow us to use fewer animals to test our hypotheses.

When possible, we will include intrinsic controls to reduce the numbers of mice required. To further reduce number of control groups, we will aim to combine experiments.

Maximum use of tissue obtained from animals will be ensured to further reduce the required numbers, and tissue samples will be archived.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 choose mice as the appropriate model organism for the proposed experiments because this species provides greatest flexibility in terms of availability of genetic modification, reagents and previous data. This provides unique opportunities to study certain immune cell interactions in a rodent system which shares many of the same features as the human immune system.

A number of approaches will be used throughout the project to minimise adverse effects and suffering to the animals, which are described below:

• We will pay careful attention to animal husbandry and provide environmental enrichment and co-housing to avoid social isolation.

• We will make every effort to reduce the number of procedures per animals and to minimize the discomfort involved during each procedure. This will be achieved through careful experimental design and by ensuring that the researcher performing the procedure is fully competent and understands the protocol and its limitations.

• We will aim to use the least painful substance and route of administration and make sure that it does not exceed the limits of the allowed amount. For instance, we are not using known irritants (such as CFA) but instead we are using less irritants molecules which still retain immune properties (such as Alum). For the route of administration, we are no longer injecting in areas such as the footpad, which is associated with soreness in the paw, inducing pain during locomotion. We have refined our protocol and we are now injecting higher up to prevent suffering.

• When possible, we will use anaesthetics to reduce temporary discomfort during a procedure. In addition, inhalation anaesthetics will be used where possible to avoid risks associated with injectable forms (complexity in maintenance, dose control).

• When surgery is involved, we will use appropriate aseptic techniques, monitor the animals before during and after the procedure. Special attention will be given to the husbandry of animals after surgery to monitor that they recover well.

• When mice are subjected to treatments that may cause them moderate pain, a human practice will be exercised to limit the length of procedure to the shortest possible time.

• In infection work, we will aim to use the least virulent pathogenic strains, in order to reduce suffering. In addition, sublethal doses of infectious agents will be supplied and define cut off points for all work will avoid unnecessary animal suffering.

• The use of genetically modified mice will reduce the requirement for application of labelling substances to visualise immune cells which will reduce frequency of injections.

• We will use prophylactic antibiotics for irradiation to limit infection, transient weakness and fatigue observed in those mice.

In all protocols, we will follow clearly defined action points, monitoring schemes and human end points to minimize suffering of animals.

PROJECT 78

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.

Word limit; 1000 words

Project Title	Rodent models to assess dependence of nicotine and related psychoactive substances
Key Words	Addiction, Conditioning
Expected duration of the project	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;
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 overall aim is to increase understanding of the dependence-producing properties of nicotine using rodent models of addiction. The aim is to advance our understanding on the processes underlying the nicotine reinforcement in order to formulate new strategies for treating nicotine dependence. Novel compounds that may be effective in treating in tobacco dependence may also have potential for other drugs such as cocaine addiction, which represents one of the biggest problems in society. The potential discovery of novel targets for treating addictions may be further evaluated using clinically-derived behavioural endpoints, such as relapse behaviours. Finally, by utilising electrophysiological processes along with brain stimulation techniques, the neural circuits relevant in mediating the dependence-producing effects can be targeted using novel techniques such as optogenetics and designer receptors. Identification of these substrates will help to formulate better medications for drug addictions.

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

It is anticipated that these approaches will lead to identification of those aspects of nicotine dependence that are important in the process of quitting, and with better insights, lead the way for the development of more effective medications in reducing relapse rates in smokers. The benefits from this project will come from further understanding how nicotine acts in the brain to mediate the satisfaction/pleasurable effects from tobacco products and e-cigarettes. Overall, this work will assist with the development of new aids for smoking cessation and thus, will reduce the burden that tobacco smoking places on society.

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

Male hooded Lister rats will be used in all experiments and the numbers requested (150 rats per year over the 5 year duration) are derived from previous experience with the behavioural techniques and anticipated continued research funds over this period. This ensures that the work is completed with an adequate subject population that may be published in an International peer-reviewed journal.

In the context of what you propose to do to 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 examine the effects of drugs on nicotine self-administration and to clarify the role of different neurotransmitter systems by comparing to other abused substances such as cocaine. In 1 hr daily sessions, rats will be free to move within a chamber to voluntarily self-administer drugs of abuse intravenously. Rats will be closely monitored during recovery from surgical implantation with an intravenous catheter. Tethered via a swivel, the rat learn to self-administer the drug, the number of lever press responses will be increased. Once stable levels of drug intake are exhibited, then the effects of compounds will be evaluated. Some subjects will receive the treatment via microinjectors, directly into the brain. For experiments involving electrical recordings, microwire electrodes will be implanted during the surgery and recordings made at various stages of the behavioural experiment. In tests focussing on drug-seeking responses, the behaviour will be reduced by eliminating cues and drugs. The persistence to respond on the lever previously associated with drug injections following a priming dose of the drug will be used as a measure of the drug-seeking response. Treatments will examine the ability of compounds to reduce this reinstatement effect. These experiments can last as long as 6 months depending largely on the patency of the catheter. Once the experiment is finished, all animals will be killed either by a schedule 1 method, whilst under non-recovery anaesthesia or by decapitation where the brains are required for analysis that have not been damaged or exposed to anaesthetic gases (20%). Given the intravenous surgeries, the severity of the procedures is classified as moderate. Regarding welfare, animals giving cause for concern will be removed from study until they recover. The animals adapt well to the task and they maintain their body weight and stay in good health. Doses of drugs to be tested will be chosen so as not to have adverse effects. Repeated exposure to compounds repeatedly produces little adverse effect. A few animals may develop an infection where catheters are implanted, which is a rare event and veterinary advice will be sought on these occasions. On a daily basis, animals will be checked by technical and/or the scientific staff. Body weights will be recorded at least twice each week.

Application of the 3Rs

Replacement

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

Replacement

The rat is the most appropriate species for this work. A large body of literature already exists on the neurobiology of drug-seeking and drug-taking behaviour in rodents.

There is no other approach to examine dependence-producing effects of psychoactive drugs – which is based upon a behavioural endpoint. The available alternatives are similar models that have been described for primates and dogs, but these species are not required for the work proposed under the proposed license. It simply cannot be done in a test tube. While computational network modelling is being introduced within the cognitive behavioural field, there really is no appropriate alternative to examine the neurobiology of drug dependence.

Reduction

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

Reduction

Experiments will be designed carefully to minimize on the subject numbers while retaining statistical power, the design of the proposed experiments have incorporated within subject tests – each animal serves as its own control. All tests with pharmacological treatments will be tested in a multi-factorial randomized sequence design. Also, pilot tests will be able to inform the number of subjects required to inform on a significant observation with confidence.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 considered to be one of the better alternative species to study reinforcing properties of drugs. In the past, non-human primates were used for this purpose and over the last 30 years significant advancements have been made in understanding the optimal conditions under which rodents can be trained and tested with psychoactive substances and also ensuring prolonged catheter patency. There is no other approach to examine dependence-producing effects of psychoactive drugs – which is based upon a behavioural endpoint. The available alternatives are similar models that have been described for primates and dogs, but these species are not required for the work proposed under the proposed license. While computational network modelling is being introduced within the cognitive behavioural field, there

really is no appropriate alternative to examine the neurobiology of drug dependence.

In terms of maintaining welfare and minimising adverse effects, most of the rodents will suffer no more than mild stress. The animals adapt to the task readily and they maintain their body weight and stay in good health. Doses of drugs to be tested will be chosen so as not to have adverse effects. Repeated exposure to compounds produces little adverse effect. A few animals may develop an infection where catheters are implanted, which is a rare event and veterinary advice will be sought on these occasions. On a daily basis, animals will be checked by technical and/or the scientific staff and body weights will be recorded frequently. As part of the experiment, the animals will also be handled and thus any adverse effects of drugs will be detected.

PROJECT 79

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.

Word limit; 1000 words

Project Title	Advanced Education in Pharmacology
Key Words	education, pharmacology, CNS, cardiovascular
Expected duration of the project	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.

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):

The purpose of the licence is to provide advanced education in pharmacology and *in vivo* skills training for undergraduates seeking a career in academia or the pharmaceutical industry.

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

Training delivered under this licence will provide students with an understanding of in vivo experimental design, application of the 3Rs and detailed knowledge of drugs affecting the central nervous system and cardiovascular system. Students will be better equipped for careers in academia or the pharmaceutical industry where they will contribute to the development of new treatments for diseases affecting both humans and animals.

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

We will use up to 2000 mice and 650 rats 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?

Experiments using mice involve (a) the injection of drugs that act as pain killers and (b) drugs that affect mood. In order to test the response to pain killers, mice will be placed on a hotplate set at 55oC which is likely to cause discomfort. However as soon as the animal reacts, it will be removed; no animal will be allowed to stand on the hotplate for more than 30 seconds. Some of the mood altering drugs may cause the animals to become aggressive; however they will be housed separately during

the period of observation so there is no opportunity for fighting. Drug injection will cause only transient pain. The level of severity is mild. Experiments using rats involve the administration, directly into a vein, of drugs affecting the heart and blood pressure. There is a risk of blood loss when inserting catheters into blood vessels which is minimised by students practicing the technique on cadavers to ensure competence. Students are supervised closely be experienced staff at all times. There is a risk that the dose of drug injected will cause blood pressure to fall or increase too much, which could lead to death of the animal. This will be minimised by starting at very low doses of the drug and only increasing to a higher dose if the response of the animal is small. These experiments are undertaken under non-recovery anaesthesia. In all cases, animals are killed by a Schedule 1 method 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 properties of drugs can be explained in lectures or by using computer simulations; however these are no substitute for the first hand observation of the effects of drugs administered to the whole animal. Students value their experience working with animals highly, as it makes them more aware of the properties of the drugs, the technical challenges and the ethical concerns of *in vivo* work. As a result they have a far better educational experience and are better equipped for future careers.

Reduction

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

Reduction

The estimated number of animals required is based on maximum class sizes, statistical advice and our experience of teaching this course over the past 20 years. Once the scientific and educational goals of a particular experiment have been achieved, no further use of animals will be authorised for that particular cohort of students.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 the effects of the drugs that will be administered are well characterised in these species. Therefore there is a high chance that the experiments will be successful and that the students will obtain the maximum educational value. We anticipate that the majority of our students will go on to a higher degree and/or progress to careers in the pharmaceutical industry where these species are still the most commonly used in drug development.

Prior to commencing experimental work, students work though computer simulations and observe a video in which a member of the teaching staff demonstrates the relevant surgical techniques so that they are familiar with both the methods that they will use and the anticipated experimental outcomes.

In testing the properties of pain killers, mice will be placed on a hotplate set at 55^{o}C which is hot enough to provoke a rapid response (e.g. withdrawal of the paw) but not so hot that permanent damage or lasting pain will be produced in the 30 second (maximum) test period. Animals that do not respond in this timescale will be removed from the heat source. This approach is used commonly in the pharmaceutical industry and is accepted as causing only mild discomfort.

In testing the properties of drugs that affect mood, some mice may become more aggressive than usual. Animals will be kept apart to prevent fighting and injury, so the chances of harm are minimal.

In testing the properties of drugs that affect the heart and blood pressure, rats will undergo surgical implantation of catheters into blood vessels while under non-recovery surgery. Students will not be allowed to progress to using live animals until they have shown that they are competent in the techniques using cadavers. They will be supervised closely by experienced staff at all times in order to ensure that the surgical procedure is successful. The depth of anaesthesia will be monitored throughout the experiment to ensure that an appropriate level is maintained such that the animals do not experience any pain or discomfort.

All animals will be killed by a Schedule 1 method at the end of the experiment.

PROJECT 80

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.

Word limit; 1000 words

Project Title	Understanding mechanisms of inherited heart disease (cardiomyopathy) and exploring treatment options
Key Words	Inherited heart disease, modelling disease, treatment of disease
Expected duration of the project	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;
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 aim of this project is to better understand a group of human inherited heart diseases called cardiomyopathies. These diseases are caused by genetic “spelling mistakes” (i.e. mutations) in the genetic blueprint of cardiac proteins. Cardiomyopathies contribute substantially to the burden of heart disease in the UK (overall costs of heart diseases are £11 billion to the NHS annually), as they can lead to heart failure. Clinical management of heart failure so far is general, there are no specific therapies (e.g. specific drugs to cure). A major risk of the disease are heart attacks (“arrhythmias”) which can lead to Sudden Cardiac Death. A recent example of undiagnosed cardiomyopathy featured in the media was the professional football player Fabrice Muamba, who collapsed during a match in 2012 and was lucky to be resuscitated successfully.

The majority of cardiomyopathies are caused by genetic “spelling mistakes” in proteins directly involved in cardiac contraction, however this project will focus on a novel group of proteins, which helps the heart muscle to sense and respond to stress the organ experiences under demand (and is called “biomechanical stress signalling proteins”) e.g. during exercise and in pregnancy. Biomechanical stress signalling is a complex network of signalling cascades and proteins involved and we are just beginning to understand which proteins are involved, but our understanding of "how it works" is very limited. We hypothesis that genetic mutations in this group of proteins cause the heart to "adapt" (e.g. to grow bigger, i.e. undergo hypertrophy) in the absence of appropriate triggers.

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

This research will help to gain insights in how mutations in a group of proteins can cause heart disease: The project will generate mouse models for human disease and our experiments will tell us which cellular processes “go wrong” in this condition. Based on the findings and our better understanding, we will test options to treat or prevent disease, e.g. by manipulating the steps which “go wrong” in the disease. In the short run, our research will be disseminated to other researchers (e.g. through scientific publication and conference abstracts). It will also help to inform Geneticists in the NHS how to interpret certain findings of genetic testing in human patients and how to advise patients and clinical practitioners on the findings. Ultimately, we hope that our project will contribute to the development of novel, specific therapies (e.g. new drugs) for this group of diseases. In this project, we aim to generate mouse models reflecting the mutations found in human patients and to perform experiments helping us to understand mechanisms of disease. In particular we are interested how the disease develops and what the earliest measurable signs of the condition are (long before the mice are visibly sick). We will also explore how the disease can be ameliorated or treated, e.g. whether substances derived from Green Tea may have a protective or therapeutic effect.

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

We will use primarily mice (6,450) and some rats (250) 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?

REDACTED we assume that the introduction of the mutations will have little effect on the majority of mice. However, in some cases, the mice may develop heart failure similar to humans. A small proportion of mice may die suddenly of arrhythmias and sudden cardiac death or develop severe heart failure. Wherever possible, we will avoid death by using humane end-points, the most important one being deep irregular abdominal breathing. We will monitor these mice vigorourisly (some up to three times daily) to spot early signs of heart failure. The animals may have several imaging sessions or other investigations, under general anaesthesia where appropriate. Some animals will have ECGs or implated ECG monitors (telemeters) to identify those at risk of arrhythmias. At the end of the studies the animals will be killed and their organs be used for molecular studies. Animals who do not show signs of cardiac disease will be challenged either with a surgical procedure called “trans-aortic constriction”, or by the administration of drugs. Both interventions will mimic the effects of high-impact endurance training on the heart and will help to reveal defects in signalling pathways, or test how drugs and/or novel compounds may ameliorate disease.

Application of the 3Rs

Replacement

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

Replacement

We use bioinformatics predictions, biochemical experiments and cellular models to understand the impacts of the genetic “spelling mistakes” on heart cells. While these experiments are informative on certain aspects of disease (e.g. they can show binding to other proteins is affected, mouse models are needed to understand the effects of the genetic “spelling mistakes” on the whole organ level, e.g. how these genetic “spelling mistakes” can cause arrhythmias.

In our in vitro experiments, we use human cardiomyocytes derived from blood or skin biopsies via an exciting novel technology called "induced pluripotent stem cells". These cells are human to reflect best real human heart cells and future work will show which aspects of animal work can be replaced by these cells.

Over the course of the project we will review and potentially incorporate alternatives as they became available.

Reduction

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

Reduction

We will use a novel technology (called “CRISPR/Cas9”) to introduce the genetic “spelling mistakes” into the mice. This new method needs less animals than conventional approaches to generate new lines. We generate all mouse models in one particular strain of mice (C57bl6) to reduce variability and preferentially use males only in in vivo investigations, as we know there is less variability among males. However, the corresponding females will be included in molecular analyses. We will design our breeding strategies in a way to avoid the production of unwanted genotypes. We will use power calculations for planning experiments and use blinded, randomised block design wherever possible.

We will use small trial studies (pilot studies) before embarking on full scale 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 will use very few rats. Mice will be used for the majority of experiments, as methods are available to introduce genetic “spelling mistakes” easily and mice are similar enough to humans in terms of cardiac physiology, e.g. their hearts have 4 chambers like the human heart. Animals are kept in a modern and well-resourced facility in individually ventilated cages, and being offered enrichment and group housing (where possible).

The team has long-standing expertise and experience in all experimental procedures; we will constantly refine techniques and apply best practice to all animal work.

We will use non-invasive imaging techniques (such as echocardiography or MRI scanners) to monitor the development of heart disease in the models. Once we have established when the animals develop symptomatic heart disease, we will perform subsequent studies at an earlier time-point (when there is some measurable change in the heart, but before the animals become symptomatic), to avoid unnecessary suffering. We have defined clear humane end-points to minimise the suffering of animals and will adhere to monitoring regimes (documented in observation sheets) when adverse effects are expected. We will used small implantable ECG monitors (telemeters) to identify those mice at risk of arrhythmias and potentially sudden cardiac death so we can monitor such animals more closely.

Where scientifically justified, we will use drug induced models of challenging the animals instead of trans-aortic constriction. The latter is the equivalent of open heart surgery, hence has quite an impact on the animals, while in the first case the drugs will be delivered via small devices (similar to insulin pumps), which require only minimal surgery to be implanted. This slow release device also means we can avoid twice-daily injections of the animals, hence reducing stress and discomfort in the mice. Moreover, this method can be tailored to certain substances and hence provide a better understanding of the signalling cascades being affected by a particular genetic “spelling mistakes”.

PROJECT 81

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.

Word limit; 1000 words

Project Title	Neurotransmitter action, ion channels and receptor mechanisms
Key Words	Neurotransmitter receptors, synaptic transmission, ion channels
Expected duration of the project	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):

This project aims to improve understanding of the action of one type of neurotransmitter in the brain: the excitatory neurotransmitter, glutamate. Glutamate has an important role in communication between nerve cells of the brain, being involved for example in learning, motivation and movement. In neurological and psychiatric disorders, including Parkinson's disease, Huntington's disease, Alzheimer's disease, drug addiction and schizophrenia, glutamate is now known to be important, and yet the reasons for this are still largely unknown. Because current treatments for these neurological conditions are inadequate in a number of ways, our objective is to make further research on the actions of glutamate in the brain which will contribute knowledge and understanding that can be used in the future to help researchers trying to meet the clinical needs of patients suffering from neurological 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)?

Results of the proposed experiments will increase fundamental knowledge of the neural mechanisms of higher brain functions, and will be communicated to the scientific community by publication in scientific journals and presentations at national and international conferences. The research findings are of importance to the pharmaceutical Industry in developing new treatments for currently incurable diseases and for improving the quality of life for patients with diseases related to the brain regions under investigation and will increase understanding of animal behaviour and welfare.

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

Rodents (rats and mice) are used for these experiments. Over the duration of the project (5 years) approximately 540 rats and 120 mice are expected to be used. with the majority of these animals being neonatal (P7).

In the context of what you propose to do to 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 possible adverse effects to the animals are mild in severity. Neonatal animals are killed by decapitation which is very fast (~1s). Any adverse effects are minimised by good training and precise application of the procedure. Older animals (P8 - adult) are deeply anaesthetized. The likely adverse effects here are mild irritation of the airways and some loss of motor control before the animal goes to sleep. The level of anaesthesia is checked by pinch of the hind paw to test for reflex withdrawal of the foot which is absent in deep anaesthesia. The severity is minimised by good training and precise application of the procedure, by carefully handling the animal to avoid stress and using a maximal concentration of anaesthetic (4-5%) which ensures rapid (~ 1 minute) loss of consciousness and reflexes. At the end of the protocol, the animal is killed by decapitation. Signs of distress in the animal are very rare (based on past experience <1%). Any animal that is observed to be in distress will be killed immediately 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 questions I aim to address specifically relate to the function and dysfunction of the mammalian nervous system, and how the physiology of the system contributes to the behaviour. These questions can only be addressed ultimately through the use of living, functioning mammalian brain tissue with relatively intact neuronal circuits that enable empirical measurements of neuronal physiology to be made. Such tissue can only be obtained from animals. The *in vitro* brain slice preparation will be used for the majority of the proposed experiments, using brain tissue removed from rats or mice. Rodents are the most appropriate species for these experiments because of the wealth of knowledge currently available on rodent glutamate receptors and rodent models of human diseases. Rodents are the lowest vertebrate species suitable for the preparation of brain tissue for physiological measurements. Key pharmacological systems share similar characteristics in both rodents and humans. Compared with using *in vivo* and behavioural models of higher brain

functions, this model reduces the number of animals required, and also minimises the severity of the procedures that will be carried out.

Reduction

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

Reduction

Each experimental animal provides several brain slices and where possible, brain slices are shared among laboratory personnel, so reducing animal usage. Because the brain has two substantia nigra regions, each brain slice can be cut into two equal pieces providing up to 12 tissue samples for use in each experiment. A single neurone recorded in the whole-cell patch-clamp configuration provides it's own 'internal' control recording before application of a test drug. This reduces measurement variation and so allows smaller drug effects to be more reliably detected, or allows less brain tissue (and so reduced animal numbers) to be used when measuring a drug effect. Each brain slice is used for only one drug application, so each new recording is made in a fresh tissue sample avoiding potential confounds due to cumulative drug effects. Thus use of brain slices allows several independent measurements of drug action to be made using tissue from a single animal, therefore minimising the number of animals used 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

Rodents are the most appropriate species for these experiments because of the wealth of knowledge currently available on rodent glutamate receptors and rodent models of human diseases. Rodents are the lowest vertebrate species suitable for the preparation of brain tissue for physiological measurements of this kind where the results can be related directly to the human brain. This is because key systems in the brain share similar characteristics in both rodents and humans. Compared with using *in vivo* and behavioural models of higher brain functions, this model reduces the number of animals required to investigate drug action and receptor mechanisms, and also minimises the severity of the procedures that will be carried out. During preparation for the experiment and when undergoing anaesthesia, rat or mouse welfare can be carefully monitored by observation and stress to the animal minimised by careful training of the experimenter.

PROJECT 82

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.

Word limit; 1000 words

Project Title	Analysis of normal chicken blood
Key Words	Blood, chicken
Expected duration of the project	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;
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 aim of this project is to allow maximal blood collection
from birds which are already being culled for use of tissues and organs for scientific purposes.

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

Enabling regular and relatively large volumes of blood to be collected from chickens will allow the chicken immune system to be further examined, and enables ongoing use of the blood for inclusion in tests for diseases such as influenza.

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

Roughly 2000 birds will be used under this project. It must be noted that these birds are already being used for tissue collection, so this project will not increase the numbers than are already in use.

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

Only mild severity is expected as the birds are all culled by cervical dislocation and then exsanguinated by decapitation

Application of the 3Rs

Replacement

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

Replacement

By its very nature, examining and characterising the cellular characteristics of the blo od of chickens requires the use of animals.

Reduction

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

Reduction

This project will allow the optimised utilisation of birds already being used to provide tissues and organs. By permitting these birds to have the maximum volume of blood be collected via this project licence, it would negate the need for any additional birds, thus keeping the number of birds used 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

Chickens are needed in order to collect chicken blood, and will be exsanguinated after having their neck dislocated.

PROJECT 83

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.

Word limit; 1000 words

Project Title	Pathophysiology of rhomboid-like proteins in mammals
Key Words	Mouse, Rhomboid, Inflammation, Growth factor, Signalling
Expected duration of the project	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;
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):

This project aims to determine the biological and medical functions of a family of proteins called rhomboids. Rhomboids were first discovered REDACTED to be unusual proteases – enzymes that cut other proteins – which specifically target membrane proteins. More recently, we and others have found that the rhomboid proteases are in fact members of a much broader family of proteins whose fundamental role is to interact with other proteins in cellular membranes.

During human and animal development and adulthood, cell fate becomes determined and this is largely governed by signals that pass between cells. Understanding the signalling between cells and what goes wrong in diseases is a major goal of modern biomedical science. Our work and that of others has shown that the rhomboid-like proteins control many important signalling events and other processes of potential medical relevance, including in cancer, inflammation, metabolic disease, infection and brain function. Much of this prior work has focused on invertebrate model systems and cell culture. Little is yet known about their function in mammals. Very recent work with mice, however, has supported the idea that members of this family are medically relevant, specifically in the field of immune system function, inflammation 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 data obtained from our experiments will lead to an understanding of the function of rhomboid-like proteins in mammals. There is a real prospect that our results will lead to significant medical insight. The data will also be very useful for other researchers studying a host of processes related to human health and disease (e.g.

inflammation, cancer, neurodegenerative diseases) and we will publish it in appropriate journals to ensure that all relevant researchers can benefit from this work. All our work is published in open access journals so that it is available to anyone for free.

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

Mice, up to 50,000 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?

We expect that greater than 75% of the mice will experience only mild if any effects. A small number (no more than 25%, in practice we expect fewer) may suffer moderate adverse effects. For example, in all our protocols, animals can exhibit behaviour indicating discomfort. Other side effects may be specific to each protocol. - In the breeding, metabolic state and ER stress assessment protocols, they might include loss of weight. - In the ageing protocols, they might include age associated conditions such as dermatitis, tumours, eye or dental diseases. - In the immune regulation protocol, they might include ulceration, anaphylaxis or acute toxic shock like response. - In the assessment of metabolic state, they might include diabetes. - As part of making genetically modified animals, some animals will have surgery. The minimise the side effect of the surgery, it will be performed aseptically, with peri and post-operative analgesia. Specific steps will be taken to minimise suffering and discomfort, including the use of anaesthesia and regular monitoring by well trained and experienced staff. At the end of experiments mice are killed humanely.

Application of the 3Rs

Replacement

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

Replacement

Mice are now well established as the best model system for identifying the function of specific genes in mammals. Usually this is done by removing or knocking out the relevant target gene, or by expressing it in abnormal places or levels. The consequent effects on the mice or on cells derived from them are observed and they give strong clues about the function of the gene/protein of interest. In fact, we do most of our research in cell culture and using the fruit fly Drosophila. This allows us to achieve many of our results without using mice, thereby minimising potential suffering. However, where we need to extend our results into mammals, to investigate their relevance to human and animal health, we need to use mice.

Reduction

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

Reduction

We carefully design all our experiments to ensure that they are as efficient as possible, so that we can produce the maximum amount of statistically rigorous data from the minimum number of animals.

The same approach is used when breeding mice. We are careful to breed as few excess mice as possible.

Where practical, we freeze mouse embryos or sperm so that we do not need to breed mice of a strain that we will not need for a long 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

Mice are the most common standard model of mammalian biology, used in biomedical science across the world. By doing all our preliminary experiments in cultured cells and/or fruit flies, we ensure that all mouse experiments are targeted and carefully designed to answer precise questions.

We take great care that all procedures done on mice are performed by state of the art methods and only by people who have appropriate training. We use a range of scientific communication methods (work published in journals, conference presentations, informal networks, online resources) to learn about best practice so that we can ensure that our methods are as refined as possible. We hold regular team meetings to ensure that this information is spread across our group. We also attend regular meetings among the wider scientific community, again to stay current with best practice.

PROJECT 84

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.

Word limit; 1000 words

Project Title	Novel therapies for Pain
Key Words	Therapy, Pain, Neuropathic, New
Expected duration of the project	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:
Yes	(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.

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)

In the UK 43% of people suffer from some kind of chronic pain, with around 12% suffering from pain to the extent that it is disabling, despite treatment. The objectives of this project are to identify new targets for treating pain disorders, and to identify compounds for treating pain disorders that are superior to current treatments. In this project, pain disorders include long-term pain caused by damage to nerves (neuropathic pain). While pain can be a normal sensation triggered in the nervous system to alert you to possible injury and the need to take care of yourself, under some circumstances, such as chronic pain, drug treatment is required to provide pain relief (analgesia). Pain is the most common complaint for which people visit health-care providers.

Patients who fail to receive adequate pain relief, or are affected by significant side effects from current treatments represent a large unmet medical need. Inadequately treated pain is associated with fear, anxiety, sleep deprivation, depression, suicidal ideation, the inability to engage in normal family life, or sustain productive work.

Experiments in this project typically follow test tube (in vitro) studies. However, in vitro methods cannot predict and replace whole animal (in vivo) models, as the technology does not exist to simulate the complexity of the whole body system. New medicines that merit testing are assessed in rat and mouse ‘models’ of human pain indications that detect clinically effective medicines.

A number of rodent models of pain are covered by this licence to reflect the various clinical pain indications (inflammatory, visceral, post-operative, neuropathic and arthritic). The reason that several models, and not just one, are employed is that pain sensation and pathological pain can involve several different physiological

processes. This licence covers models of pain, each of which involves different phenomena involved in the sensation of pain and the development of a variety of types of pathological pain seen in human patients.

Animals are typically tested in protocols that last a day, but sometimes they are tested for longer, and occasionally animals may be tested for up to 70 days.

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

The major benefit of this work is the discovery of new, effective drugs for the treatment of pain indications, an area of unmet medical need. This project licence will enable drug discovery projects that are aiming to produce treatments which are superior, in terms of effectiveness and side-effect liability, to current treatments.

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

Rats and mice will be used on this licence, as they are the lowest sentient species that can be used. As an estimate, a novel compound will be tested at 3 doses with 2 controls per experiment (a vehicle treated group and a clinically relevant control) with 10 animals per group, and an average of 4 experiments will be carried out per month. Over the 5 year timeframe of the project, this would equate a total of 12,000 rodents.

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

Possible adverse effects include those associated with testing novel compound from early stage drug discovery projects (eg mild sedation and salivation), but the likelihood of occurrence is low. The use of pilot studies, and close monitoring will help to keep the incidence of adverse effects to a minimum. The models covered by this licence are largely designed to generate painful sensation upon stimulation, and not obvious signs of pain in an animal in a sedentary state. Possible adverse effects associated with pain will be monitored closely. Animals will be killed humanely 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

The in vivo models described in this licence application are employed to generate information about how the whole body responds once it has been given a compound. It is neither possible, nor ethical, to use human volunteers in early drug discovery. It

is therefore necessary to use other whole body systems, animals, to find out how a living organism responds.

The studies covered by this licence typically follow on from in vitro models/assays performed by our clients. The in vitro models provide useful information about which are the best chemical leads from a particular chemistry program to be select for further study.

However, at present, in vitro methods cannot entirely predict and replace the in vivo models described by this licence, as the technology does not exist to simulate the complexity and diversity of the whole body system.

Reduction

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

Reduction

The number of animals required per group and experimental design are determined on the basis of power calculations, advice from statisticians, published data and previous results that have consistently identified target effects in a clear and unambiguous manner.

- Whenever possible repeated measure analyses will be employed to increase precision, maintain smaller group sizes, and reduce animal usage. For example behavioural measures from one subject might be recorded, and compared, both before and after drug administration, or on multiple occasions over time.

- Within each experiment a positive control is included to provide an internal control to compare the relative efficacy of the test compound and to assess the sensitivity/validity of the test procedure on a given test occasion. This good experimental design principle will avoid unnecessary replication of experiments.

Opportunities for further reduction will be sought constantly.

Refinement

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

Refinement

Purpose bred, adult free living animals of assured health and genetic status will be obtained from commercial suppliers, or from breeding colonies.

Animal suffering will be minimised by the following;

- Conditions in the animal house follow current best practice, and items such as bone chews are placed in rodent cages for their stimulation.

- Competent personnel will perform all studies on this project licence and adverse effects will be minimised by careful handling and the application of good technique.

- Guidelines on the limit of volumes of administration of substances and blood sampling will be strictly adhered to.

Clear-cut end points are described in the possible adverse event description for the protocol covered by this licence.

Opportunities for further refinement will be sought constantly.

PROJECT 85

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.

Word limit; 1000 words

Project Title	Mouse cancer models for understanding disease mechanisms and interventions
Key Words	cancer, oncogenes, drugs, mice, transgenic
Expected duration of the project	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;
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 overall aim of this project is to gain a greater understanding of the mechanisms underpinning cancer initiation and progression and to design improved therapeutic interventions. The objectives are three-fold:

1. To characterise the mechanisms underpinning cancer initiation and progression by key cancer driver genes using mouse models.
2. To characterise the role of novel signal transduction pathways and “non-oncogenes” upon which driver cancer genes are dependent
3. To determine the efficacy of anticancer agents in suppressing cancer initiation and progression and to characterise their mechanisms of action.

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

Through this work we will have greater knowledge of how cancer is initiated and progresses. This will in turn allow us to develop novel interventional and diagnostic strategies, which can be tested in the mouse models as a preclinical platform before clinical investigations are initiated. The potential benefit is that we will be able to improve the survival of cancer patients by developing improved therapies and biomarkers.

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

We propose to use approximately 5000 mice during the course of this work 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 vast majority of these mice represent those used for breeding purposes only and therefore will only suffer mild adverse effects. Occasionally, mice will be subjected to tumour induction, which could result in more moderate adverse effects including weight loss, hunched posture, under-activity, pallor and tremors. The treatment of tumour-bearing mice with anticancer agents will have a good chance of improving these adverse effects. In situations where tamoxifen is utilised, moderate adverse effects may develop in the form of skin or stomach ulcerations. Animals will be culled humanely before suffering clinical signs according to this PPL.

Application of the 3Rs

Replacement

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

Replacement

Although cancer is initiated from the loss of growth control of cancer initiating cells, the further development of tumours and their metastasis involves a complex interaction with the tumour microenvironment. It is not possible to recapitulate the complexity of this tumour-stroma interaction using any *ex vivo* or *in vitro* model system and so we have to use mouse models in order to gain the most relevant mechanistic insight. In addition, in recent years it has become clear that the testing of therapeutic agents on cell lines grown in vitro is not predictive of clinical efficacy and this has led to some high profile failures of anticancer drugs in Phase III clinical trials. Patient-relevant model systems are vital for improving the efficacy of anticancer drugs, and, for this, mouse models are essential.

Reduction

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

Reduction

Animal numbers will be kept to a minimum by close monitoring of backcross breeding colonies to avoid “overbreeding” and use of the appropriate animal group size required in order to obtain statistically meaningful data. We will also use *in vivo* imaging to monitor tumour development, which will reduce number of animals to be used for longitudinal 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 animal we will use for all of our studies will be the mouse. The main reason for using the mouse is that the development of cancer accurately mimics that in humans. For many human cancers, it is not possible to get access to early lesions that represent the initial stages of cancer, from which malignancy arises; the mouse circumvents this problem. We are fully aware of the adverse effects of the majority of strains to be used under this PPL and will not allow any to go beyond the severity limits. In addition, we intend to utilise a number of in vivo imaging modalities to more closely monitor tumour development within internal organs which will further help minimise suffering.

PROJECT 86

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.

Word limit; 1000 words

Project Title	Investigating the role of platelets in haemostasis, thrombosis and beyond.
Key Words	Platelets, Thrombosis, Haemostasis
Expected duration of the project	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):

Platelets are small cells inside blood vessels that protect the body from bleeding. The inappropriate activation of platelets leads to formation of blood clots or thrombi, which cause heart attacks and stocks. The objective of our work is to

(1) Identify proteins that control platelet function.

(2) Identify proteins that curb unwanted platelet activation

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

Cardiac vascular diseases (CVDs) associated with inappropriate activation of platelets are the leading cause of death in the UK. In addition, changes in modern diet and lifestyle have drastically increased the risk of developing CVDs. While current anti-platelet drugs have been successful in reducing disease burden, the death rates are still unacceptably high. Our project is designed to identify new potential therapeutic targets under the complex conditions that resemble human disease.

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

3700

In the context of what you propose to do to 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 continue, and have refined our protocols, however we expect some animals may develop adverse effects. These may include hypothermia and/or irregularity in breathing rhythm. In some cases animals may develop reactions to administered substances, although we don’t anticipate such reaction. We will anesthetise animals to protect them from adverse effects during bleeding time measurement and intravital microscopy procedures. These procedures will be performed on heat pads to offset hypothermia. Animals that show signs of sever distress will be humanely and swiftly culled using a Schedule 1 method. The level of severity of all the experiments is classified as mild or non-recovery, with all animals being 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

There are two major reasons for the proposed use of mice.

(1) It is not possible to grow platelets in culture and therefore they must be harvested for each individual experiment.

(2) Platelets lack a nucleus and therefore are not amenable to standard genetic manipulation protocols.

Therefore the only realistic method for the examination of individual proteins in platelets is to use genetically modified mice. However, whenever possible we will use specific pharmacological inhibitors and in vitro models to establish the role of these proteins in platelet function.

Reduction

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

Reduction

We used statistical procedures such as power calculations to ascertain the minimum number of animals required to use in a single experiment. We have also used a 2X2 factorial design to establish the role of drug intervention in platelet function. We are also using equipment that measure multiple readouts of platelet function ex vivo, therefore reducing the overall number of animals need. For invasive experimentation we have refined our procedure to assess clotting in multiple blood vessels. This will reduce the overall number of animals these procedures.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 breeding protocols that require the genotyping of small set of animals, therefore eliminating the need to subject all animals to the procedure. We have also examined the use of hair clipping as an alternative method for genotyping animals. We have refined our method of labeling mouse platelets by injecting fluorescent dye via tail vein, which is quicker and less invasive. All invasive procedure will be completed under anesthesia from which the animal will not recover. Moreover, should methods become available for the culture of blood platelets to maintain cell populations in vitro or the generation of platelets in vitro, we will move our studies in this direction.

PROJECT 87

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.

Word limit; 1000 words

Project Title	Neurophysiological mechanisms of sleep regulation
Key Words	Sleep, memory, brain activity, torpor
Expected duration of the project	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):

Sleep is a vital process, necessary for well-being, but its neurophysiological substrates and specific functions are poorly understood. This is especially relevant since sleep disturbances are common in most neurological, psychiatric and metabolic disorders. The overarching aim of this project is to attain better understanding of the effects of sleep deprivation on the brain and the body, and of the benefits of sleep for metabolic regulation and cognitive 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)?

This project will have high impact for society, the economy and well-being because it will lead to a greater understanding of the mechanisms which regulate circadian entrainment, sleep/wake timing and sleep quality. Circadian rhythms and sleep/wake timing are commonly disturbed by social and lifestyle factors, such as jet lag and shift work. Poor sleep is among the most prevalent complaints observed in many epidemiological studies, and the second most common overall complaint reported in primary care settings after pain. Disturbances in circadian rhythms and sleep/wake timing have a major impact on quality of life, resulting in impaired cognitive performance, increased risk of accidents as well as effects on immune function, hormone levels and cardiovascular performance. The amount of sleep and its quality also deteriorate with increasing age. As a result, elderly people are the main consumers of hypnotics, which have various side effects. The work detailed in this program has therefore direct relevance to clinical conditions. A greater understanding of how circadian and sleep responses are disturbed by disease is necessary to enable therapeutic intervention. For example, data from this project are expected to provide a greater understanding of how sleep is affected by

dysfunctional neurotransmission, typical for a range of neuropsychiatric disorders. In addition, there is a well-known trend for increased incidence of obesity, diabetes and metabolic dysregulation, which is often associated with reduced sleep quantity and quality, or disrupted wake/sleep patterns. Finally, this project will determine neurophysiological links between brain mechanisms underlying sleep and torpor. Inducing a reversible hypometabolism that mimics natural torpor in humans could have important influences on critical medical situations, including myocardial or cerebral ischemia, haemorrhagic shock, septicaemia, and organ transplantation. Controlled hypothermia and metabolism are already widely used in clinical practice, such as during cardiac surgery and to protect tissues from damage when blood flow is reduced, such as after a stroke. The main difficulty with replicating spontaneous torpor is that we do not know how animals start and maintain the process, and our research should provide important insights.

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

In this project I will use mice and Djungarian hamsters. It is expected that over 5-year period, up to 10,000 mice will be used for breeding/maintenance purposes. Approximately 1500 animals will undergo procedures, such as behavioural training/testing, surgery for implantation of electrodes in the brain, sleep deprivation or stimulation.

In the context of what you propose to do to 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 procedures used in this project are well established, and are not expected to result in any adverse effects exceeding Moderate level of severity. Recording of brain signals involves surgically implanting devices in the brain, which will be the highest severity procedure in this project (severity: moderate). Special care will be taken to minimise suffering related to the surgical procedure. The surgical procedure can last approximately 3-4 hours and may be performed in aged or transgenic mice that may be more susceptible to potential complications. Surgery can result in a transient post-operative pain or discomfort, which will be treated with analgesics, and single housing of the animals. Behavioural tests require the use of food as a reward, which requires limiting the animal’s normal food intake. Animals will be weighed daily, and the target body weight will be 85% of baseline body weight. We will also examine the effects of drug treatments, which involves administration of substances to modify circadian or sleep signalling pathways throughout the body. In addition, we will study the effects of genetic mutations on sleep, brain activity and behaviour. Some of the mutant/transgenic mice used in this project are established or emerging models of neuropsychiatric disorders, such as schizophrenia, neurological and neurodegenerative disorders, such as Parkinson’s disease, ocular diseases or sleep/circadian disturbances. We do not expect, however, that the full-fledged disease will develop in any of these models, but only specific, relatively subtle

symptoms will be apparent. In some animals, torpor will be induced by food restriction in mice, or by shortening the photoperiod in hamsters. Weight loss by up to 40% associated with short photoperiod in Djungarian hamsters is physiological, and occurs even when food is provided ad libitum. Sleep deprivation will be performed by the most ethologically relevant way, i.e. by providing naturalistic stimulation. Humane endpoints as described in the Project Licence will be closely observed, and 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

Sleep, torpor and waking are complex behavioural states, which require use of live animals. Numerous brain areas are implicated in various aspects of memory, cognition and sleep-wake control and cannot be fully emulated in an vitro preparation or using computer modelling. The processes associated with cognition involve multiple distributed brain systems and therefore can also only be studied in alive freely-behaving conscious animals. Laboratory mice and Djungarian hamsters are the best species for this project, as the techniques for chronic neuronal recordings and sleep studies have been well established in these species. These are also the phylogenetically lowest species of mammals commonly used in the laboratory with a brain sufficiently large enough to accommodate the recording electrodes as required by this project.

Reduction

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

Reduction

We will use two different species, to be able to address specific question using animals, most suited for a specific goal. Biostatisticians will be consulted regularly while specific experiments are being designed. Breeding of genetically altered animals will be closely monitored to prevent over-production. Animal numbers will be reduced by careful experimental design (e.g. power calculation). Finally, we will employ randomisation and blinding to ensure our results are reproducible, as well as employ within-subjects experimental designs where each animal can act as its own control.

Refinement

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

Refinement

A variety of measures will be used to reduce the amount of suffering, pain and distress to the absolute minimum. This is critical for the current project, as sleep and behaviour are greatly affected by pain and distress and so this could also confound the results. The animals kept in isolation will have appropriate environmental enrichment provided. All experimental procedures used in this project are well established and routinely used in the field and in my laboratory, and we will routinely evaluate technical improvements to improve experimental conditions, specific procedures and animal welfare. All surgical procedures will be conducted with aseptic techniques with appropriate analgesia and post-operative monitoring. Transgenic mice will be monitored closely to identify signs of genotype-related adverse effects. Animals subjected to aging will be also monitored carefully, including the identification of any signs age-related health impairments.

PROJECT 88

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.

Word limit; 1000 words

Project Title	VIRAL VACCINES (RESEARCH)
Key Words	Vaccine, Virus, Disease
Expected duration of the project	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;
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.

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 objective is to make the materials and perform tests to be able to assess the quality and effectiveness of new and existing biological products, such as vaccines. These tests are essential to ensure the vaccines are safe and effective before being administered 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)?

One part of the work will be to develop tests that can replace the use of animals and in one case a test in rats that could replace a test currently performed in primates. Other tests could lead to improvements in vaccines so that are better at preventing disease and help to make vaccines for new diseases or for existing diseases that do not currently have effective vaccines. Safety tests of viruses used in biological products could improve their safety or potentially reduce the time taken to make urgently needed vaccines The consequences of using a vaccine of low potency or inappropriate strain are that it will fail to protect recipients and disease burden in the human population could increase. Serological surveillance of the population is dependent upon robust assays, those used in Rubella diagnostics have evolved faster than the qualifying standards. A better understanding of the interpretation of the assays available will ensure a safe balance preventing unnecessary vaccination or the loss of immunity against this pathogen in the human population.

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

Mouse 4700 Rat 3000 Rabbit 100 Ferret 550 Chicken 20 5 years Mice are used because they make good immune responses to many test materials and there are a

large range of commercially available materials to analyse the responses. Rats, chickens and for some tests ferrets are used because it is a regulatory requirement to use a particular animal for that test. Rabbits are used if a large quantity of serum is needed to make material for an in vitro test. Ferrets are used for influenza tests because the immune response developed and the illness they experience both closely resemble those seen 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?

Animals may be injected with substances by the intraperitoneal, intramuscular, subcutaneous, intradermal or intravenous routes, or dosed with substances by the intranasal route. Newly born rats will be injected intracranially with mumps viruses. Some animals will have a microchip implanted under the skin for the purpose of identification and to monitor temperature. Samples may be collected e.g. blood, nasal washings, or mouth or eye swabs. Injection and dosing procedures, microchip implantation, and sample collection procedures are expected to cause no more than mild and transient discomfort. Where appropriate anaesthesia is provided to limit distress. Repeated anaesthesia may be given to immobilise animals for non-invasive procedures e.g. imaging. For animals being immunised there may be some local irritation at the site of inoculations particularly where adjuvants are used. Any animal showing signs of adverse effects as a result of the regulated procedures will be humanely killed unless there is a rapid return to normal using no more than minor medical treatment. Some animals will be infected with influenza viruses and will experience influenza like illness. If possible animals that become ill will be treated with medicines to alleviate symptoms according to a regime recommended by the vet. Ferrets and mice infected with virulent influenza may become seriously ill, experiencing weight loss and impaired movement and may be at risk of death from the disease unless there is prompt intervention. Where possible animals will be treated with anti-viral medicines to prevent development of serious illness. The outcome of these infections can be unpredictable and so animals will be monitored very closely by experienced staff with knowledge of humane end points. Rubella is not thought to generate adverse effects in mice. Mice infected with related viruses have been known to develop arthritis 6-8 days post infection. Injections at the sites chosen for this work will not lead to arthritis; however mice will be monitored for signs of distress suggesting these complications. Any animal that has any significant adverse effect will be humanely killed using an overdose of anaesthetic. All animals used under this licence will be humanely killed at the end of the study, or before if it is necessary for the welfare of the animal.

Application of the 3Rs

Replacement

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

Replacement

The data concern the immune response and in some cases the protective immune response to viruses and vaccines and the pathogenesis of disease, which cannot be generated without the use of protected animals.

In some cases data will be generated to validate *in vitro* assays with a view to eliminating the use of protected species.

Reduction

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

Reduction

The types of experiment proposed have been conducted for many years and the numbers of animals required in each approach to give a successful outcome are well established by experience. Where appropriate, statistical input is sought on animal experiments so that the numbers of animals used are the minimum needed to produce statistically reliable results. Sometimes the numbers used are based on regulatory requirements, for example to test a vaccines strength or for safety 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

Ferrets are among the few animals other than primates whose response to infection with influenza reflects that of humans; both the immune response and the clinical signs closely resemble that seen in humans. Methods for observation of clinical signs have been developed for recognition of onset of disease allowing earlier intervention with the use of anti-viral drugs or medication to relieve symptoms or to identify and humanely kill animals before progression to severe disease states .

It is recognised that group housing is preferable for optimum well-being of ferrets and wherever possible they will be group housed. There are situations where single housing is required due to husbandry needs or for safety reasons. In these situations wherever possible animals will be housed in cages in rooms with other ferrets.

Best husbandry practices will be employed to reduce the possibility of rejection of the rat pups by their mothers. Mothers and pups will be closely observed following injections and any rejected pup will be humanely killed immediately

Anaesthetics will be used for procedures where there is potential to cause pain or distress to an animal

Immunisation with adjuvants suitable for use in humans will be used. Freund’s adjuvants will no longer be included for use under this licence. Studies under previous versions of this licence have established that alternative adjuvants are at least as effective in achieving the required outcomes.

PROJECT 89

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.

Word limit; 1000 words

Project Title	Improving mouse models of intratumour heterogeneity
Key Words	Tumour evolution, intratumour heterogeneity, resistance, targeted therapy, selection
Expected duration of the project	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):

Analyses of cancers have revealed evidence for diversifying genetic evolution and intratumour heterogeneity (ITH) within the cell populations of cancers. This heterogeneity may account for resistance to therapy, and ultimately disease relapse and progression. The current project aims to identify how heterogeneity is generated and sustained in cancer populations, and also to develop animal models of ITH to study the evolutionary forces driving cancer changes during disease progression. Our current research integrates clinical, genomic and basic science data to understand how cancers develop and evolve over time By understanding how cancers evolve, we aim to not only predict therapy response and disease progression, but to ultimately control how cancers evolve and to channel them towards a trajectory that gives better disease outcome. By generating animals that exhibit the same kind of genetic instability observed in cancers, we will study the roles of genetic instability in multiple cancer types such as lung, colorectal and kidney cancers. We will characterise the evolutionary changes that occur in animal cancer models, tracking cancer cell populations as the animal progress through 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)?

This project could answer key questions about cancer growth, spread and treatment response, laying the foundation for potential novel treatments, personalised cancer therapies and improving patient outcomes.

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

We expect to use up to 10,000 mice during the course of this project. While a significant number, we keep numbers relatively low by using efficient statistical analyses and protocols in what is a complex genetic 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?

As we are studying intra tumour heterogeneity and the evolution of cancer, different tumour models will be studied in the mice. We do not expect the severity to exceed moderate as we closely monitor the welfare of the animals and work in close contact with experienced animal technicians and veterinaries in house.

Application of the 3Rs

Replacement

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

Replacement

The studies and experiments in this project track living, evolving cancer cells as the disease progresses and changes in response to cancer therapies. Tracking the changes in the tumours and manipulating them through genetic engineering or therapeutic intervention precludes the use of lower models or human volunteers. We complement these studies with cells from the mice as well as data obtained from patients in clinical trials.

Reduction

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

Reduction

We minimise animal numbers where possible by adhering to the best practices in animal breeding, statistics and experimental design that allow us to extract maximal useful data from the minimal number of animals. We use expertise from collaborators and within the project to continually monitor and analyse experimental strategies before, during and after the experiments, to refine and reduce if possible. For example, data from one experiment might inform and reduce the number of animals in subsequent experiments. Where feasible, we generate cell lines, tissue surveys and other resource archives in anticipation of downstream and future analyses REDACTED.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 recreating the mutations from human cancers, we predispose our genetically engineered mice to cancers. The desired data is obtained by looking at how the different combinations of genetic mutations affect the characteristics of resulting tumours and their response to cancer therapeutics. While these mice do get tumours, welfare is closely monitored throughout the entire process and study animals are euthanised and data collected before pain and distress arises. We are able to track the continued evolution of the tumours and therapy responses without causing pain by serially transplanting tumours from euthanised mice to recipient mice before onset of pain and distress.

PROJECT 90

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.

Word limit; 1000 words

Project Title	Light and electrically driven intervention in visual tasks
Key Words	
Expected duration of the project	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):

Recent developments in molecular biology have delivered an interesting technology that may have the capacity to be an important research tool and even (ultimately) a targeted therapeutic intervention. Specific neurons in the animal's brain can now be "switched on" or "switched off" with the appropriate combination of light and molecular intervention. This technique is becoming a powerful tool for demonstrating a causal role of specific groups of neurons. It is especially effective in cases where the experimenter wishes to target a functional class of neurons that are anatomically intermingled with neurons from different functional classes. By comparison with the earlier methods of electrical stimulation, the use of light-activated molecular interventions potentially allows a much more differentiated approach to identified neuronal types.

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

This method has enormous potential but we are a long way from realising this. Clearly, if a specific group of dysfunctional neurons could be stimulated or quietised selectively, this might have significant impact on treating the symptoms of neurodegenerative diseases; Parkinson's and Huntington's diseases are obvious and well known examples. There are many hurdles to overcome before this approach could enter clinical use. Sooner or later, somewhere in the world, novel techniques like this will need to be trialled in NHP models before they can be applied to humans: without this step, the techniques will lack scientific credibility.

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

Up to ten rhesus macaque monkeys (Macaca mulatta) will be used in pairs or groups, each pair/group being used for about 4 years 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 animals will be trained to carry out tests of visual performance. They will be introduced to the tests slowly and progressively. Initially they will be trained with 'treats' but, to be sure that the limits of performance have been explored, the animals will eventually be trained to earn their daily fluid intake. The animals will be carefully monitored for health and well-being. The optical and electrical stimulation and the neurophysiological recording and testing require that the animals' heads are restrained to remain still, so the animals will also be gradually accustomed to achieve this. Surgery will be required to insert recording and stimulation devices and these devices will be placed based on data collected using magnetic resonance imaging (MRI). Similar devices have been used in different brain areas of human clinical patients and the standards of surgery and care are closely similar to human clinical standards. The research programme is planned at the Moderate level of severity as defined in DIRECTIVE 2010/63/EU. Current UK regulatory practice requires an assessment of the greatest level of harm that might conceivably be experienced by even just one of the 10 animals to be used. Accordingly, this licence is banded as SEVERE, with the expectation that the level of harm encountered by most or all animals will be MODERATE. At the end of the procedures, animals will normally enter a procedure with terminal anaesthesia, at the end of which they will be killed whilst under anaesthesia and the brain recovered for analysis of the tissue.

Application of the 3Rs

Replacement

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

Replacement

This research project can only be carried out through stimulation and neurophysiological recordings in awake behaving macaque monkeys, because this is the only available research method for directly linking the activity in cortical or thalamic sites to behavioural performance in sophisticated perceptual tasks. Non-invasive human techniques, e.g. fMRI or MEG, are currently neither temporally nor spatially precise enough by themselves to study neuronal mechanisms, but specifically they do not permit intervention of the kind proposed here.

Reduction

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

Reduction

For each identified part of the brain to be studied, results will be typically collected from two animals. A third animal is needed if findings in two animals are inconclusive. Additional animals may be needed if an animal cannot be trained in the specified time or if neurophysiological cannot be completed in an animal for welfare reasons. In these cases, although the data collected will be useful, our objectives will require additional animals. All behavioural experiments are based on the same perceptual task.

Therefore, by carefully staging different parts of Protocol 1, the primary objectives can potentially be achieved with between 8-10 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

There are currently no other, non-invasive methods available to test the capacity for causal intervention in neuronal function and to elucidate neuronal mechanisms and functional circuitry at the level of single neurons in real-time. In order to characterize a brain cell and relate its firing statistically to visual stimulus and behaviour requires many trials. The cognitive tasks are subtle and sophisticated, thus requiring the use of macaque monkeys. Animals will be carefully assessed for their suitability and will be socially housed. They carry out these tasks for fluid rewards in the context of restricted access to fluid at other times. Training schedules and rewards are tailored to the individual animal. The risks associated with general anaesthesia and surgery for skull implants are similar to those for humans; we work to the same aseptic standards. Animals will be regularly monitored for well-being by researchers and veterinary staff.

PROJECT 91

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Determining dynamic changes in tumour infiltrating lymphocytes to enhance checkpoint blockade therapies
Key Words	Tumours, checkpoint inhibition, T cell, migration
Expected duration of the project	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;
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 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 2500 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. REDACTED.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. REDACTED. 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.

PROJECT 92

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.

Word limit; 1000 words

Project Title	Epigenetic control of mechanotransduction in the spatial organization of (vulnerable) plaques
Key Words	Atherosclerosis, therapy, diagnostic
Expected duration of the project	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;
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 aim of the current programme is to identify the role of blood flow in the formation of an heart attack and stroke. In order to do so, we will

- Study the reaction of the inner layer of the blood vessels with modern genomic techniques that focus on how the switching on or off of certain genes in specific tissues leads to arterial disease
- Develop new computing-based analytical strategies to validate these genomic techniques.
- Identify new targets for imaging and treatment strategies that target specific molecules involved in the progression of arterial disease: molecular imaging and/or molecular medicines

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

The direct benefits from this project will be: - identification of new gene networks during development of atherosclerosis : the interactions between various atherogenic genes that regulate cellular behaviour during their transition from healthy vessels to diseased vessels will be discovered - identification of new epigenetic (miRNA) drivers for atherosclerosis : the switching on and off of certain atherogenic genes will be studied - identification of new epigenetic interventions for treatment of Atherosclerosis: Once identified, key steps in the sequence of genes switching on/off can be targeted with new drugs

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

1500 mice over a period of 5 years. Specifically, we will be mainly using ApoE-/- mice, which are a well-validated mouse model for atherosclerosis. Most importantly, the plaques they develop are closely comparable to those found in humans so discoveries made in mice will be transferable to human medicine. With the growing number of transgenic mouse strains available, using mice in our research allows us to reap the benefits of any future new strains as they become available as any techniques we develop in our studies will be directly transferable.

In the context of what you propose to do to 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 intervention is the placement of a cuff around a blood vessel in the neck. This will induce the formation of vulnerable plaques over 9 weeks. The cuff itself produces no adverse affects in the mouse other than the induction of the plaques so the surgically altered animals will continue normal activity after the initial intervention. All intervention will be performed under general anaesthesia. After 3-9 weeks the animals are humanely killed and their blood vessels studied with histology. In the rare (<5%) case of side effects (lack of growth, obesity, wound infection) the animals will be humanely killed. Extra nesting material will be provided aiming to prevent this and animals monitored closely for changes in hair coat or skin condition. If problems are seen, a NACWO and NVS will be consulted and treatment provided as recommended. All experiments will be planned so they can be published in accordance with the NC3Rs' ARRIVE guidelines.

Application of the 3Rs

Replacement

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

Replacement

Atherosclerosis only occurs in animals placed on a high fat diet. Other methods (computer modelling, cell culture) suffer from restrictions as Atherosclerosis is a highly complex disease which is difficult to mimic in models different than chronic studies in animals: As atherosclerosis involves multiple cell types (endothelial, muscle, macrophage etc.), purely cell-culture based research fails to address the inter-cellular interactions that characterise the disease. Co-culture methods where multiple cell types are grown in close proximity have a significant drawback that the geometric arrangement of these cells is not representative of the arrangement present in arterial tissue and often lacks the extracellular components which provide significant biological cues in the molecular progression of the disease. Computational multiscale modelling has progressed rapidly in the last decade and is

now capable of integrating molecular and cellular models with fluid and solid mechanics models at the tissue and artery-scales. We are actively pursuing work in this field. However the quality of these models is still limited by the quality of physiological data on which they are based, hence the continued need for in vivo investigation. The output of these computational models also needs to be validated against physiological data.

The strength of cell culture studies is the simplification of a highly complex disease by their design. We use cell culture studies are performed as a screening tool and to obtain data that elucidates cellular molecular details of mechanisms occuring in the intact animal.

Genomics analysis on tissues isolated from experimental animals will be used to obtain information on how endothelial cells adapt in Atherosclerosis at a molecular scale. . A detailed Bioinformatics analysis is associated with this analysis, enabling to maximize quantitative information output of this analysis.

Both cell culture and bioinformatics reduces the need for usage of animals through a reduction of interventions, and animals per intervention as they increase the amount of quantitative data acquired per animal

Reduction

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

Reduction

We use non-invasive imaging techniques to acquire the data required for subsequent computer simulations to obtain information on time-dependent processes of Atherosclerosis development, without the usage of extra mice. Bespoke, statistical techniques are used to optimize assimilation of information from a single animal, and correct for confounding factors.

The very nature of non-invasive imaging allows repetitive imaging of the disease in a single animal, allowing us to perform studies on disease progression without needing multiple mice for each time point

A minimal number is used for each study (based on a detailed power analysis) while achieving realistic outcome. This number is derived from medical statistics that balances the repeatability and amplitude of effect of a certain intervention against the number of repeat observations required to demonstrate a statistically significant result. Eg: if we were looking for a very small effect in a very unreliable experiment, we would need more repeats than if we were looking for a large effect in a highly reliable 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

ApoE-/- mice are used as they rapidly develop human-like atherosclerosis after cuff placement. Other mouse models, are slower, less relevant and produce only plaques after longer duration of studies. To minimize harm, we will only allow trained postdocs to perform studies, shortening the surgical procedure.

We are in constant consultation with veterinary surgeons on how the surgical techniques can be refined to minimise recovery times eg: we have recently replaced simple suturing with intradermal suturing as the standard skin closure method. This has eliminated the problem of wound dehiscence due to the animal scratching or biting its own sutures. Additionally, this has allowed us to re-house animals after surgery in group conditions immediately after recovery as there is no risk of cage mates interfering with each other’s sutures, allowing the mice to resume normal social behaviour.

Anaesthetics and analgesia will be used to mitigate harms (pain,etc) during and after interventions

PROJECT 93

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.

Word limit; 1000 words

Project Title	Bone Tissue Engineering
Key Words	Bone, Biomaterials, Tissue engineering, Fractures, Repair
Expected duration of the project	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;
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):

Our project aims to utilise tissue engineering to develop optimal conditions for bone cell growth on biocompatible scaffolds to repair bone loss in skeletal trauma and diseases. The overarching aim is to develop new clinical approaches that tackle current unmet clinical orthopaedic needs in an ever increasing ageing population. In 2010 there were 69,000 primary hip replacements performed in the UK (>£250m cost p.a.), a significant increase from the 30,000 performed in 2003 (Smith et al 2011). The number of primary hip replacements in the UK is now over 90,000 (financial year 2014/15) which will continue to increase. The scientific unknowns in bone disease and trauma is the ability to repair Ph.D. cartilage and bone defects successfully. It is a clear aspiration that this programme of work will provide a real opportunity to find new medical/clinical technologies that can be subsequently used to repair a multitude of skeletal diseases and trauma.

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

Simple approaches to rebuild and repair bone as proposed are very important. With an increasingly elderly population, the socio-economic consequences of treating failing bone implants and bone fractures are a major concern for all. We believe beneficiaries from the outcomes of the proposed research will include patients undergoing fracture repair as well as the science/academia/veterinary professions in their pursuit of generating new avenues and techniques for stem cell research and orthopaedic repair.

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

The estimated numbers of mice/rats/rabbits to be used in this is study is 960, 224 and 60 respectively. With the ovine studies, the numbers of animals to be used is 144. The mice, rats and rabbit models for skeletal tissue engineering are well characterised and valuable tools in understanding skeletal engineered constructs in the ability to repair bone, this is why they are the first models to be employed to investigate the potentially clinical value of new bone building biomaterials. If a biomaterial is very favourable in the smaller animal models then a model with similar properties to humans is required to test the regenerative strategy before possible translation to the clinic. Hence, the sheep models are used to answer many of these questions particularly the potential pitfalls of scaling up regenerative materials so that they can be employed successfully in larger species. Rigorous, in vitro studies will be carried out before any material is tested in vivo. In addition, to further reduce the numbers of implant models we will test the safety of the test substance(s) in the chick egg model, which provides the functioning blood vessel system (similar to the mouse implant model) without harming the growing chick. With some of the bone fracture models, we have successfully reduced numbers by employing a defect on each limb which is well tolerated thus reducing our numbers of animals used by half. In addition, we have new scanning techniques which allow us to visualise the bone repair over time without the need to cull the animals at certain time points. This has had a dramatic reduction in the animals required for these types of 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?

Apart from the subcutaneous/intramuscular implant model, the main procedures involve the creation of a defect in a bone. The expected adverse effects will be pain from the defect. In the case of the limb bones, there may be an initial reduction in weight bearing and movement. Apart from the implant model (severity - mild) because of the creation of a bone defect all procedures are classified as moderate. However, with the experience gained in previous studies, the likely levels of these models can be categorised as Mild. All animal will humanely euthanized and tissue samples collected for scientific analysis.

Application of the 3Rs

Replacement

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

Replacement

Using animal alternatives is always preferable for a number of reasons including moral and ethical considerations. Some of the proposed work will be achieved using computer modelling and cell and tissue tests in the laboratory. However, laboratory bench testing cannot mimic the complex biological systems such as blood vessel

growth and inflammation that occur in the body that contribute to fixing bone. Other models such as the chick egg model are a bridging step to understanding how bone repairs but the problem with using these non-protected animals is that they are unable to test for bone loading a critical factor for successful bone repair.

Access to human bone tissue will allow us to do most experimental work in the laboratory. Our mathematical/computer models for bone growth and repair have provided us with valuable experimental data which with more advancements will be able to replace a lot of the animal experimental work that is carried out.

This proposal necessitates the use of animals to test an organism's ability to generate new bone using the bone tissue engineering principles following extensive cellular experimental analysis in the laboratory prior to application in human or veterinary clinical settings.

Reduction

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

Reduction

Where possible and with better experimental techniques and analysing systems we aim to reduce the number of animals used per experiment or studies. Experimental laboratory studies using cellular/molecular techniques and the development of bone organ/organoid culture assays have significantly reduced the numbers of animals used in our studies. Better techniques and collaborations with other research groups have maximised the amount of data we can achieve from one animal. This has reduced the number of animals used in other studies where the data could be accessed from our studies. From the experience gained from previous studies, careful experimental design and planning will significantly reduce the numbers of animals used. Along with power calculations and statistically powered experimental designs prior (using statistical calculators used in the NC3Rs 'Experimental Design Assistant') to any planned in vivo work to be undertaken, the minimum number of animals will be used to achieve significance have been efficiently calculated

In addition, new scanning technologies allows at intermediate time points, x-rays or other scanning modalities to assess bone growth/repair and chronological time points These external monitoring systems have enabled us to reduce the usage of animals in some experimental designs by a factor of 4.

Choosing the right model and animal; is critical for reduction of animal use. Therefore, non-load bearing bone defect models can assess gel-like biomaterials to repair the bone, whereby in the larger load-bearing fracture models stronger biomaterials will be required. Every animal will be closely monitored at regular intervals particularly after the procedures, and therein weekly after that. Every possible means will be employed to ensure the well-being and health of the animal

and to reduce any avoidable complications that might arise. In so doing, this rigorous aftercare will give every chance for the animal to complete the study and in doing so reduce any further experimental repeats and therefore reduce 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 animal models proposed have already been extensively detailed in the scientific research field and have been used to demonstrate bone formation using human bone cells. Specially bred mice/rats will allow us to investigate the potential of human cells to form and repair bone fractures which cannot be achieved in ordinary bred mice/rats. In addition, specific models will be used to understand how different types of bone of the body repairs i.e. skull bones grow and repair differently from leg bones. Larger animal models such as the sheep bone defect model are well characterised to assess the potential of bone repair techniques to be translatable for clinical applications in humans.

Our programme of research is continually being refined to improve surgical procedures and aftercare, where new techniques are continually being developed. Inserting multiple implants or creating two small defects in each limb of larger animals have proven to be a successful refinement which is well tolerated by the animals with minimal suffering. The use of live imaging techniques allows us to assess the repair of the bone over time without culling the animal to visualise/analyse the levels of bone repair. Every animal will be closely monitored at regular intervals particularly after the procedures, and therein weekly after that. Every possible means will be employed to ensure the well-being and health of the animal and to reduce any avoidable complications that might arise. To minimise suffering, all animals will be assessed daily for signs of distress or ill health. Any animals exhibiting a reduction in weight gain of 10 % body weight over a 3 day period, or showing signs of distress and/or pain will be humanely killed. Handling will be minimised to routine husbandry and procedures required for the project

PROJECT 94

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.

Word limit; 1000 words

Project Title	Preclinical development and assessment of medical interventions
Key Words	microbiology, vaccine, antibiotic, intervention, countermeasure
Expected duration of the project	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;
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.

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 overall objective of this licence is to develop new vaccines, therapies and treatments (interventions) against several infectious diseases which are considered to be a public health threat in the UK and globally (e.g. Anthrax, Plague, Melioidosis). New vaccines and other interventions are required to be developed against these diseases but human trials are ethically and practically very difficult to conduct due to the infrequency as well as severity of the diseases caused by these public health threats. This project aims to assist in the assessment of the new interventions using animal models of these diseases. Candidate interventions that show promise in these models will be taken forward by sponsors in order to provide doctors with new interventions to protect the health of the public against 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)?

This project will provide the capability to assess new interventions against the most serious forms of infectious disease. Human trials will not be 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 against serious forms of infectious disease. 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?

Over the next five years, it is possible that a variety of species to develop models of infections and to assess the properties of new interventions. 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 each year. 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. As a result, we predict to use the following; Mice 7450 Rabbits 800 Guinea pigs 1000 Sheep 10

In the context of what you propose to do to 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. In addition, some of these experimental drugs may cause harm for example by disrupting the normal community of beneficial bacteria in the gut. Respiratory infection in animals is likely to develop very quickly and may overwhelm the animals due to a rapid septicaemia. The likely adverse effects include a period of fever, a combination of clinical signs of infection including ruffled fur and eye closure. If clinical signs include signs of imminent death, the animals will be euthanised in accordance with Schedule 1 procedure. 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 regulatory restraint associated with the infectious diseases we are studying, all animals will be humanely euthanised 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

Human studies of infection with these diseases are too dangerous. We will, however, try to use human sera, wherever possible to assess human vaccines for biological activity. In addition, although insect models of infection are available, the relatively simple nature of these models makes them only suitable for early stage high throughput screening. If however, insect models suggest a therapy or intervention may show promise, mammalian studies of efficacy will need to be conducted to assure regulators that the candidate is effective in more complex animals.

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

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 as closely matched to human disease in which case we need to use other species such as rabbit.

Our staff work in shift patterns to ensure that animals considered to be in the critical phase of an experiment are regularly monitored. This high frequency hands-on monitoring has been shown to effectively minimise the welfare costs to animals hence our overall severity rating of moderate.

PROJECT 95

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.

Word limit; 1000 words

Project Title	Identification of endocrine disrupting chemicals in fish for regulatory purposes
Key Words	chemicals, hormones, sexual disruption, regulation, wildlife
Expected duration of the project	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.

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);
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):

The aim of this licence application is to safeguard wildlife (and humans) from the effects of certain type of chemicals.

Some man-made chemicals interfere with the endocrine (hormonal) system that controls, amongst other functions, reproduction. These chemicals are called Endocrine Disrupting Chemicals (EDCs). As well as posing potential risks to humans (there are several studies that attempt to link exposure to EDCs with the decline in sperm counts, the increased incidence of certain cancers, diabetes, obesity and developmental abnormalities), EDCs are of particular concern for fish. This is because the aquatic environment is often an important sink for man-made chemicals and sewage waste.

Field studies during the 1990's revealed an extensive feminisation of wildlife. In the UK both roach from rivers and flounder from estuaries were heavily affected. Factors contributing to this include large population size, small geographic area, heavy industrialisation, low riverine flow and inefficient effluent treatment. Importantly in the 1990s there was no information on the ability of certain chemicals to interfere with the hormonal system of fish and other aquatic wildlife.

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

Chemicals are essential to life on Earth. Humans have developed chemicals to use for sanitation, preserving foods, treating diseases, and increasing crop yields to allow food security. Chemicals are also produced for less essential to life activities (i.e. recreational, travelling, communication and for personal care products); these chemicals add value to human life by increasing welfare and the sense of well-being.

Although many chemicals are simply beneficial, others are harmful to humans and wildlife or present both benefits and risks. The benefit therefore derived from this project is to provide the regulatory evidence needed for discriminating between harmful and not harmful chemicals. In this way we can enjoy the benefits from the use of not harmful chemicals and prevent the harmful chemicals entering our environment. Once harmful chemicals enter the environment, they can harm both wildlife and humans.

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

In the 1990's there was no information on the impact of chemicals with endocrine disrupting properties. This led to the development of internationally standardised tests under the management of the OECD, all aimed at assessing the hazard and/or the risk of old and new chemicals in terms of endocrine disrupting potential. These tests have now been validated and are used to assess the chemicals to avoid further harm to wildlife and indirectly to humans. The validated species (each to a different degree for different protocols) include the Fathead minnow, the Japanese Medaka, the Zebrafish and the Three-spined Stickleback. The numbers we will use depends largely on the requested we receive from the relevant stakeholders (in this case primarily the chemical industry). To avoid licence amendments, the numbers requested are most likely overestimated in relation to the numbers we will use. In total, we request the authority to use 18,000 fish during a five year period.

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

There is a regulatory need to assess chemicals properties in terms of endocrine potential in order to protect both wildlife and humans from their potentially harmful effects. When regulators suspect endocrine disrupting activity they can ask for these highly specific tests to be conducted. Suspicion is usually raised by a series of relevant non animal tests. Chemicals with endocrine disrupting activity are not toxic, so they don't present adversity from this point of view. The harm they can do is very different; they can completely prevent for example sexual maturation or they can block spawning. These are not perceived as effects of high severity because they don't involve any suffering; yet, their endocrine modulating activity can result in population level effects via impaired growth and reproduction. All fish will be humanely killed at the end of each experiment. All protocols are of mild severity.

Application of the 3Rs

Replacement

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

Replacement

The regulatory requirement is currently involving vertebrate (fish) testing which can confirm the endocrine activity beyond *in vitro* (non animal) tests. If regulation was based solely on *in vitro* tests then many useful chemicals would have to be either removed from the market or never allowed to be placed in the market. This is because the false positive rate of most relevant in vitro tests is particularly high.

Nevertheless, we believe that the collection of more data from the exposed fish (as we plan to do) may allow in the future replacement (via computational modelling techniques).

Reduction

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

Reduction

The tests relevant to this licence application have been validated following international efforts and interlaboratory testing; as such the number of animals needed and the number of treatments have been determined in a robust manner and are highly descriptive.

Refinement

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

Refinement

Like in any testing involving a protected vertebrate, there is plenty of scope for refinement, even if the test conditions and aquaria housing are highly descriptive. There are four species involve in this particular test guideline programme, namely, Fathead Minnow, Japanese Medaka, Zebrafish and three-spined stickleback.

The procedures are mild (i.e. we don’t expect mortalities or long and lasting suffering) but we have controls in place, should it happen unexpectedly. The main tool we will is close monitoring.

The fish are sourced from our own breeding establishment to ensure disease-free, high quality animals acclimated to experimental tank conditions and being in the right stage of age and sexual maturity. We have a dedicated, high-tech aquarium facility, with monitoring and call-out alarms. Named persons oversee staff training and performance, care of fish, and dissemination of information. Close links with the international fish research and regulatory community ensures we are aware of any developments. Consideration is given to all aspects of the environment including

husbandry where a dedicated team of specialist aquarists complemented by long-standing experience operate. We believe we have a strong institutional culture of care and have review processes to identify where further improvements in care can be made.

PROJECT 96

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.

Word limit; 1000 words

Project Title	Provision of an outsourced Drug Discovery platform for diseases with an inflammatory component
Key Words	Out-sourced drug development platform, Inflammation
Expected duration of the project	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;
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):

Discovering new and better medicines has become increasingly challenging and sadly many potential new drugs have failed when tested in man. Improved understanding of how diseases start in the body (including the role of faulty genes) and the development of new technology has come together to provide new ways of looking to treat disease. Under this project licence our aim is to provide a service to smart thinking clients by working with them on novel targets they have identified. Combining our knowledge of animal models (and what their limitations are) with their understanding behind the target and the disease it is aimed to treat, we should help achieve a better clinical outcome. One such disease we have a real hope in a new treatment being successful for is osteoarthritis (OA), a condition that affects your joints causing pain and stiffness. Our focus is on looking at how other novel treatments for this disease have not been as successful as predicted and on how by teasing apart the progression of disease in the animal models used, may make them more clinically relevant for testing the next generation of related medicines. Secondly, some targets have the potential to treat more than one illness. For example stimulating a mechanism that helps the body rid itself of unwanted molecules may be beneficial in treating both lupus, which is an autoimmune disease in which the body’s immune system mistakenly attacks healthy tissue in many parts of the body leading to symptoms such as a feeling of tiredness (it is often associated with a red rash on the face), and in a serious liver disease, that is not caused by over consumption of alcohol but by a build up of fat in liver, called non-alcoholic steatohepatitis (NASH). This disease may be associated with our unwitting desire to consume a fast food diet but once the damage has been done it is difficult to reverse. In these cases of potential multi therapies we will focus on the disease with the biggest un-met clinical need first (NASH) and if we find a potential drug that has

a positive outcome in our animal model of that disease then we will go on to evaluate that compound's effects in models of other relevant diseases, such as lupus. The third area we hope to make a difference in is an example of where genetic mutations have identified a link with a lung disease called pulmonary arterial hypertension (PAH). Here the small arteries in the lungs become narrow, making it harder for blood to flow through so the heart has to work harder, eventually becomes weak and may even fail. There is a chance that understanding the outcome of this genetic alteration and how to moderate it with a biological agent could be of benefit to these patients.

These are just three examples of where we are providing a service to our clients to work towards a common goal of developing new and better drugs. However, because we want to do more we have other collaborations in the early stages of drug discovery that may lead to the treatment of additional diseases such as Alzheimer's disease and Alpha-1 Antitrypsin disease. Because targets for these diseases are developing we are mostly focusing on helping the client with proof of concept studies, developing cell based assays to test their compounds in and then looking at how well these compounds enter the blood stream so we know that drug will reach the right part of the body before going on to test them in the relevant animal model of disease.

Whilst drugs are available to help treat all the above diseases none are perfect meaning there is still an unmet clinical need to find better medicines. Animal models that were developed in the past have been invaluable in taking current drugs in to the clinic but they may no longer be the most appropriate ones in which to test the effectiveness of drugs with a novel mechanism of action. Our aim is to provide animal models of disease that will be able to answer the question of whether a drug with a novel mechanism of action will be able to treat that disease when tested 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)?

Advances in our understanding of the science behind diseases that have an unmet clinical need such as OA, lupus and PAH will lead to new and better treatments. By 2050 there will be around 130 million OA sufferers, 40 million of whom will be severely disabled by the disease. There are significant unmet needs in the early diagnosis, monitoring and treatment of the disorder that could bring relief to suffering patients. Similarly, whilst the management of lupus has progressed enormously in the last 10 years, there are patients who do not respond to the most widely prescribed drugs and addressing this major unmet need by developing new drugs remains a priority. Non-alcoholic fatty liver disease (NAFLD) has reached epidemic proportions and is becoming the most common cause of chronic liver disease. NASH, a severe form NAFLD, is most likely to become the primary reason why patients will require a liver transplant over the next 10-20 years. An improvement in

diet and increasing physical exercise is the first line taken to help these patients but this is known to be of limited effect. Hence, there is an urgent need for new and safe drugs that successfully reverses or prevents progression of liver injury in patients with NASH. Once diagnosed with PAH a patient has a 30% chance of dying within three years. Despite improvements in the diagnosis and management of PAH over the past two decades, with the introduction of targeted medical therapies leading to improved survival, current treatments only manage the symptoms and the prognosis remains poor. By understanding and refining our animal models of OA, lupus and NASH we can learn more about progression of disease and how to design experiments in which to test new medicines in the most appropriate way for each target, and advise on how best to run a clinical trial in humans with the disease. Likewise for the targets in the early phase of drug discovery our scientific driven approach to the service we provide will help prove whether these targets are worth pursuing by our clients. In all cases we will enable milestones to be met more efficiently for our sponsors (academics, the Pharmaceutical industry, clinicians, venture capitalists) so key ‘go’ / ‘no go’ decisions can be made using the minimal number of animals possible and assure a better success rate in the drug discovery process than has been seen previously.

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

We will use normal mice and rats and mice that have been genetically altered to investigate the disease of interest or the role of a specific gene in the development of that disease. We anticipate we will use 12450 mice and 8250 rats during the five years of this licence.Rats have now been added to Protocol 9 so the total number of rats during the course of this licence has now increased by 500 to 8750.

In the context of what you propose to do to 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 involve either rodents that have a predisposition to exhibit disease or those that undergo a regulated procedure to induce disease. For example we breed mice that have been genetically modified as a model of lupus. These mice spontaneously develop antibodies to their own tissues in the same way as humans with lupus do but they appear outwardly normal. We measure levels of these antibodies in the blood by taking a small sample from a vein in the tail, which causes minimal pain or distress. As these mice age they develop kidney damage, which we can detect by measuring how much protein is excreted in their urine by allowing the mice to urinate onto a special stick that measures protein. Animals are humanely killed before the disease is likely to cause anything other than moderate distress. To model osteoarthritis we either inject an agent into one knee of a rat or mouse that causes inflammation or we carry out a surgical procedure that mimics a tear in the knee caused by a sports injury. Gradually the architecture of the knee is destroyed, as is seen in humans with OA, and as a consequence the animal may experience

mild to moderate pain. We monitor this using the same techniques as used in humans. For example by measuring how much weight an animal puts on its affected limb compared to its healthy limb. We know from our experience of looking at histology of the knees how the disease progresses and we do not allow any experiment to go beyond a certain point to ensure that no animal experiences severe pain or distress. In our model of fatty liver disease we mimic the human condition by feeding normal mice with a diet that is high in fat and putting sugars in their drinking water. This “fast food diet” is similar to that many humans consume who have an unhealthy life style and who eventually develop fatty liver disease. Whilst some of these patients do become very sick none of our mice experience anything other than mild or moderate distress before they are humanely killed and the degree of liver damage assessed. To reproduce PAH in our animal models we either, expose them to air containing low levels of oxygen for up to four weeks and then put them back into normal air or we inject them with a plant extract. These procedures result in changes in the lungs, similar to those seen in humans with the disease, which we measure at the end of a study when the animal is anaesthetised and from which it is not allowed to recover. In some cases we make measurements using non-invasive imaging techniques so we can follow the course of the disease with time. Prior to the non-recovery step in the protocol animals will suffer no more than mild to moderate distress. We use drugs or other substances to try and prevent or reverse disease. The techniques used for dosing with test agents and sampling to measure outcomes are chosen to cause the minimal pain and distress to the animal, whilst achieving the desired outcome. At the end of any programme of work, animals are humanely killed and in most cases, tissues taken and analysed to answer questions around that programme of work.

Application of the 3Rs

Replacement

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

Replacement

All the diseases we are working on have an inflammatory component. Inflammation is very complex with a web of interactions that unfold as the result of provoking an inflammatory stimulus. As such animal models of disease in which there is an inflammatory component are equally complex, with no one model being able to mimic the human condition and there is no *in vitro* model that recapitulates this complexity. Where they are available, we obtain human cell lines from patients with diseases to help support the hypothesis that a potential new target is likely to lead to the development of a new drug for that disease prior to testing in animals. We have also developed some new techniques in cells and tissues for measuring key biological processes that a target with a novel mechanism of action should affect.

When testing new drugs, we can support the use of our animal models by applying these cell/tissue based techniques to the animal model of disease.

Reduction

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

Reduction

The number of animals we use depends on the desired outcome, the study design, the number of groups needed (including appropriate positive and negative controls (both animals and test agents) and how large a difference we hope to be able to detect. Pilot studies that are used to refine procedures and to discover potential problems before the main study begins uses relatively small numbers of animals based on experience and judgement but numbers are large enough to provide needed estimates for future sample size analysis. We use experienced scientists with a range of skills to design experiments and interpret data. Their experience in the challenges of animal experiments helps with determining the minimum number of animals needed to generate data that is meaningful both biologically, and statistically for each measurement made within a study (e.g. the use of careful power calculations to ensure that a significant effect can be detected with the number of animals assigned to an experiment). Good planning ensures that within any series of studies we can control any variability that might be introduced. This includes using animals of a similar age/weight range; testing different batches of test agents *in vitro* first; using the same source of reagents; keeping records of observations made and standardising as many components of an *in vivo* model as practicable. Wherever possible our *in vivo* workers are blinded to any treatment thus reducing bias. Likewise those that carryout downstream analysis is blinded to the treatment. If an appropriate genetically altered animal is not available commercially then we manage our breeding programmes, as far as is reasonably practical so no animals are wasted. In most cases excess animals are used to provide blood or tissues to support target validation or are offered to others. If a programme of work on a particular GA strain is no longer required then the strain would be cryopreserved (frozen at very low temperatures) so they can be re-derived in the future.

Refinement

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

Refinement

With any new programme of work we scrutinise the literature for information and work closely with our clients to identify which animal model is most appropriate for their target. When new models or experimental designs are being developed we use

data from preliminary studies to guide us on refinements, points at which to intervene and what our humane end points should be. Likewise, where signs of harm are difficult to predict, for example when testing novel compounds, we carry out preliminary studies at an expected therapeutic dose and route in small number of animals first (typically 3) and continually monitor them over a 2 hour time period. If acute adverse effects are seen then the dose is titrated down to a no effect level before pharmacokinetic or efficacy studies are conducted.

We seek input from others, such as clinicians and experts in other disciplines (such as toxicology); look to useful websites such as the NC3Rs, RSPCA and advisory bodies such as FELASA. We also follow the local rules for the animal facilities we use with respect to husbandry, housing, transport and acclimatisation periods.

We employ a consultant clinical histopathologist for assessing blind any pathology data in our models. This increases our knowledge of the models and where along the pathway of disease progression novel targets are most likely to be effective. This in turns supports the validity of our models relative to the human condition.

PROJECT 97

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.

Word limit; 1000 words

Project Title	Mechanisms of Infectious Disease
Key Words	Infectious disease, respiratory pathogens, co-infections, host pathogen interactions, vaccines
Expected duration of the project	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;
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):

We aim to explore the mechanisms of interaction between disease-causing microorganisms and parasitic worms, and their hosts in models of respiratory colonisation, brain infection, intestinal infection, and invasive disease. Our objective is to determine the key microbial virulence factors that lead to disease and the key host factors that provide protection against disease. In doing so we aim to discover novel therapeutics and potential new vaccine candidates against human pathogens of significant disease burden globally.

We will do this in two ways; [1] by investigating the characteristics of the microorganism that enable it to cause disease and [2] by examining elements of the host immune response that determine how the disease progresses. The overall aim of these studies is to uncover new targets for therapy. These may come about through identifying genes or proteins of the microorganism that can be targeted to inhibit its ability to cause disease, or else by examining essential or counter-active components of the immune system that can be boosted or inhibited in immunotherapy.

What 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, we would anticipate that our research will be of direct benefit to human health in a number of ways. Improved understanding of the mechanisms of disease may improve diagnostics and disease monitoring procedures. Identification of microbial and host factors that influence disease progression will inform and aid us in the design of new therapeutics and vaccines to target infectious diseases. Finally, discoveries of host immune mechanisms that contribute to disease resistance in

microbial infection may be more widely applicable and the dissemination of such findings within the scientific community may open up new avenues of research for researchers in other fields such as virology, parasitology and cancer biology.

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

Mice and rats. We have been conducting this research for the past 20 years and will continue to do so for the foreseeable future. We typically use several thousand animals per year. REDACTED

In the context of what you propose to do to 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 infect animals by different routes to induce different disease characteristics. For example, to study sepsis, an intra-venous administration of bacteria provides the most effective route to disease and minimises the suffering of the animal as compared to allowing a progression from pneumonia to sepsis following intra-nasal injection. REDACTED This is a highly attractive model for a number of reasons: [1] Nasal carriage is extremely common in human populations but is poorly understood. Improved understanding of the factors that control carriage vs. invasive disease may influence the design of vaccines and therapeutics. [2] This model involves very little suffering for the animals. We do not observe any adverse effects during carriage experiments and the animals remain active throughout.

Application of the 3Rs

Replacement

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

Replacement

Animal models of disease are an essential component of our plan of work and we have extensive experience in developing and characterising disease models that closely mirror clinical phenotypes including pneumonia, meningitis, sepsis and asymptomatic nasopharyngeal carriage in the nasopharynx. These model systems can be easily manipulated and have a well-defined progression and so we are confident that they represent the best route to producing robust data with relevance to human disease. Furthermore, we have multiple checks in place to ensure that animal suffering is kept to an absolute minimum. Any animal infected with a pathogen is monitored for the duration of the experiment.

Reduction

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

Reduction

We have been reducing our overall numbers of mice over the past 20 years by refining the models we use, by introducing in vitro cell- and tissue culture-based models prior to in vivo mouse models and by accurately powered statistical analysis. By these means we only use the minimum of mice that is enough to allow statistically significant differences.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 our disease models involve mice. Mice represent the ideal species for our studies due to the extensive similarities in mouse and human immune systems and the high reproducibility of disease phenotypes following experimental infection. We will always endeavour to use the minimum number of mice per experiment and are well aware and have implemented the 3Rs in our current and previous project licence work.

PROJECT 98

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.

Word limit; 1000 words

Project Title	Orthopaedic Infection
Key Words	Orthopaedic, Infection, Implant, Trauma
Expected duration of the project	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:
Yes	(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.

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):

Infection following repair of broken bones or joint (e.g. hip and knee) replacement is recognised as a significant problem in orthopaedic surgery. Despite the care taken during surgery bacteria can occasionally invade the implant surface resulting in failure to heal the fracture or new joint being unstable and the need for further surgery to rectify the situation.

Current failure rates are up to 2% of initial implantation surgeries (and significantly higher in secondary implant replacement procedures following previous infection), with treatment methods involving removal, prolonged strong antibiotic treatment and eventual replacement of the implant through further surgery. The impact of these infections is wide reaching with significant patient discomfort, increased healthcare costs and in severe cases, amputation of the infected limb.

The aim of this project is to develop devices and device coatings that demonstrate the ability to eliminate any bacteria that adhere to the implant, preventing the development of bacterial infection. The resulting decrease in infection rates and the subsequent human and financial costs would be substantial justifying the use of animals in technology development.

In time the intention would also be to further disseminate the scientific knowledge gained through peer reviewed publications.

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

This project aims to ensure that anti-infective implants can be developed and demonstrated as effective for clinical evaluation and eventually to launch. 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 burden to already stretched healthcare systems.

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 project it is estimated that no more than 400 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 project will involve the surgical implantation of a metal pin into a bone canal, which may lead to some degree of discomfort following surgery, although this will be reduced by the use of a minimally invasive technique and the absence of a fracture in the bone which is not required for this work. Any discomfort will be minimised with the use of appropriate pain relief. The establishment of a bacterial infection in the bone of the test animals may lead to some moderate discomfort, however efforts will be made to minimise this with the use of circulating antibiotics and appropriate pain relief. At the end of the studies the animals will be humanely euthanised. 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 euthanised.

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 safety and microbiology testing regime to select the most appropriate anti-infective coating for implant surfaces (e.g. an intermedullary nail), allowing selection of the best 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**.**

Reduction

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

Reduction

Prior to studies covered by this project licence extensive laboratory based microbiology testing will be used to ensure that only the most promising technologies will put forward for testing in animals. During the planning of these studies appropriate statistical techniques will be used to ensure that the studies yield appropriately reliable results with the use of 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 species of choice for this work is the rabbit, chosen for its susceptibility to infection, allowing a lower, more clinically relevant level of bacteria to be applied than would be required for other species.

During surgery rabbits will be under general anaesthesia. Following implantation surgery the rabbits will be treated with antibiotics and appropriate pain relief. Suffering will be kept to a minimum through the enforcement of short time lines, relatively low bacterial doses and use of systemic antibiotics restricting the chance of a systemic infection developing.

PROJECT 99

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.

Word limit; 1000 words

Project Title	Development of Models of Parkinson's and Lewy body disorders
Key Words	Parkinson's disease, Dementia, Chemical, Therapy
Expected duration of the project	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;
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 aim of this work is to understand more about the biology of Parkinson's disease (PD), the main cause of degenerative movement disorder in the UK and the related disorder dementia with Lewy bodies (DLB), collectively termed Lewy body disorders (LBD). Parkinson's disease affects about 120,000 people in the UK, and similarly DLB affects about 50,000 people in the UK. We have no long term cures for these conditions. In PD it is thought that, in addition to growing older, chemicals in the environment and also the genes we inherit from our parents, contribute to developing this disorder. For DLB there is a strong genetic effect, but it isn't known how the environment might affect DLB.

We want to understand what changes in the brain lead to LBD, and what genes and chemicals might alter these processes. By having a greater understanding of LBD, we aim to identify treatments. Currently there are several animal versions (models) of LBD, but none of these really looks like what is found in the human situation. This is probably because we haven't identified all the factors that cause LBD. As part of this project we want to produce more accurate models of LBD, so that these are more likely to create findings that are relevant for patients.

We have been able to identify certain chemicals that might cause LBD, and some of these seem to work by affecting mitochondria (the parts of cells which produce energy). We also know that in people with LBD these mitochondria are faulty. We now want to see how mitochondria work in a model of LBD. Looking at the genes which cause LBD, we have also been able to find that some of these relate to the function of another small compartment within the cell called the lysosome which normally removes any damaged cell parts, including mitochondria.

What 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 produce more realistic models of LBD. These models will more accurately reproduce the biochemistry, pathology, and symptoms of these complex human disorders. At the same time we will be able understand how mitochondria function in nerve cells, and also what happens when the mitochondria become damaged. This might be because of specific genes or perhaps because of environmental chemicals. Having a better model will allow us to try new drugs which might modify the disease in the animals and which would ultimately lead to better treatments for people with these conditions and improve their quality of life.

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

We will use mice and would expect to breed up to 800 mice. Only about half of the animals we breed will be used since the genes the mice carry will only be passed on to half of the offspring. Most of the normal mice will be humanely killed but we will also try to use these mice where we possibly can.

In the context of what you propose to do to 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 are studying LBD, the two main adverse effects we expect to see would be impaired movement and an inability to remember things. Both of these we regard as being of moderate severity. Because the ability to move about affects so many aspects of behaviour, at the first signs of any problems we would seek advice from a vet and as necessary. humanely kill the animal. For problems with memory, this really only becomes apparent when the mice begin to stop looking after themselves such as forgetting to eat. Again, we would ask the advice of a vet and humanely kill the animal. In all cases, once the animal is humanely killed we will take multiple tissue samples to allow a comprehensive analysis of the animal’s pathology and biochemistry.

Application of the 3Rs

Replacement

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

Replacement

Neurodegenerative disorders like LBD are uniquely human conditions which affect specific parts of the brain and consequently have many different aspects giving rise to several clinical symptoms. What defines these disorders is that only specific nerve cells are damaged and it is these changes that produce the specific symptoms. Whilst we can understand at a cell level what might lead to these disorders, this type

of work doesn’t tell us why only certain groups of nerve cells are damaged or provide us with complex symptoms. For example, why do nerve cells in the basal forebrain become abnormal in LBD and cause memory problems? These questions can only be answered using complex animals who have brain structures similar to those found in humans and have complex behaviours such as memory for objects or can undertake complex tasks such as gripping an object.

Reduction

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

Reduction

Because we have considerable experience of mice and how they behave in certain tests we have a good understanding of how many animals are the minimum needed to give us an accurate answer to an experiment. We have calculated for each test the minimum numbers of animals needed and these are the figures we base all of our work on. This allows us to reduce as much as possible the numbers of animals in each study.

Additionally, before we do any work with animals, we try to find out what the effects in animals might be using cell models. Here we can quickly treat a cell to find out what changes could occur before we try the experiment in an animal. If the cell experiment provided a suitable answer, then we would proceed to using the animal with some assurance that we might see similar things.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 because they have similar brain structure and function to humans, and damage to the same nerve cells which are damaged in LBD causes symptoms similar to those seen clinically. These animals are used because we can relatively easily change their genes, but they are still complex enough to produce the same types of symptoms. In all cases, we will continuously monitor the mice so that we do not allow the symptoms to become so bad that they interfere with an animals normal daily activities within the home cage.

PROJECT 100

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.

Word limit; 1000 words

Project Title	Assessment of intersex and sex-reversal in wild roach (Rutilus rutilus) in English rivers
Key Words	pollution, environment, fish, micro-plastics, oestrogenic
Expected duration of the project	1 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.

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):

Chemicals from wastewater treatment works effluents (WwTW) entering rivers are known to negatively impact fish living in them by causing male feminisation, this can reduce their fertility. The last national survey of this fish feminisation was conducted over a decade ago, since then some feminising chemicals have been banned and others are being monitored. Improving WwTW effluent quality to a level which very low levels of chemicals escape in to the environment is expensive. To understand if the health of fish living in rivers has improved, stayed the same or worsened in the last 10 years we need to conduct another survey. This information will help legislators decide if we need more environmental controls (which can be expensive) or if what we've have already achieved is good enough to protect fish and aquatic environmental health.

Many other chemicals (such as prescription drugs) entering rivers from WwTW are also now being considered as a possible threat to wild fish health. We are staring to understand how these chemicals might impact fish behaviour or immune systems using laboratory tests. To assess the threat to wild fish we need to know which chemicals and at what concentrations they are found in fish, as the blood or tissue concentration will tell us if the chemical(s) are getting into the fish at a 'dose' which could cause an negative effect.

Another emerging environmental problem is micro-plastics. These tiny plastics can be ingested by animals. There has been growing research about micro-plastics being eaten by marine animals (birds, turtles, fish, etc.) however, there is far less known about freshwater animals. To minimise animal use we will combine the fish feminisation survey with the chemical analysis of fish tissues and the fish micro-plastics survey. This information will be very useful for the water industry,

environmental regulators, environmental groups, members of the public and other researchers.

What 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 robust and up to date survey of roach (fish) feminisation, chemical (e.g. drug) and plastic contamination will help inform environmental regulators of the impacts of WwTW effluent entering rivers is having on native fish species. This will help them decide on the best policies for protecting the environment.

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

Our chosen fish species is the native roach, we expect to use 700 fish over a one 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 main adverse effects expected will be due to handling and restraining wild animals. We need to capture the fish at river locations and then transport them back to our labs for analysis. The capture and transport can cause mild adverse effects (stress, abrasions, scale loss). Experts in fish capture will be used to collect the fish from the river. Fish will then be held in large aerated tanks for transport and given mild anti-infection treatment via the water to minimise effects of small abrasions or scale loss. When fish arrive at the lab they will be housed in large tanks in a temperature controlled room, prior to being sampled. All fish with be anesthetized during the sampling procedure, they will not wake up from the aesthetic, and will be killed by humane methods. All fish will be regularly observed for signs of stress or trauma, especially directly after capture and transport. Any fish found to be injured or to be un-well will be killed immediately by humane methods.

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 wild fish living in English rivers is central to this research project, as these fish have spent their lives in rivers impacted by WwTW effluent being exposed to a huge variety of chemical (e.g. drugs, personal care products) and wastes (e.g. plastics). Only by studying real wild animals can we get the full information we require to inform future scientific research and provide robust information to policy makers.

Therefore, owing to the nature of this environmental survey work no non-animals models or methods such as cell based assays can be used instead of using real fish.

The information gained during this research will help researchers in the future to use less animals in research. The data obtained from the feminisation survey will be included in a roach population computer model, which in the future could be used to assess the risk of different conditions (such as WwWT effluent levels) to real fish populations.

The data produced on chemical contamination (drugs, personal care products etc.), identification and concentration of wild fish will be used to help inform and test another computer model, which in future could predict the types of chemical pollution found in fish, and whether they would be at high enough concentrations to negatively impact fish health. This type of model would reduce the need of animals in the future and help researchers and regulators understand the threat of these ‘emerging’, as yet unregulated, pollutants.

Reduction

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

Reduction

We have a lot of historic data on the frequency of feminisation in this species i.e. how many feminised males we might expect to find at a given location due to the level of WwTW effluent. We have conducted special statistical analysis, called 'power analysis' to determine the minimum number of fish required to detect male feminisation. We will use a maximum of 50 roach per site, a total of 700 fish for the whole 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

The roach is our chosen fish model for this project. Roach normally have separate genetically determined sexes i.e. males and females. The feminisation/intersex condition is therefore not normal and is caused by external stressors (chemicals/pollution).

The roach has been used in the majority of our and other researchers work on this topic in the past, proving a wealth of knowledge on this species, and a significant historic data set with which to compare our current research. Roach live in lowland rivers, which are most frequently impacted by WwTW effluents. Therefore, they are

one of the species most at risk of this and other types of aquatic pollution. Their biology is well studied.

Roach are known to feed on a variety of food items: plankton, a range of insects, algae and plant material, etc. so they are likely to encounter plastics in the environment whilst feeding, either directly or via food items that themselves have ingested plastics. There broad diet makes them a good test species of this type of analysis.

Roach will be captured and looked after by highly trained people. Once they are captured they will be transferred to aerated buckets of river water to aid initial capture recovery. When transporting fish, treatments will be dosed in to the transport tank water to aid recovery from handling/transport stress. During capture, transport and while housed in the lab roach will be observed to check they are healthy. If at any time roach are seen to be stressed or un-well (not able to swim properly, missing scales, injured) they will be humanely euthanized. Once at our lab fish will be housed in a temperature controlled room, prior to being sampled. Fish will be anaesthetised for blood sampling and will not be allowed to recover consciousness, i.e. the fish will be killed while under anaesthetic to minimise any potential suffering. All other tissue samples will be taken once the fish is dead.

PROJECT 101

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.

Word limit; 1000 words

Project Title	Development of Nanomedicines as Theranostics
Key Words	nanomedicine, nanotechnology, gene therapy, drug delivery, theranostics
Expected duration of the project	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;
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):

A recent field using nanotechnology to diagnose and treat diseases called ‘Nanomedicine’ has recently emerged. Nanomedicines utilise nanoparticles (i.e. in nano-size scale) as a carriers to deliver drugs, protein/peptides or gene, serving as therapeutic and diagnostic modalities. The overall goal of this project is to use nanomedicine strategy to increase the gap between a therapeutic and a toxic dose as a result of keeping the cargos out of tissues where toxicity may result, and concentrating them in the areas of interest to do the greatest good. In particular, cancers, neurological disorders, cardiovascular diseases are of interest as such diseases are rapidly increasing in the European community. Developing new therapeutics by improving the formulation and delivery is of extreme importance.

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

First of all, this project will advance our knowledge of nanomedicines. Material, bioengineering and pharmaceutic science will be bridged together to investigate and explore how layering different materials (formulation) can influence the drug delivery efficiency and what key factors are important to achieve specific targeting. Improvement on therapeutic index (increased therapeutic effect with lower toxicity) is also expected. It is also part of the findings from our previous studies that using nano-formulation can enhance drug loading with reduced side effects or off-target effects, making the resulting nanomedicine more effective compared to drugs their own or with current formulation. Moreover, we aim to develop novel and clinically applicable nanomedicines for therapy/diagnosis purposes in this project. The results obtained should have immediate application in treatments or diagnosis for diseased animals and can be translated into applications in patients in future. The developed

nanomedicines and nanotechnologies will be valuable to other peer scientists and clinicians in a basic engineering/formulation point of view or their perspective clinical translation.

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

In most cases, mice will be used in these studies and when not possible rats will be used as alternatives. A maximum of 400-600 animals per annum is estimated for each protocol depending on the protocol aim and experimental design. This number was allocated to ensure that the data obtained is truly meaningful and can be trusted for further progress into the topic. The study periods varied depending on the type of protocols. Disease models range from 14 weeks to 6-7 months. Toxicity assessment studies in heathy animals can last up to 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?

After the initial tests in cells, candidate nanomedicines which prove to be safe and effective in cells will be investigated for their behaviour in animals. Selected nanomedicines will be administered in healthy animals to assess the distribution and toxicity profiles. Only the most promising nanomedicines will be further tested in diseased animals which are used to model therapy in clinic. Some animals will develop tumours and some will develop early signs of Parkinson's disease. Some animals may have surgery to induce a heart defect that reflects heart disease in people and this will make animals develop signs of heart disease over time e.g. breathing changes. Diseased animals will be exposed to developed nanomedicines to assess their ability to accumulate at the diseased site and the consequent therapeutic/diagnostic effects. Animals may be imaged under anaesthesia. They may undergo behavioural testing. The majority of the animals are not expected to show signs of adverse effects that impact significantly on their general well-being. The adverse effects in diseased animals will therefore be the development of the diseases and the negative side effect of therapy. Some animals may experience moderate severity, such as discomfort and abnormal behaviour that does not prevent eating, drinking and other normal activities. However animals will receive supportive treatment and will be humanely killed at the earliest opportunity when study objective has been reached or if the animals begin to deteriorate,whichever comes first. Most of the protocols are designated with moderate levels of severity due to the possibly involvement of surgery. Only one of the protocols (i.e. ischaemic myocardium model, when the heart receive reduced supply of oxygenated blood) is designated the severity limit as substantial in this project due to the involvement of heart surgery and the nature of the disease(high mortality rate). To alleviate the risk of adverse effects, all recipient animals will be carefully monitored to assess their health status. Animals will be killed humanely 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

It is an unfortunate truth that testing drugs in cultured cells does not shed much light on their behaviours in animals following administration. Many biological barriers (e.g. blood to tissues and tissues to tissues) and animals' physical conditions (e.g. immune system), for instance, have great influence on the transport/delivery of drugs. While we will make every effort to study the efficiency of the developed nanomedicines using cells cultured in dishes, it will be still necessary to study their biological performance in animals The biodistribution profiles, therapeutics efficacy and toxicity are complex outcomes which depend on the interaction of the developed nanomedicine with blood, the immune system, and multiple biological barriers (e.g. How long they stay in the blood? How much they accumulate in different parts of the body? Could they reach the target tissues with sufficient amount to be effective? Will the administration of nanomedicines induce immune responses that are not favourable?). It is also impossible to pursue the programme without the use of animal models of diseases such as cancers, neurological disorders and cardiovascular diseases. These models are properly designed in this project to mimic the human disease condition while reducing suffering to the animals.

Reduction

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

Reduction

Several imaging technologies are employed in this project to obtain comprehensive information in animals that can be translated into the clinic to treat humans. The distribution of the nanomedicine in the body can be captured and semi-quantified at different time points after administration using the same animal. Imaging approaches thus hugely reduce the number of animals needed to study the dynamic changes of nanomedicines in animals. The experiments and the analysis methods are designed to 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

Mice are the main animal chosen for this project for many reasons, primarily as they are the least sentient species that give data applicable to human clinical studies. Immunocompromised mice are sometimes required when human cancer models are to be used. For the development of Parkinson’s disease model, rats are sometimes preferred over mice as their behavioural assessment, as a measure of therapy success, can be easily monitored.

Animal suffering will be reduced by appropriate environmental care, the use of general anaesthesia and analgesics in surgical procedures and implementation of humane endpoints. Several imaging techniques will be carried out to monitor the disease progression qualitatively. Diseased animals will be monitored by daily physically examination during the expected critical periods.

PROJECT 102

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.

Word limit; 1000 words

Project Title	Humanised mouse models for public health research
Key Words	Humanised mice, immunology, Biologics, Pathogens
Expected duration of the project	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;
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.

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 main objective of this licence is to explore various humanised mouse models for their suitability as predictive pre-clinical models for testing novel biological medicines and pathogens. The aim is to enable earlier and better detection of undesired biological activities and to evaluate the humanised mouse model as a suitable replacement for pre-clinical testing in non-human primates.

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

Science will benefit from this project through a greater understanding of the mechanisms of responses to new biological medicines. People will benefit from this project through the use of proven safe and effective biological medicines. Animals will benefit from this project through the further development and validation of safe alternative assays that do not require animal testing.

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

The number of animals required per dose and numbers of groups will be determined by statisticians in order to consider the minimum required to give reliable results of satisfactory precision. On average we expect to use per year approximately 3000 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?

Mice will be engrafted with human cells or tissues. Humanised mice might develop mild graft-versus host disease (approximately 10-20% of animals), surgical tissue

implantation may cause surgical or post-surgical complications (approximately 2%). The procedures utilised involve administration of human biological medicines by the intravenous, subcutaneous or the intraperitoneal route, withdrawal of blood from superficial vessels. The licence contains 1 protocol classified as mild and 8 classified as moderate. Typically the majority of animals used in protocols are not expected to experience more than transient discomfort. All animals will be frequently inspected by experienced personnel and/or a veterinary surgeon. Any animals showing signs of adverse effects will be appropriately managed either treated under veterinary direction or humanely killed.

Application of the 3Rs

Replacement

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

Replacement

In vitro tests could form an alternative in the future but may not always be suitable for thorough assessment of therapeutic intervention strategies.

For example, for the evaluation of immune responses to biotherapeutics, the activation of immune cells is complex and requires the correct cell types to be spatially and temporally arranged within a suitable microenvironment.

It is important that the assays reflect the human body as closely as possible. A typical example of wrong prediction of toxicity happened in 2006 during the preclinical study of the superagonist TGN1412. Both preclinical safety testings *in vitro* and in macaques failed to predict an adverse response to TGN1412 at clinical trial. Therefore, better assays that are equally suitable for a wide range of biotherapeutics could be instrumental in harmonising the immunotoxicological characterisation of novel biotherapeutics. The humanised mouse models have the potential to revolutionize immunotoxicology by making other animal models less important (e.g reduction of the use of Non-Human Primate) or even obsolete.

In addition, for a number of human infectious diseases the immune correlates are not known (e.g. malaria, tuberculosis) and therefore, currently no suitable *in vitro* tests to determine protective efficacy can be used. In addition, some pathogens have complex life-cycles including several hosts or several tissues within a host; it is not currently possible to determine the effect of several life-cycle stages in vitro. As a result, it is often not possible to reproduce this *in vitro*.

The data that will be generated will inform us on whether these protocols are more or less predictive than alternatives such as in vitro assays or non-human primate studies and will allow us to make decisions on whether humanised mice represent a suitable model to be used to assess efficacy and safety of biologics and vaccines.

Reduction

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

Reduction

Experiments will be discussed with biostatisticians to ensure that meaningful data will be obtained from each experiment with a minimum number of animals used. All new studies or areas of work will be first subjected to pilot studies to refine procedures and experimental groups.

Steps will be taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. operator is blind). To reduce potential variability, the same source of hematopoietic stem cells will also be used for engraftment whenever possible.

In vivo imaging system such as optical imaging will be used in a number of protocols to observe changes, either at the organ, tissue, cell, or molecular level by measuring any variation in light emitting specific targets. This will allow to determine if animals are responding to biologics or vaccines without sacrificing the animals at different time points and therefore reducing the number of animals required for particular 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

Immunocompromised mice with deficient or non-functional immune cells are used to generate humanised mice because their immunity is compromised making them unable to attack foreign cells. These mice are therefore the best recipients for engraftment for human cells or tissues. Such humanised mice can be used to model the human immune system and to study the effect and safety profile of biologics and vaccines.

Mice will be housed in a dedicated facility and well cared for by professional animal care staff. Environmental stimuli necessary for optimal psychological and physiological well-being of the mice will be provided (e.g. shelter, wheel). Mice will be housed in groups when appropriate and scientifically valid. Score sheets and monitoring regimes will be routinely applied to fully record the experiment.

When repeated blood sampling is needed only a very small amount of blood will be taken from individual mouse at different time point of the study. This way of blood sampling, called microsampling is quicker and less stressful for the animal than

blood vessel cannulation. Blood samples will be taken from the main study animals and therefore avoid the use of extra animals, allowing direct comparison of efficacy and adverse event study in the same animal.

If we can establish the suitability of humanised mice as predictive pre-clinical models for testing novel biological medicines and pathogens it will be a powerful argument in favour of its adoption and will encourage a reduction in the use of macaques in preclinical studies.

PROJECT 103

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.

Word limit; 1000 words

Project Title	The role of Gas6 in myelination in the mouse central nervous system
Key Words	Multiple sclerosis, Myelin, Repair, Immune, Gene knockout
Expected duration of the project	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;
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.

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):

Multiple Sclerosis (MS) results from damage to myelin, which insulates nerve fibres in the central nervous system (CNS), or to oligodendrocytes, the specialised brain cells that produce myelin. The brain and spinal cord of adults contain many “stem” cells which can divide, making oligodendrocytes and thus repair the damage in MS. We have recently observed that a particular protein (here we will call it proteinM) is able to promote this repair, by activating receptors on the surface of CNS cells. ProteinM also alters the immune system to support this repair.

We aim to discover 1) how the immune system is manipulated to encourage re-myelination and 2) whether we can stimulate the brain to make more proteinM locally to repair the damaged brain cells.

The information obtained through the project should bring us closer to realising the true potential of the proteinM as a therapeutic stimulant of repair in MS. This would be an alternative approach to artificial drugs, where instead the body’s own capacity for repair is exploited.

What 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’s data will confirm proteinM, and its accessory molecules, as stimulants of repair in MS. In addition to advancing the understanding of MS, our findings would enable us to work with clinicians to then extend this project to new pre-clinical and subsequent clinical studies. The MS Society and its alliances enables us to have the network of clinicians for this translational work.

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

The project period is 5 years. The study will utilise mice as the sole model. The types of mice that will be used will include so-called “wildtype”, i.e. a strain that is not genetically altered. In addition, genetically altered, “knockout”, mice will be used, which will enable characterisation of the role of specific molecules in the CNS repair process to be performed. In total, ca 460 mice are expected to be used.

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

The majority of procedures will be “mild” or “sub-threshold” severity level, as the animals will be bred and maintained, without any phenotypic differences to wildtype mice. They will be a source of tissue for in vitro experiments. Daily administration of the drug of interest into the mouse abdominal cavity or orally is expected to be of no more than mild severity.

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 since access to complete CNS tissue in all of its complexity is essential. This is because precursors of oligodendrocytes (the cells that make myelin) must be present, together with the signal-generating and receiving cells involved in the repair process, some of which have yet to be identified. This complexity is beyond that which can currently be supplied by using cell culture. Unprotected, larval animal forms (e.g. frog and fish) are also unsuited to these particular studies since, at these stages, their embryos do not have myelinated neurons.

The most useful information from the planned experimental models will come from working with knockout mice. These mice are uniquely deficient in specific proteins of interest to this study, and so will be invaluable in understanding the roles of those molecules in the CNS re-myelination process activated by proteinM.

The majority of the work will utilise *ex vivo* tissue culture, thus minimising the use of live animals in experiments, as well as reducing the numbers of animals required in the project. In the *in vivo* experiments where we study the effects of administration of a drug of interest on changes in the brain, use of live mice over a defined period will allow reliable monitoring of the drug’s effects and metabolism.

Reduction

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

Reduction

The breeding of the animals will be conducted efficiently to minimise their numbers and maximise the efficiency of their use (i.e. experiments or further breeding). For each planned experiment, we shall use the minimum mouse numbers required to provide statistical significance. This will allow us to answer the research questions whilst reducing animal numbers to a minimum. Throughout the investigation, experimental rigour will be applied and maintained consistently so as to minimise confounding factors such as experimental bias. The experimental design, incorporating the optimal numbers of animals used in order to obtain robust data, will be guided by the NC3Rs ARRIVE Guidelines as well as principles taught during PPL Holder training and, wherever possible, will be double-blinded.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 mice is justified above. The knockout mice used show no reported differences in physiological measures when compared to wildtype mice under normal conditions.

For refinement, we will mainly use *ex vivo* experimental models, which avoids any procedure on the mice prior to Schedule 1 killing. The mice will be housed in accordance with the HO Code of Practice for the Housing and Care of Animals. Appropriate housing systems are available to use for the mice, with regular health status monitoring and a dedicated team of trained animal technicians providing 365 days a year care and welfare.

PROJECT 104

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.

Word limit; 1000 words

Project Title	Investigating the microbiotal influence upon health in zebrafish
Key Words	zebrafish, microbiome, immunity, development, mechanism
Expected duration of the project	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):

This project aims to increase our understanding of the role that the microbiome plays in health and disease, with particular reference to the teleost but with application to other animals. It also aims to investigate the extent that the microbiome is altered by environmental factors such as diet and the consequence of this alteration upon development, immunity and disease. The beneficial effects of supplementing the microbiome by faecal transfer or feeding probiotics on the health, development, immune responses and resistance to infection will be assessed.

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

We expect to increase our understanding of the teleost microbiome and the role that it plays in the development of the gut and the immune system. This may allow us to develop new strategies for managing the health of teleosts in the aquaculture industry and to expand such ideas into the investigation of the role of the microbiome in health of other animals and man.

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

We use the zebrafish as our experimental model. The embryos develop externally from the mother and do not become free-feeding and regulated under ASPA until 5 days post-fertilisation. The majority of the work will use embryos at unregulated stages, so most of our studies are not regulated. However, we do need to use larvae and adults after 5 days post fertilisation to fully understand the role of the microbiome in immune function, which continues to develop after the embryonic stages. These fish will come under regulation. At regulated ages, we expect to use

9620 zebrafish over a 5 year period, some of which will be transgenic. Any embryos produced by these zebrafish that are employed in experiments as opposed to breeding will only be used up to 5 days post fertilisation.

In the context of what you propose to do to 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 effects are largely related to the creation of germ free embryos and larvae. Expected effects are epidermal sloughing and a maximal survival time of 30dpf has been reported. Other adverse effects include enteritis from vegetal diets and short term effects from i.p or i.m injections. Apart from the two pilot studies to assess the severity limits, all other protocols are moderate. The two severe protocols will use a small number of animals to ensure the protocol severity parameters are determined for our laboratory setting. All animals 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 role that the microbiome plays in the development and health of an animal is complex, involving multiple organ systems. While it is possible to model simple aspects of development and immunity in vitro, it is impossible to model the complex events that occur between tissues and organs to form a fully functional system. Furthermore, pathogenic processes during disease, and attempts to treat them, involve many cell types and tissues and this cannot be recreated in vitro. It is for these reasons we must undertake experiments upon animals. Our species of choice is the zebrafish which are unregulated up to 5 days post-fertilisation. Most of our experiments are performed on these unregulated animals.

Reduction

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

Reduction

To ensure minimum numbers are used, we will perform pilot studies and use power calculations to determine the number of animals required and take those numbers into account when deciding on the most appropriate assays to 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

We use the zebrafish model as a substantive body of work shows that developmental processes and immune function are highly conserved between vertebrates and because it is the vertebrate model with the lowest neurophysiological sensitivity. Thus, data derived from our models can be extrapolated to humans and other animals.

The protocols to be used are largely standard methods in zebrafish research. With the exception of the minor surgery of regenerating fin tissues in order to genotype, all of the treatments are non-surgical.

Latest knowledge on analgesia will be applied.

PROJECT 105

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.

Word limit; 1000 words

Project Title	Thrombus formation and its resolution
Key Words	Thrombosis, Venous, Arterial
Expected duration of the project	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):

Blood clots (thrombosis) in the legs are a major cause of death and may cause long term conditions including leg ulceration and limb loss. Treatments include surgery and drugs that break up the clot or stop it developing further, allowing its natural removal by the body. Surgery can lead to further clotting, while the drugs used can cause fatal bleeding (e.g. stroke).

The aims of this project are to better understand the mechanisms that regulate formation and natural removal of a clot and to use these to design experiments that aim to reveal: (i) new targets for treatment that prevent clots forming and promote their removal and the restoration of blood flow through a vessel, without posing a serious risk of bleeding; (ii) new more specific and sensitive markers of the presence of a clot and its composition; and (iii) novel imaging targets that can be used to non-invasively assess clots

What 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 of clots is mainly by the use of anti-clotting agents that can lead to unwanted bleeding (e.g. a stroke). Markers of the presence of a clot, especially in smaller blood vessels (e.g. calf veins) are not very specific and can lead to unnecessary treatment or lack of treatment. A better understanding of how clots form may reveal novel treatment targets that do not have the potentially fatal side effect of excessive bleeding and may also reveal more specific markers of the presence of a clot. Treatment of more extensive clot involves surgery and the use of clot-dissolving enzymes (lysis), which can also lead to a fatal bleed. Young clots can more readily

be dissolved than older ‘fibrous’ clots, but aging of a clot is currently dependent on a subjective examination of the patient. More objective methods of determining the composition of a clot are therefore needed. Understanding the cellular and molecular changes that take place in the clot and circulation during clot formation and resolution could help us to develop novel imaging (e.g. based on MRI) and biochemical measurements that are informative of clot structure and susceptibility to lysis. These would better inform treatment options for patients and reduce the risk of potential serious bleeding.

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

The complex and dynamic cell environment in blood and surrounding blood vessel cannot be replicated in the laboratory. Mice and rats will be used in this project as models of thrombosis are well established in these animals. All the tools necessary to study clots in these animals are readily available. Genetically modified mice, in particular, allow us to study the effect of enhancing or inhibiting particular factors in our model. We will carry out parallel studies in patients with blood clots, as well as more detailed work in the laboratory to investigate the importance of specific cells and molecules revealed by the animal studies. We expect to use up to 9000 animals over the 5yr period of this 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?

Our long experience with these models is that rats and mice tolerate blockage and manipulation of blood flow in their blood vessels very well. If pain is anticipated during a procedure (e.g. surgery) then the procedure will be carried out with appropriate anaesthesia. Adverse effects are most commonly associated with post-procedure discomfort. This will be minimised by administration of pain relief until the animal has fully recovered. Animals will be humanely killed at the end of an experiment. Some animals will be used for to breed animals for use in study protocols. We will only breed genetically manipulated animals that have at most a mild effect on normal behaviour and health. If we create an animal with a more severe effect, the animal will be immediately killed.

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 used because the complex molecular and cellular environment that regulates clot development and its removal cannot be replicated in the

laboratory. These processes involve a dynamic interaction between cells and molecules in the blood and local vessel wall.

We will use mouse and rat models of thrombosis in the veins and arteries, that we and others have developed, to investigate the cells and molecules responsible for clot formation and its natural removal by the body. Genetically modified animals will help us to highlight the importance of specific cells and factors in these processes. These studies will provide the platform from where we can design interventional studies aimed at stimulating specific beneficial processes, as well new imaging methods and analytical methods that are informative of the presence, size and composition of a clot. These studies will be supported by laboratory-based analysis of the function cells and factors revealed in the animal models to be involved in the processes that give rise to clots and dissolve clots.

Reduction

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

Reduction

Animal numbers will be minimised by in the following ways:

(i) Carrying out appropriate calculations (based on our experience of the variability in various end points such as clot size) of the requisite numbers of animals needed to provide a robust statistical analysis of these end points.

(ii) Where possible, longitudinal analysis (changes over time) in, e.g. clot size in the same animal using [LJN 28/04 non invasive] scanning techniques such as microCT.

(iii) Following humane killing, single animals can be used as donors to provide cells, from tissues such as bone marrow, that can be grown in the lab, to provide sufficient numbers of cells for experiments in a number of other animals. In some circumstances we will use the much larger reservoir of cells obtainable from human blood for experiments in mice that will accept human cells without rejecting them, thereby removing the need for donor mice.in these instances.

(iv) Laboratory-based techniques to screen the activity or function of cells or factors revealed by the observational studies to be involved in clot formation and removal. e.g. the activity of factors that affect the growth of small blood vessels (that form inside clots to restore blood flow through the blood vessel) can be assessed using cells that form blood vessels, grown in the laboratory.

(v) Using animal models in which multiple factors or cells can be screened prior to administration into the more complicated and severe clot model;

(vi) Carrying out parallel studies of the measurement of cells and blood borne factors in patients with blood clots.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 will be used, as they are the least sentient and smallest animals in which we are able to reproducibly induce clot formation and assess its prevention/removal by the body or by our interventions. All the tools necessary to study clots in these animals are readily available. Genetically modified mice, in particular, allow us to study the effect of removing or inhibiting particular factors in our model. We will carry out parallel studies in patients with blood clots, as well as more detailed work in the laboratory to investigate the importance of specific cells and molecules revealed by the animal studies.

Adverse events from procedures carried out in these studies will be minimal and mostly relate to post-surgical discomfort, which is controlled by the use of appropriate pain relief until the animal has recovered.

PROJECT 106

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.

Word limit; 1000 words

Project Title	Mechanisms of cancer progression and metastasis
Key Words	Cancer metastasis, Cancer spreading, Blood vessels, Cytoskeleton, Platelets
Expected duration of the project	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;
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):

Cancer affects 1 in 3 people in the UK. When cancers spread to other sites in the body they are very difficult to treat and normally cannot be removed by surgery. During cancer spreading (known as metastasis), cells from the cancer enter the bloodstream and are carried to different tissues, where they attach to the blood vessel wall and then leave the bloodstream and start to divide to form a new tumour.

Our project aims to identify genes that are important in cancer spreading and hence could be targets for treating patients to reduce metastatic disease. We first identify which genes contribute to cancer spreading by using cancer cells derived from human cancers. We study how these genes contribute to the attachment to cells lining the bloodstream, called endothelial cells, and ability of cancer cells to move across endothelial cells. We also test whether small cells in the blood called platelets increase the attachment of cancer cells to endothelial cells.

This work is done with cultured cells that we grow in incubators in the laboratory. The genes we work on code for proteins, and for some of these proteins there are chemicals that are known to inhibit their activity in cells. We will test these chemical inhibitors in our cell culture systems to find out if they reduce the interaction of cancer cells.

To find out whether these genes are important for cancer growth and metastasis, it is essential to test their roles in animals that have intact blood vessels and circulating blood as well as many other types of cells. For example, it is not possible to grow complete blood vessels in culture that include all of the cell types that circulate in the blood. Only by using a whole organism can we know whether these genes could be used to develop therapies to treat cancer patients.

We will use mice because they have been studied for many years for tumour growth and metastasis. We will use two types of approaches:

1. We will use mice that express tumour-promoting genes in either the prostate gland or breast, and develop tumours in these tissues. After the tumours develop, some of them will spread to other tissues, particularly to the lung. We will test whether chemical inhibitors of protein activity reduce the growth and spread of tumours in these mice.
2. We will inject cancer cells into the blood or tissues of mice. These experiments aim to test the roles of specific genes in the spread of tumours from one tissue to another, and for the establishment and growth of metastases. We will also test whether the chemical inhibitors reduce the growth and spread of cancer cells.

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

In the cancer clinic, there are no treatments available to patients that specifically reduce cancer metastasis. The results of our work will provide new insight into how molecules inside and outside cells contribute to cancer progression and metastasis. It will identify which proteins are likely to be the best targets for therapeutic intervention to treat human cancers in the future. This work will also provide information that could lead to new treatments for animals that get cancer, including pets. Before testing therapies in patients, it is essential to test them in animals to determine whether they reduce cancer growth and spreading. A first step in this process is to identify genes and chemical inhibitors that reduce cancer spreading, which could then be developed into therapies by chemists in the future.

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

We will use approximately 1900 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 severity is mild to medium. Most of the mice will develop tumours and some of these tumours will spread to other parts of the body. We know approximately when the tumours will grow to a size when we can measure them but they will not cause distress to the mice. This is from our own experience, from experts that we consult with regularly, and from published information from other research groups. In some cases we will use imaging to monitor the growth of tumours and metastases. This imaging will provide us with information to reduce the suffering of the mice. We will test chemical inhibitors in mice that we expect to reduce tumour growth and spreading. The chemical inhibitors we will use have already been tested in mice for treatment of cancer and/or other diseases, and the concentrations we use will be chosen to not be toxic. However, it is possible that the inhibitors in combination with

the methods we use to induce tumours may have unanticipated side-effects. The mice will be examined at least 3 times per week for signs of any general distress or signs associated with tumour development or inhibitor toxicity, including failure to eat or drink, a hunched posture or inactivity. All mice will be killed humanely by a Schedule 1 method or terminal anaesthesia at the end of experiments, which is before they show signs of distress. If they show any symptoms of distress, they will be killed humanely as soon as they are detected so that they do not suffer any further.

Application of the 3Rs

Replacement

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

Replacement

We have used *in vitro* cancer cell culture systems for many years to identify signalling molecules important for cancer cell migration, invasion and transendothelial migration, focusing on Rho GTPases and their signalling partners. However, little is known about whether these molecules also affect cancer progression *in vivo*. If our *in vitro* work is going to be translated ultimately to patient treatments, it is critical that we carry out pre-clinical studies in animals. It is not currently possible to create *in vitro* cell culture models that completely recapitulate the environment in which tumour cells grow *in vivo*. We will continue studies *in vitro* to complement the animal work, and we will also analyse human cancer samples for expression levels and distribution of the proteins we are studying.

Reduction

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

Reduction

Our *in vitro* work with cultured cells reduces the number of animals used because it allows us to select the best genes and chemical inhibitors to test *in vivo*. Where possible, we will monitor tumour growth with bioluminescence to reduce the number of mice used to measure tumour burden.

We will use the minimum number of mice, typically 6-10 mice per condition, to allow statistical analysis of results, based on our previous experience.

Minimising the number by monitoring tumour growth and metastasis using luminescent markers, and where possible by co-injecting control and treated cells simultaneously into one mouse. We have chosen the experimental metastasis (monitoring metastases in tissues) and tumour prostate and subcutaneous

implantation models because the time-courses of tumour growth are very consistent and we know when to kill the mice humanely to quantify tumours, before the mice suffer substantially from the tumour burden.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 xenograft models where human cancer cells are injected into mice, because the human cancer cell types are very well characterised for their growth as primary tumours and metastases in mice. We will also use syngeneic and spontaneous tumour models to allow studies in the presence of an immune system. Optimal doses of chemical inhibitors and chemotherapeutic agents are also well known in mice. We will co-inject control and treated cancer cells labelled with two different fluorescent dyes. This procedure halves the number of mice used.

PROJECT 107

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.

Word limit; 1000 words

Project Title	Identification of interventions to maintain health and resilience with age
Key Words	Stem cells, Ageing, Healthspan, Multimorbidity, Resilience
Expected duration of the project	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;
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 number of people aged over 65 is increasing rapidly due to life extension. However, even though we live longer we do not necessarily live healthier for longer. The average time a woman suffers from diseases in old age is 19 years and 15 years for men. Therefore, there is a need to find new strategies to maintain health for longer periods of time. Current strategies treat each disease individually but this has problems related to taking multiple drugs. They interfere with each other, have more side effects and are more costly to administer. With age stem cells, a small groups of cells in the body able to maintain tissues functional, stop working efficiently. This causes tissues to work less well and to be more prone to disease. There are drugs which can keep stem cells in a youthful state by interfering with the causes of ageing. Here we want to test these drugs to determine whether by keeping stem cells in a youthful state, tissues remain healthy with age, less prone to become diseased and more able to recover from injury. Because the causes of ageing are the same in the stem cells of each tissue, it is possible that one of these drugs may maintain more than one tissue in a healthy state thereby preventing the development or progression of more than one 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 will understand whether keeping stem cells youthful improves tissue health and make tissues more resistant to develop disease. This knowledge will be important to influence policy makers to fund more of this research so that progress can be faster. This will also provide the evidence for clinicians and industry to change the way they

think about treating diseases of older age and encourage them to test these interventions in patients, once the appropriate preclinical studies have been performed. Finally with these drugs older people can stay healthy for longer, reducing the costs of health and social care resulting in a better quality of life.

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

We expect to use 7500 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?

We do not expect adverse events for most procedures. Occasionally the animals may be sick (similarly to cancer patients undergoing radiotherapy or with age). They may display diarrhoea, infections, weight loss and problems of mobility. With advancing age mice accumulate multiple health problems similar to older people. If the effects are too severe we will sacrifice the animals. At the end of the experiment the animals will be killed and their tissues examined and analysed.

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 in vitro test which can assess the ability of stem cells to maintain healthy tissues in an appropriate 3D structure. This is too complex to be faithfully reproduced in vitro. The complexity increases when studying ageing because it affects many tissues and they influence each other. For example we know that the health of the intestine or of the immune system can influence the activity of other tissues such as the brain. In addition, testing of the efficacy and safety of drugs, or how much of the drugs reach the interested tissues can only be done in a leaving organism with a circulatory system. However, our group is working with engineers to create computer models, which mimic the 3D tissue structure and the relation among the different structure, like a digital mouse. It is hope that, in the future we will be able to perform some of the experiments, which requires animals by using computer models.

Reduction

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

Reduction

We will plan the experiment carefully and predict the number of animals using statistical tests. We will take professional advice from experimental design experts. We also have a colony management database that allows optimal breeding strategies to be performed. In addition we will develop computational approaches which will help to reduce animal usage in the future by increasing the accuracy of the measurements we do so that we can use less 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 the most commonly used animals to study regeneration and ageing and for drug testing. This means we have lots of information about them, which allows us to design better experiments. We will use extensive in vitro testing before animal testing. Only the most promising drugs will be tested in animals. We will monitor the adverse effects very closely, give pain killers to the animals if required or sacrifice the animal if the adverse effects are too serious

PROJECT 108

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.

Word limit; 1000 words

Project Title	Oxidative stress
Key Words	oxidative stress, degenerative disease, raging
Expected duration of the project	2 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;
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):

Just as iron implements becomes rusty over time and stop performing correctly, the molecules that comprise our bodies (biomolecules) also degrade over time and fail to function as they should. This molecular failure ramifies upwards: cells that are built from these molecules start to misbehave, and the organs that are built from these cells fail as a consequence of this. For example, muscle cells (myoblasts) lose flexibility and you lose grip and strength. Brain cells (neurons) fail to propagate electrical currents and Parkinson's disease results. Cardiac cells start to pulse out of sync with each other and you suffer a heart attack.

Scientists use the formal term 'oxidative stress' to refer to degradation of biomolecules.

As the frequency of death due to infectious diseases diminishes through improved hygiene and use of antibiotics, so diseases of old age have emerged as the major cause of mortality in the Western world. These diseases, which include Parkinson's- and Alzheimer's diseases, diabetes, cardiovascular disease and cancers, and indeed aging itself, are believed to be the direct consequence of oxidative stress. At first glance, this might appear to be a grim diagnosis as oxidative stress is irreversible and accumulates over time at an ever accelerating rate. However, the situation is less bleak than might at first appear; while oxidative stress is indeed irreversible, the rate at which it accumulates might be decelerated by, for example, treating the population with anti-oxidants that prevent biomolecular damage

Unfortunately, the 'holy-grail' of aging research – the identification of effective anti-oxidants that slow down the aging process – has been hindered by the lack of appropriate models for measuring oxidative stress in living organisms. For, if we

cannot measure oxidative stress, how can we tell whether an anti-oxidant has been effective? The work we are performing is aimed at generating novel mouse models that will allow us for the first time to evaluate anti-oxidants and assess whether the retard the aging process. We refer to these models as reporter models as they measure (‘report’ on) oxidative stress.

What 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 addition to the moral imperative to alleviate the suffering of patients and their families, age-related diseases represent an increasing and unsustainable economic burden on society; it is estimated that these diseases will cost the NHS £13bn a year by 2022. The animal models that we are establishing will be instrumental in identifying drugs and behaviours that can reduce oxidative stress and so the extend the healthy lifespan of the population. A healthier population will allow scarce NHS resources to be targeted to other pressing health problems, such as the increasing problem of resistance to antibiotics.

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

We will be using mice. We expect to use ~3000 animals over a period of two 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 most cases, the interventions being trialled in the animals are actually expected to be beneficial and to improve their health and lifespan of the mice. Thus, in most cases the expected level of severity is zero (formally mild). We are however proposing to use one strain of mouse that suffers accelerated aging. We intend to use these ice before they become symptomatic; however, we cannot rule out that in symptoms of moderate severity might become manifest in rare and unexpected cases. Mice will be killed in a humane manner at the end of experiments.I

Application of the 3Rs

Replacement

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

Replacement

Aging affects multiple organs and can only be explored in an intact organism. Although in an ideal world, some of these studies would be performed *ex vivo* using human cell lines, these lines are invariably cancerous in origin. Unfortunately, once cells have become cancerous, they suffer oxidative stress in a different manner to normal cells. This makes them unacceptable for our purposes.

Reduction

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

Reduction

Our laboratory has been at the forefront of devising clever experimental approaches that reduces the numbers of animals required for any one experiment. The details are necessarily technical and here we provide but a single example to exemplify our approach: we have engineered our reporter mice to emit visible light in amounts that rise-and-fall with oxidative stress. The amount of light can be measured with a sensitive external camera. For this reason, we do not have to kill the mice but can re-use them. This will lead to a significant reduction in the number of mice used in our 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

Of the model organisms commonly accepted as surrogates for humans, the mouse is the closest in evolutionary terms to humans. Simpler, more socially-acceptable alternatives, such as the worm species *C. elegans,* have well-documented differences with humans in how they handle oxidative stress and this makes them unacceptable for this project.

PROJECT 109

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.

Word limit; 1000 words

Project Title	TARGETING EPIGENETIC FACTORS AS A THERAPEUTIC APPROACH IN PANCREATIC CANCER
Key Words	Cancer, Epigenetics, microRNAs, Therapy
Expected duration of the project	3 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;
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):

While the diagnostics of pancreatic cancer has improved and animal models mimicking the disease have also advanced in the past 15 years, the bottom line is that pancreatic cancer remains a deadly and incurable disease. Standard chemotherapy and new adjuvant targeted therapies show some promise in the pre-clinical setting, yet they fail to impact the dismal survival statistics in patients. The current project proposes a new approach that can help in the design of new efficacious stand-alone or combinatorial treatments for this deadly 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)?

Pancreatic cancer is a devastating and almost uniformly lethal malignancy that accounts for approximately 9,000 deaths in the UK every year, and is the fourth most common cause of cancer-related mortality. The overall median 5-year survival rate for pancreatic cancer remains at around only 5%, and is among the worst of all human malignancies. Due to the absence of early symptoms and lack of reliable diagnostic tools for early detection, the vast majority of pancreatic cancers are diagnosed at locally advanced or metastatic stages, precluding curative surgical resection. Understanding the mechanisms underlying pancreatic cancer growth would allow for the development of effective approaches to reduce pancreatic cancer related mortality.

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

Immunodeficient mice will be used for the investigation of new therapeutic approaches for human pancreatic cancer growth and aggressiveness. All

experiments will be designed and performed according to the 3Rs principles, the animal usage will be minimised and experiments refined. We expect that the project will require approximately 600 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?

All protocols have been designed in order to achieve the desired objectives without compromising the animal's welfare. Although adverse effects such as transient discomfort from the injections and the presence of tumours may be expected, these are likely to be of mild severity. Subcutaneous tumours are not expected to ulcerate or grow too fast to impede movement and normal behaviour. The overall severity of the project is expected to be 'Moderate', and all animals will be killed 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

Although this research employs an extensive portfolio of human tissue analyses and in vitro laboratory techniques, these cannot adequately model the complete array of biological events that are involved in tumour growth. Tumour growth and aggressiveness are complex multistep phenomena that are controlled by different cell types, molecules and biological processes. Mice are remarkably similar to humans in terms of their genetic make-up, so studying them helps us understand cancer in humans. Thus, it is essential for us to use mammals (like mice), because the complex interactions between cells and tissues in the human body cannot be modelled using simpler, non-mammalian animals.

Reduction

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

Reduction

Pilot studies, which intend to investigate the biology of the tumour, will provide information that will significantly reduce the number of animals to be used in the therapeutic studies. The therapeutic studies will be designed according to our experience and by using statistical analysis for achieving significance. We will further minimise the number of animals, since multiple treated groups, treated with different compounds of interest or their combinations, will be compared to one control group. We will discuss our experimental design and statistical analysis with the in-house

biostatistician who will provide guidance on the statistical components of our experimental protocols.

Furthermore, we will employ as much *in silico* and *in vitro* analyses as possible. Molecular analyses will address the most efficient concentration of the candidate medicines with the minimum toxic effects. Thus, the use of mice for titration of compound doses will be avoided or significantly reduced.

Upon completion of the experiments, mouse tissues and fluids will be collected to verify the lack of toxic effects. Combination of the evaluation of drug efficiency with the analysis of toxicity in the same animals, further reduces the number of mice used.

The variation between individual mice and the variable development of the disease has been taken into consideration as to provide a valid result. Experiments have been designed to include the minimum possible number of mice needed in order to reach statistical significance in the anticipated 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 mouse model is our chosen species based on previous literature and our previous work. Immunodeficient mice offer the best platforms on which to effectively evaluate the therapeutic efficacy of compounds on tumour growth. The tumours will be measured using callipers. The subcutaneous tumour models are not expected to metastasise, ulcerate or impede any of the animal's normal movements or behavioural repertoire. The therapeutic compounds to be used have well characterised toxicity profiles, thus we do not expect any adverse clinical effects at the doses that we intend to administer to the animals. We will be monitoring our animals to make sure that the tumour will not affect the animal’s general well-being.

PROJECT 110

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

Project Title	Immune Mechanisms Controlling Toxoplasma gondii
Key Words	Toxoplasma gondii, infection
Expected duration of the project	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):

We will identify novel pathways and mechanisms of host defence to the protozoan parasite *Toxoplasma gondii*. *Toxoplasma* infects a broad range of warm-blooded hosts and is arguably the most common parasitic infection in man. Also, *Toxoplasma* infection is generally combated by a similar immune response as other pathogen infections, such as Tuberculosis, which causes a significant human disease burden.

In normal immunocompetent individuals, the acute phase of infection generally causes no symptoms and proceeds to the chronic phase silently. *Toxoplasma* infection is incurable and tissue cyst of the parasite reside in the brain and heart muscle tissue of people for their whole lives. Immunocompromised individuals, such as HIV patients or organ transplant recipients, are at risk of reactivating the dormant cyst form and dying of brain disease called toxoplasmic encephalitis. Normal people can become blind when infected with certain types of *Toxoplasma* and an unborn child can have birth defects if the mother becomes infected during pregnancy.

Infection with *Toxoplasma* causes a defence reaction through the production of a protein called gamma interferon. Certain immune cells produce gamma interferon. Gamma interferon activates mechanisms that restrict parasite growth or eliminates *Toxoplasma*.

Specifically, we will elucidate which immune factors controlled by gamma interferon combat *Toxoplasma* infection and how parasite proteins interact with the host's immune system. We also study the immune cells that produce gamma interferon and how they control the parasite in the acute and chronic phase.

Mice are natural intermediate hosts of the parasite just like humans and will be used as a source of parasites and to dissect host-parasite interactions and immune 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 programme will lead to publications in peer-reviewed journals and provide insight into the molecular mechanisms regulating the immune response to Toxoplasma. These studies will pave the way to follow-up studies in humans to design counter-measures or a vaccine strategy. Also, since the immune response to Toxoplasma is similar to the response to pathogens that cause higher human morbidity and mortality (e.g. Tuberculosis, Salmonella, Chlamydia), lessons learnt from this study can help guide countermeasures to these diseases.

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

In total we propose to use maximally 13600 mice of which around 75 % of animals will be used for breeding, maintenance of our GA mouse lines and tissue and parasite provision. We will make sure to use the minimum amount of animals per experiment to achieve statistical significance.

In the context of what you propose to do to 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 infect mice with Toxoplasma and monitor their immune status during the acute infection. Mice having reached the chronic phase of infection, we will supply with specific CD8 T cells and monitor their progression. For some experiments we can use Toxoplasma engineered to express firefly luciferase, thus being able to in vivo image the parasite load without harming the mice. Like this, we will be able to administer a minimal dose of the parasite and still be able to assess differences in parasite load between wild-type and genetically altered mice. In experiments which could potentially result in severe adverse effects for the animals due to a nature of infection, we will monitor weight and body condition regularly and intervene when necessary.

Application of the 3Rs

Replacement

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

Replacement

Once infected with *Toxoplasma*, all hosts including humans establish a chronic infection that is kept asymptomatic by the host’s immune system. If the immune

system fails, *Toxoplasma* can kill the host. To study the aspects of the immune system mediating this control *in vitro* is not possible at all, since it relies on the structural integrity and connectedness of lymphoid organs and other tissues.

Reduction

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

Reduction

Our biggest advantage is that we can often use *Toxoplasma gondii* engineered to express firefly luciferase, thus being able to *in vivo* image the parasite load. Using this method, we can follow the infection in each animal in real time and do not need to sacrifice animals to analyse each time point.

Power calculation is used in our experimental design in order to use appropriate number of animals to achieve scientific significance. We routinely review our breeding strategies and cryopreserve any strains of mice not being actively investigated.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 model is currently the only extensively refined animal model of *Toxoplasma* infection. As we are studying how the immune system restricts the parasite in both the acute and chronic phase of the infection, we are reliant on a whole body organismal system to understand the complex interplay of different cellular players of host defence.

Using the *in vivo* parasite imaging approach, we are able to administer a minimal dose of the parasite and still be able to assess differences in parasite load between wild-type and genetically altered mice. We have developed mouse body condition scoring sheet aiming for robust monitoring of any adverse effects of infection and prompt intervention where necessary.

PROJECT 111

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.

Word limit; 1000 words

Project Title	The Synaptic Plasticity of Epilepsy and Sleep
Key Words	Seizures, Epileptogenesis, Plasticity, Sleep
Expected duration of the project	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):

We are investigating how the process of synaptic plasticity – how our brains change the strength of connections between brain cells – is involved in the development of epilepsy, and how it is gated by the natural process of sleep. We will make recordings of the brain activity of rats and mice using, for example, wireless EEG systems or by perfoming recordings from cells in the brain under anaesthesia. We will generate a model of epilepsy in rats by giving them a low dose of drugs.

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

We aim to aid the development of medical interventions to prevent the occurrence of acquired epilepsy which may arise after head trauma or neurological disease. We also aim to reduce the problems with disrupted sleep experienced by people with psychiatric and neurological disease. Our research will aid understanding of how the brain works to further research into epilepsy, Parkinson’s and other related conditions.

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

Rats and mice, about 500-1000 over a period of 5 years.

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

The procedures will be mild or moderate (e.g. trimming whiskers to induce plasticity, giving drugs to alter neuronal transmission) and some of the epilepsy work involving implanted electrodes will have several stages (implantation of electrodes to record

the brain activity, and epilepsy induction) but the animals will not experience more than a moderate level of severity. The epilepsy procedure may cause rats to lose some weight over 48 hours. The endpoint of the experiment will be the production of brain slices to maximise the data obtained from each animal.

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 accurately record from neural circuits similar to those found in humans, so rodents are the smallest and simplest animals we can use for this purpose. Sleep and epilepsy are complex, but the advantage of rodents is that we can induce plasticity simply by giving their whiskers a trim.

Reduction

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

Reduction

We will use the minimum consistent with our statistical calculations, and maximise the use of each animal through procedures designed to keep brain tissue alive for as long as possible. Our protocols are designed to maximise data collection from each animal meaning we need to use far fewer animals to get valid 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 epilepsy model being used was developed with refinement as the primary goal, through NC3Rs funding. The model minimises harms through reduction of time spent having epileptic seizures, and better drug-control of seizures to prevent spread of uncontrollable epilepsy to the brainstem (causing death). The model does not cause gross damage to the brain, unlike many models. Using rodents for plasticity studies means that we only have to trim whiskers to induce plasticity, a painless and non-invasive procedure.

PROJECT 112

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

Project Title	Breeding genetically altered mice
Key Words	Mouse, breeding, genetically altered
Expected duration of the project	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):

DNA (a molecule that makes up genes) has been studied in great detail over the past few decades and the entire sequence of DNA in both humans and mice has been sequenced many times. However, the function of many of the genes are still unknown and the underlying cause of many genetic diseases is not understood. To address these problems, the DNA of mice can be altered and the effects of these alteration studied. This can give us insight into biological mechanisms as well as help us understand how modified genes cause disease.

This project is designed to provide an efficient service for breeding genetically altered mice and transferring them to researchers for further study.

Very little work to assess the changes in these mice will be carried out on this licence, other than some initial welfare assessments.

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

This project will benefit science through the rearing and distribution of genetically altered mouse strains that can be used to better understand how genes control all aspects of mammalian biology. In particular, this project will help scientist around the world study new models of human (and animal) disease which will lead to better treatments and reduced human and animal suffering. We aim to breed over 400 new mouse lines over the next five years. This will be a huge resource for researchers to be able to study many different aspects of disease. Doing all the breeding in one place under one project allows us to carefully control the quality of the cohorts of mice. We have lots of experience breeding mice with controlled genetics, at specific times with the welfare of the mice as our highest priority. This means the research

carried out later will be more likely to show reproducible, useful results. We will also breed and maintain colonies of mice that are widely used by several researchers. This means we can keep one colony and supply everyone, rather than researchers all holding their own mice. This reduces the overall number of mice, as well as allowing us to maintain the quality of the colony.

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

All animals used on this licence will be mice and we expect to breed around 80,000 over the next five 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?

The mice on this licence will be used for breeding or held as stock to be transferred to other licences. They will have very few procedures carried out and most are related to general breeding and housing, for example taking an ear clip for genotyping and identification. A large proportion of the mice will be carrying genetic alterations so there is a possibility of some of them experiencing discomfort due to the genes that have been changed. There could be a wide variety of unknown effects as the function of many of these genes is not understood. For example, the mice may not survive until birth as the gene is necessary for development, or they may exhibit decreased hearing, a change in sleep pattern or change in activity level, as well as many other possible effects. We do not aim to breed or maintain animals which show symptoms of significant ill-health, pain or distress and we will employ careful monitoring systems to ensure any mice are killed humanely before this such signs develop. Some mice will have a microchip inserted to allow us to identify them during video tracking. These videos will provide us with extra information on their behaviour and allow us to better assess any welfare issues. All mice will be humanely killed or exported to other projects 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

Mice will be bred on this licence only when the alternatives are not appropriate. For example, many of the projects these mice will go on to are looking at the systemic effects of genes, how they effect different areas of the body, or at complex organs such as the brain. There are currently no systems outside of living animals that can provide this information accurately.

Mice are used because they are mammals and have very similar or identical physiology to humans, which is not the case for non-protected alternatives such as fruit flies. In some cases initial work is done on unprotected species before researchers need to move to mice to look at the more complex picture.

Reduction

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

Reduction

Reducing animal numbers is one of the key aims of this project licence. For each project, calculations will be carried out to work out how to breed the cohort with the minimum number of mice. We will use our knowledge, along with statistics from previous work to inform our calculations and ensure we do not breed any excess 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 most appropriate animal model for this project because our intended aim is to work out the function of all mammalian genes and proteins. Animal models are important because we are able to manipulate their genes using genetic engineering and investigate the consequences for the organism. The mouse occupies a unique position in determining gene function and the genetics of disease for a number of reasons. Firstly, as a mammal it demonstrates a remarkably similar development, physiology and biochemistry to the human. Secondly, mouse geneticists have developed a very extensive genetic toolkit that enables very specific alteration of genes in the mouse. Thirdly, we now know the complete sequence of all the DNA the mouse carries.

We will minimise the welfare costs to the animals by using the minimum number of animals at all times.

Use of a new home cage monitoring system allows us to observe mice in their home environment. This enables early detection of phenotypes in new lines and provides information on additional control points that can be put in place to reduce suffering.

PROJECT 113

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Control of cerebral and systemic circulation in health and disease
Key Words	heart, circulation, brain blood flow, neurodegenerative disease, hypertension
Expected duration of the project	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):

This project is designed to address fundamental, yet unanswered questions as to how cerebral and systemic circulations are controlled to ensure homeostatic regulation of brain metabolic environment. Proposed experiments will study the mechanisms of how the dynamic changes in brain neuronal activity are supported by appropriate changes in blood flow and how the regional brain blood flow and systemic arterial blood pressure are concomitantly adjusted to protect neuronal function in conditions of decreased pH, oxygen availability and perfusion.

What 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 proposed research are expected to contribute towards our understanding of the fundamental physiological mechanisms controlling cerebral and systemic circulations and may be relevant for revealing causes of common pathological alterations in brain control mechanisms contributing and exacerbating development of cardiovascular (e.g. hypertension) and neurodegenerative (e.g. Alzheimer’s) diseases.

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

We estimate to use 1900 rats and 1100 mice during 5 years 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?

Animal suffering is minimised or avoided by implementing the appropriate experimental routines with analgesia +/- anaesthesia. The severity limit of this project

is “moderate” with the least possible invasive surgical procedures involving neurosurgery and induction of cardiac failure as experimental models of human disease. Not more than two surgical procedures with recovery will be applied. These will be followed by either non-recovery procedures or procedures which do not produce pain, suffering, discomfort or lasting harm. The majority of the experiments are performed under deep terminal anaesthesia and therefore the severity limit for these protocols is “non-recovery”. These experimental models are the least severe and were proven by our track record to produce high quality, physiologically meaningful and significant results. At the end of the experimental studies the animals are humanely euthanized.

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 a programme of research of this type as physiological mechanisms controlling cerebral and systemic circulations are extremely complex and cannot be adequately described or studied in reduced systems such as tissue/cell culture or brain slices. However, where appropriate, *in vitro* methods have been and will continue to be used.

Reduction

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

Reduction

To minimize the number of animals used, the experiments in this laboratory are carefully designed and executed to reduce their inherent variability. Furthermore, all practically possible means are used to minimise any adverse effects on the animals. Sample sizes in all experimental groups will initially be six, but these numbers may be increased if the data indicates that this would make the observations statistically significant. Data are analysed using appropriate statistical 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

The questions addressed in this project are common to all mammalian species, including man. Laboratory rat and mouse have been used extensively to study central and peripheral mechanisms controlling cerebral and systemic circulations and the main body of data revealing these mechanisms was obtained in experiments using these animal species. Moreover, all critical molecular biology tools including targeted genetic modifications have been developed and established in mice and rats. In our laboratory all key experimental electrophysiological and imaging techniques that are relevant to the current proposal have been established, tested and verified in rats and mice, thus eliminating the need for the methodology-setting additional experimental animal studies. Animal suffering is minimised or avoided by implementing the experimental routines with analgesia +/- anaesthesia as appropriate for the procedure.

PROJECT 114

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.

Word limit; 1000 words

Project Title	Optimising gene transfer technologies for the treatment of monogenetic disorders and cancer
Key Words	Mouse, gene therapy, rare disorders, cancer
Expected duration of the project	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;
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.

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 main aim of this research programme is to develop new therapies for NHS priority areas by exploiting recent advances in gene therapy technologies made by our group and that of others. Gene therapy, involves the introduction of genes into a target cell, with the aim of restoring, modifying or enhancing the cell's function. Over the last 5 years' gene therapy has gradually begun to make a significant impact on medical practice REDACT?. We would like to build on this success in the clinic by addressing some of the limitations, which include working out a solution that enable us to administer gene therapy again if the effects of the first administration begin to wane. In addition, we want to develop and evaluate new gene therapy strategies that can be applied to a wide range of inherited disorders such a lysosomal storage disease including Fabry and Gaucher disease as well as more challenging bleeding disorders such as haemophilia A. We are also planning to apply our skills and expertise to the treatment of cancer by using gene transfer methods to provide the body's immune cells with a new method to recognise and kill cancer cells without damaging normal 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)?

An important advantage of gene therapy over current therapies is the potential for cure disease through replacement of malfunctioning genes or empowering immune cells to kill cancer cells. Gene therapy, therefore, changes the treatment paradigm from palliation to cure. The net effect of this can be dramatic as illustrated by our successful REDACTED whilst dispensing with the need for life long, intravenous injections, 2-3 times/week with concentrates of the missing protein. Gene therapy has improved the quality of life of all participants in our trial and saved the NHS more

than £5M over a 6-year period from reduction in use of protein concentrates. Successful gene therapy can also have positive benefits for the society at large with for example reduction in absenteeism from work or school and reduction in demands on social services. Additionally, we want to develop novel gene therapy approaches for other disorders, especially those (e.g. cancer) where current treatments are suboptimal. We are confident that during the course of this PPL we will report the outcome of other early phase gene therapy trials, which we are planning to open in early 2017.

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

We plan to use approximately 2500 mice over the five year period developing treatments for five different diseases; hard to treat bleeding disorders, Fabry disease, chronic lymphocytic leukaemia, pancreatic cancer.

In the context of what you propose to do to 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 expected to be moderate or less for all animals used. All protocols described have been used before by our research group. In the majority of cases, mice are expected to experience no or minimal discomfort as a result of procedures, and animals are killed, using a humane method, at the end of an experiment to allow full characterisation of the safety and efficacy effects of gene therapy. It is acknowledged that mice used in the cancer studies may, however, develop a number of symptoms and adverse clinical signs as a direct result of tumour growth. They may also experience side effects from the immune cells being tested as new treatments for cancer. Common symptoms will include weight gain/loss, loss of appetite, reduced mobility, tumour development (with or without ulceration), ascites and pain or discomfort at the sites of injection or immunisation. In addition, some mice will also undergo repeated general anaesthesia e.g. for the purposes of imaging to assess tumour growth. Mice will be closely monitored for development of such signs and clear endpoints are defined in all protocols in order to minimise discomfort experienced by individual animals. Mice with progressive tumour growth will be killed humanely before specific endpoints have been reached, allowing for the isolation of tumour cells and immune cells for ex vivo analysis. All mice will be killed at well specified humane endpoints of at the end of the experiments by a schedule 1 methods.

Application of the 3Rs

Replacement

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

Replacement

Murine models of human disease serve as an important tool for establishing pre-clinical proof-of-concepts, and for assessing the efficacy and safety profile and immunological consequences of our gene therapy approaches. The body's response to gene therapy vectors involves multiple systems, organs and cell types. Additionally, the complexity of the immune response cannot be assessed in in-vitro settings. The use of in vivo models is essential to refine existing and develop new immunotherapies as they allow us to detect in vivo homing and persistence of transferred cells, their efficacy in modifying/preventing disease and off target toxicities. *In vitro* systems, while useful, do not fully replicate the complexity of immune interactions or disease pathogenesis *in vivo* and it is essential to use appropriate and robust animal models to understand these processes.

In general, we discuss the preclinical plan for each vector with the MHRA when possible prior to conducting studies in animal to ensure the most appropriate and judicious use of animals using well appropriately defined humane endpoints. While it is not possible to avoid using animals for many aspects of our research studies, we are committed to the 3Rs and where possible the vectors and transgenic cellular products we develop will be assessed in-vitro to ensure efficacy. We will use non-invasive imaging in scientific research to reduce and refine use of animals.

Reduction

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

Reduction

Our reduction strategy is as follows:

1. Use Power calculations to reduce the number of animals being used whilst ensuring that sufficient data is obtained to answer the research question, or maximise the information obtained per animal and thus potentially limiting or avoiding the subsequent use of additional animals, without compromising animal welfare.
2. Non-invasive imaging to maximizing the information obtained per animal with longitudinal follow-up of the same animals over a period and thus potentially limit or avoiding the subsequent use of additional animals
3. Store frozen mice sperm and embryos to reducing the number of the number of mice that are kept alive for specific disease 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 chosen to work with mice as they are the lowest vertebrate group with well-characterised disease models.

Genetic modification of mice is well-defined and their immune system has been intensively studied and bears extensive similarities to that of humans.

The majority of reagents and tools required for the proposed plan of work have been designed for use in mouse models and for establishing safety and efficacy. 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 proposed mouse models are already well established in our previous projects. All the protocols have clearly defined humane endpoints and the majority of mice will be humanely killed with an appropriate Schedule 1 method within 12 months of receiving gene therapy vector or gene modified immune cells, or earlier, whenever endpoints are reached. All experimental procedures are carefully monitored by experienced staff within the group and in the animal facility. Animals exhibiting any unexpected harmful clinical signs will be killed using a humane method.

PROJECT 115

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.

Word limit; 1000 words

Project Title	Sleep apnoea and cognition
Key Words	Brain, behaviour, sleep apnoea, cognition, neurodegeneration, Alzheimer's disease
Expected duration of the project	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):

Sleep-disordered breathing is linked to many other illnesses and disorders, such as heart failure and cancer. It also has the potential to effect brain function, including cognition, though both sleep deprivation and neuroinflammation.

We wish to understand the influence of sleep on cognitive function and neurodegenerative disorders. To do this we will expose wild-type and genetically-modified rodents to behavioural analysis in learning and memory tasks. We will do this in models of sleep apnoea to indicate whether this disease has a detrimental effect on cognitive function. We will also study cognition in models of Alzheimers disease with and without sleep apnoea, to indicate whether sleep apnoea hastens the progression 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)?

The human brain, like other organs, is affected by ageing. In severe case it can be effected by neurodegenerative disease which puts the person at risk of harm and jeopardises independent living, placing heavy burdens on families and society. Neuodegenerative disorders may be worsened by sleep apnoea, which increases in prevalence with age. There is therefore a great need to both understand the effects of sleep anoea on normal cognition and it’s effect on the progression of neurodegenerative disease, and to develop strategies to limit the impact of sleep apnoea on brain function. An understanding of the factors and mechanisms that influence the degeneration of the mammalian brain will have impact on the care of vulnerable adults. Mechanistic insight into the influence of sleep apnoea on neurodegeneration provided through this project will reinforce – and explain - the

importance of monitoring patients for what is considered a seemingly innocuous disease, but which is in fact is quite insipidous.

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

10,000 Rodents 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 learning of a new behavioural task may initially be moderately stressful as the animal will be exposed to a novel, unfamiliar environment. After the behavioural testing animals will be killed humanely and brain tissue will be used for various in vitro analyses. To induce sleep apnoea we are going to kill cells in the brain. As most people with sleep apnoea are not aware that they have this disorder, this should not cause any stress to the rats and mice who only have apnoea during sleep. There are obviously other possible complications of general surgery, infection, pain, haemorrhage etc. We reduce these complications by making sure everyone on the project is extensively trained in how to perform the procedure and animal care. We also have pain medication and antibiotics readily available to treat rats and mice when it is needed. We also work very closely with a veterinarian and other highly trained technicians to make sure that the animals are well looked after and do not suffer in any way. At the end of the experiment all 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

This study assesses how entire physiological systems interact over a period of time to lead to, or exacerbate, disease states. Unfortunately for this initial study we must use animals, as we cannot replicate complex organ systems outside of the body. We are also required to study cognitive behaviour, which again cannot be studied in a dish. As it would also be extremely unethical to perform experiments on human subjects, there are no suitable alternatives to experiments in animals. However, when we begin to home in on a mechanism, we may be able to replace some of the whole animal work with other experimental material, such as cell lines.

Reduction

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

Reduction

We will use pilot studies to reduce the number of experimental groups, by only moving forward with experiments that lead to significant results in preliminary work. Using carefully design experiments to change specific parameters, the optimisation process will use the minimum number of experimental animals possible.

The experiments are designed so that following the initial surgeries the different diseases can be assessed using non-invasive methods. Therefore, a single animal can be used for all experimental time points, thus significantly reducing the number of animals used. In addition, at the end of the study, animals will be used in either non-recovery experiment or their tissue will be collected for studying *in vitro*, to identify the mechanisms that link these diseases. By using a single animal for both the behaving and non-recovery/*in vitro* experiments we can halve the number of animal used. Also by assessing the animals before the terminal/*in vitro* experiment, we can assure that diseases we wish to study have occurred before the final experiment takes place, thus all terminal experiments will provide meaningful data, again reducing the numbers of animals used in this project.

We will also make all tissue, not used by the primary investigator in the respective studies, available to other investigators, so as to reduce the need to repeat procedures for different studies. We base our sample size calculations on both our prior experience and that of others. This will allow us to generate robust, statistically significant data upon which to draw firm conclusions and in doing so both advance the field and iteratively adjust the sample size of 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

The process of refinement will be ongoing and assessed continually as new data arrive. We also take great care to keep up to date with animal welfare literature and work closely with animal welfare professionals to provide the best care possible.

We will use pilot data from different models to find the best model in terms of both experimental and animal welfare. If less invasive models provide good quality data, then they will be used to carry the project forward.

The use of terminal experiments will also help us to determine possible regions of interest in the brain, and may also help us to identify the mechanisms linking sleep apnoea to other disorders. We will use this data to refine our techniques, and to better design future experiments

Wherever possible, we will endeavour to design the experiments that use the least number of animals possible, and that cause the least amount of pain, suffering, distress, and lasting harm to those that we do use.

PROJECT 116

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.

Word limit; 1000 words

Project Title	Molecular genetics of trypanosomatids to understand parasite virulence
Key Words	Leishmaniasis, Human African Trypanosomiasis, sleeping sickness
Expected duration of the project	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;
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):

Neglected tropical diseases (NTDs) remain a significant health problem for many people in developing countries. The therapies that are available to treat two of these NTDs, sleeping sickness and leishmaniasis, are inadequate, so research is needed to discover new drugs and vaccines. This project aims firstly to study the biology of the single-celled parasites that cause the diseases in order to identify differences between humans and the parasite that can be exploited to develop new drugs. Secondly, the project aims to test new drugs to find out if they can kill the parasite in their mammalian host.

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

Knowledge will be gained about important biological processes that allow parasites to infect animals and humans. Identification of new drug targets will potentially allow the development of new drugs and vaccines.

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

We expect to use up to 4,600 animals over a 5 year period; 4,400 mice, 100 rats and 100 Hamsters.

In the context of what you propose to do to 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 infected with either trypanosome or Leishmania parasites, which is a moderate level of severity. Infection with parasites can sometimes lead to

adverse effects such as anaemia, weight loss and lethargy but these will be monitored carefully and appropriate action taken. Animals will be culled at the end.

Application of the 3Rs

Replacement

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

Replacement

Parasites can be grown in flasks in the laboratory, but these forms have different biochemistry to those that grow in animals, which can influence virulence and drug sensitivity assays. It is only possible to evaluate whether a new drug will kill the parasite in a mammalian host by testing in animals

Reduction

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

Reduction

We use statistics to design experiments such that the minimum number of animals is used to obtain the maximum information. We use non-invasive in vivo imaging to allow longitudinal assessment of parasite infection in an 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

Rodent models are well established and described for both *Leishmania* and *Trypanosoma*. Mice can be used in most of the work, but rats are occasionally used when large number of parasites is required for biochemical analysis. All personal license holders are trained to a high standard to minimise animal suffering.

PROJECT 117

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.

Word limit; 1000 words

Project Title	Understanding the mechanisms of hearing loss
Key Words	Genetics of deafness
Expected duration of the project	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):

Hearing loss is very common in the human population, but the only remedies are cochlear implants or hearing aids which do not replace normal hearing function. There is an unmet need for medical approaches to treatments to slow down or stop progression of hearing loss. The overall aim of this project is to determine the molecular mechanisms underlying hearing loss and investigate therapeutic approaches to preventing progression of hearing loss. This involves identifying the genes underlying hearing loss and building an understanding of what parts of the auditory system fail. We plan to investigate ways of reducing the progression of hearing loss using suitable drugs, to discover new routes for therapies. Finally, we will develop diagnostic criteria for different types of pathology affecting hearing, including approaches that could be used for diagnosis 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)?

The major potential benefit in the long term will be the discovery of a medical treatment for hearing loss. The aim of the current project is to provide proof-of-concept that progressive hearing loss can be avoided. We will identify new genes involved in deafness based on both human and mouse research and develop better diagnostic tests for different types of hearing loss caused by weaknesses at different sites within the ear.

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

We expect to use up to 20,000 mice for breeding, including up to 7,000 mice used for different hearing tests over the next 5 years. These mice include some carrying

genetic changes that lead to progressive hearing loss. Mice at all stages of life 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?

Most of the tests we plan to carry out will have only very minor, temporary effects on the mice (mild level of severity). Mice will sometimes be injected, leading to temporary discomfort. In some cases we will use minor surgery to place drugs in the middle ear, next to the inner ear, and this will be carried out under full anaesthesia and with appropriate pain-killers (moderate level of severity). At the end of the experiments, the mice are killed using a humane procedure and samples of inner ear tissues are taken for further study to make the best use of each mouse.

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 of investigating the development and function of hearing apart from using a living animal. We investigate humans with hearing impairment to discover candidate genetic mutations that may explain the deafness, but animals carrying the same genetic mutation are needed to confirm the role of these genes and for understanding the ways in which the auditory system fails, leading to deafness.

Hearing loss is often a progressive disorder and using a mouse we can study the early stages of the dysfunction and identify the main part of the ear affected, but this level of intrusion would never be possible in a human. We need to know the primary site of dysfunction in order to select and assess suitable drugs. It is possible to make some recordings in cultures of samples from the inner ear, but these are not helpful in explaining how the system works as a whole and still require breeding of a mouse to provide the sample to study. It is possible to expose these samples to drugs to test their effects, but this does not tell us anything about the ability of the drug to cross into the inner ear – for this a whole living animal is required.

Reduction

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

Reduction

We use the minimum number of animals to give us a robust and statistically-valid result using power calculations based on our previous experience of how variable the data are likely to be. For most experiments, we use 6 mice of each genetic type and age. We adjust the number needed if the variability is larger than we expected. We also consider size of the effect. Small effects (minor worsening of hearing) require large numbers of mice, so we focus upon severe effects on hearing to enable clear interpretation. We minimize environmental influences on findings by studying control mice raised in the same litter as the mutant mice. For drug treatments we use control mice that have undergone the same procedure but without the drug. We collect samples at the same time of day, as daily rhythms can influence the results. Whenever possible we test animals before disclosing the genetic status, to avoid any experimenter bias. Minimising variability in the results means we can use fewer mice to get a statistically-sound result. Some measures can be recorded in the same mouse at different ages, reducing the number of animals we use and allowing tracking of progressive hearing loss in the same mouse.

For statistical testing, we consult experts and websites that give guidance to the appropriate test to 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

We use the mouse because the inner ear of mammals has unique structures involved in progressive hearing loss that are not found in other vertebrates like birds or reptiles. The mouse is the most suitable mammal for the genetic manipulations we need to carry out and there are many existing mouse lines available avoiding the need to use additional animals to generate new model systems.

Any possibility of suffering is minimised by daily inspection of new mutants and breeding potentially affected mutants only when needed for experiments. Mice used for recovery experiments are observed until they recover fully from anaesthesia to ensure there are no signs of distress. Pain relief will be provided as needed. Surgery will be carried out in only a small number of mice, and will cause minimal pain or distress but we will monitor this and adapt procedures as necessary to reduce potential suffering.

We have introduced new refinements during the past 5 years, including these examples:

- Reducing the time of noise exposure from two hours to one hour;

- Refining the method of recording auditory responses so that the mouse can be allowed to recover;
- Reducing the time taken for auditory response recording to allow faster recovery;
- Reducing the number of mice needed for certain breeding procedures such as mapping mutations to specific chromosomes

PROJECT 118

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.

Word limit; 1000 words

Project Title	Evaluation of stem cells potential in arthritis
Key Words	Rheumatoid arthritis, stem cells, immune cells
Expected duration of the project	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;
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):

Rheumatoid arthritis (RA) is a debilitating and painful disease leading to increased morbidity and mortality and novel therapeutic approaches are needed. Recent advances using biologic drugs, such as anti- tumour necrosis factor (TNF), have made a significant impact on the treatment of RA patients although many patients do not respond and 50% discontinue the drug after 2 years. For that reason, it is vital to develop a new and more effective therapy for rheumatoid arthritis.

Stem cells are an ideal candidate cell type for tissue engineering and cell therapy to repair damaged structures in various arthritic conditions. Stem cells possess anti-inflammatory and immunosuppressive properties modulated by the secretion of biologically active molecules and since rheumatoid arthritis is a chronic inflammatory autoimmune disease involving tissue destruction, the anti-inflammatory and regenerative functions of stem cells could be exploited as a 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)?

The ultimate goal of this project is to develop cell-based treatment for patients with rheumatoid arthritis. Current treatments are often insufficient. For patients with rheumatoid arthritis, medications can halt inflammation but are unable to achieve regeneration of the damaged tissues. Our research could lead to novel cell-based therapeutic strategies for repair of damaged tissue via transplantation of stem cells or via the administration of substances that target the stem cells that are naturally present in our body.

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

We will use mice and estimate use of up to 1,000 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?

We will induce arthritis by injecting an antigen into the knee joints of pre-immunized mice and inject stem cells or stem cells conditioned medium to treat the arthritis. Mice may experience moderate discomfort as they develop symptoms of arthritis, such as swollen joints. Another possible side effect is local inflammation at the site of injection. This is rare and does not cause any signs of distress to the animals. Overall the level of severity for procedures in this project is moderate. At the end of experiments 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

Rheumatoid arthritis is complex disease that involves interaction between tissues and various cell types. This complexity cannot be mimicked *in vitro* hence the need to assess the therapeutic and regenerative effects of stem cells and biomaterials in animal models. *In vivo* studies are necessary to identify and characterise the native stem cells naturally present in the joint and define their role in joint tissue maintenance and repair.

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 first conduct experiments with a relatively small number of animals to establish the correct number of injected cells and the frequency at which cells are administered for the maximum therapeutic effect. 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

We have carefully selected the species and models to use in our study, based on previous published evidence. The *in vivo* procedure in this study is internationally well-established and routinely used by academic institutions and industry for preclinical studies and assessments of novel treatments. The procedures performed in a way that provides maximum information but minimal distress to animals. When needed, anaesthesia and analgesia are ensured. All animals undergoing procedures are monitored closely by trained staff that works closely with a Named Veterinary Surgeon. Humane endpoints are employed to limit suffering and disease burden.

PROJECT 119

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.

Word limit; 1000 words

Project Title	Synaptic physiology in mammalian central nervous system
Key Words	cortex, hippocampus, interneurones, synapses, neurones
Expected duration of the project	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):

The ultimate aim of this work is to understand how the human brain develops and functions in health and how and why it malfunctions. Detailed studies of the basic building blocks of the brain and their normal function are used to explain the malfunctions that can be linked to specific genetic defects and environmental factors as well as to the wide diversity of human capabilities and experience.

What 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 describe, in important detail, the fundamental building blocks of the circuits that form the brain; the circuits that acquire and process information, define cognitive abilities, control memory storage and recall and how these circuits fail in disease. Neurones communicate with each other via connections called synapses. These are highly complex structures that form and function with exquisite precision, each type of synapse developing its own unique set of dynamic and pharmacological proper-ties. Pharmacological differences are often exploited in drug design. Dynamic properties are under intense investigation currently, as they help to shape the brain’s ability to process many streams of information, simultaneously and in real time. We know these properties are important, that even tiny changes in the genetic code that defines the molecules mediating these properties can lead to tragically debilitating disease, but many of the fine details are still missing. By employing a wide range of traditional and novel techniques to explore the structure and function of the neurones and the synapses between them, we will provide important information that will help us understand how these circuits perform their vital functions and what happens when they malfunction.

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

Rats and mice will be used in this project. Power calculations predict that approximately 150WT rats and 150 WT mice and 500GM mice p.a. 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?

Mice and rats will not suffer from any side effects from these experiments. All animals will be culled either by Schedule 1 or 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

To provide information that can be applied directly to questions relating to normal human brain function, human brain development and disease, it is important to study mammalian brains in which many of the fundamental building blocks are similar to those in the human brain. Invaluable work that has identified basic mechanisms of life, has used much ‘lower’ species or immortal cell lines, but to extrapolate from single cells to principles of human brain organisation and function is too big a step. These studies are therefore dependent upon the use of mammals. Small rodents, which adapt well to life in captivity, are housed in optimally controlled environments and all precautions to ensure their health and well-being are taken.

Reduction

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

Reduction

To reduce the numbers of animals used in these studies we have designed multi-disciplinary protocols and use sophisticated data collection and analysis techniques that gain the maximum possible information from each experiment. We also employ cultures of immortal cell lines or human pluripotent stem cells wherever possible. When animals are used, they do not feel any pain.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 human neocortex is huge in comparison with rodent neocortex, but despite human-specific transcriptional networks, the local circuit organisation and the neuronal classes that are present suggest many similarities; the more sophisticated computation in humans resulting, in part, from the dedication of many more, similar small circuits to a given set of tasks. Much can therefore be learned from other mammals: how these circuits are constructed, what controls their development, how this is influenced by incoming information and the alterations associated with devastating neurological and psychiatric disease. The cross-species comparisons (rat vs mice) from this work will complement and add to studies of comparative neuronal anatomy supplemented by details of synaptic connections and their classspecific properties. The use of GM mice in which a specific subclass can be highlighted and/or activated with light make the recording easier and allow the characterisation of distinct sparse neurones.

We do not expect any side effects from this study. All researchers are highly trained to ensure optimal handling skills in performing the procedures.

PROJECT 120

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.

Word limit; 1000 words

Project Title	Novel treatments for kidney disease
Key Words	Chronic Kidney Disease, Renal Fibrosis, non-coding RNA
Expected duration of the project	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):

Kidney disease is a common disease that has significant impact on quality of life and lacks effective treatments. This project aims to identify novel treatments for patients with chronic kidney disease by understanding at the cellular level what happens when people get damaged kidneys. In particular we have focussed on important molecules called non-coding RNA that are involved in the initiation and/or progression of kidney disease. These molecules work by changing expression of defined proteins that affect kidney function in cells. These molecules are essential for kidney function but they become abnormally active in kidney disease. Recently we have found they may also be important in repairing the kidney too.

We are also optimising in parallel the production of substances that will carry genes into the kidney which can alter the expression of specific proteins involved in kidney damage which may slow or halt progression of kidney 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)?

Chronic kidney disease (CKD) is common and the number of people suffering from this disease is increasing. People with CKD have kidneys that are not functioning properly where the normal kidney tissue has been replaced by scar tissue. Having CKD puts you at a greater risk of a cardiovascular related premature death. Patients with chronic kidney disease can progress to have kidney failure which ultimately results in the patient requiring dialysis and eventually a kidney transplant. The cost of treating these patients is a burden on the NHS. Treatments that can target this scarring of the kidney could reduce the numbers of patients with kidney failure, reduce NHS costs and potentially reduce premature cardiovascular deaths.

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

Rats and mice (wild-type and genetically altered) have been proposed to be used in these studies as they most published species when examining renal fibrosis and chronic kidney disease. Furthermore the results from these models in mice and rats have been translated to humans. We will use less than 650 rats and less than 1100 mice over a 5 year period. To allow us to perform studies in genetically altered animals we propose to breed less than 4000 mice and 1000 rats. In order to examine kidney disease an animal model has to be used as the disease process involves several cell types and an inflammatory response which we are unable to replicate in cell culture. However, were possible we will use in vitro models of kidney damage to investigate the mechanism of disease and for testing our gene transfer vehicles prior to use in 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?

Animals under this licence will develop renal disease under some of the protocols as a result of having to create models of kidney disease in order to identify novel treatments and to investigate and test potential treatments. In order to induce kidney disease animals may undergo surgery or be injected with a compound that damages the kidney. As a result of the kidney damage the animals may lose their appetite, have protein in their urine, develop high blood pressure and may lose weight as the kidney damage develops. Unfortunately, some animals may reach a severe level as a result of some of the models within this project licence but every effort will be taken to minimise this happening by having monitoring systems in place to minimise any animal suffering. Painkillers are used where required and animals are very closely monitored throughout these studies which are not long term.

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 complexity of renal disease and the involvement of multiple cell types it is not possible to mimic renal disease in cell culture. However, we have several cell culture models that we can mimic parts of the process for example tubular cells and fibroblast cell lines and we will use these to carry out some of our mechanistic studies prior to carrying out studies in animals.

We now have the ability to examine other researchers published data (all published datasets are required to be placed in a freely available public website) and we are

able to compare this to our own historical data sets we have. Therefore we are able to use bioinformatics to examine any potential targets by in silico means rather than having to perform additional animal experiments.

Reduction

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

Reduction

Any experiments using our established protocols have power calculations from initial pilot studies performed by ourselves or collaborators identifying the lowest appropriate group sizes for each procedure.

We use fully blinded, randomized studies (using computer generated randomization) and littermate controls were possible to ensure no bias in our experimental results and to control for variability. We use the NC3Rs experimental design assistant to help us improve the design of our experimental studies. Animal genetic backgrounds are also considered when designing experiments to ensure we are controlling variability.

We actively use historical tissue we carefully bank when we cull animals on procedure. This ensures we have good quality tissue and histological blocks available which allows pilot studies for new avenues of research to be conducted without the use of new animals thus reducing the number of animals we use.

All our gene therapy vehicles are extensive quality controlled (QC) before being used in in vitro models to assess the functionality and efficacy of the viral vector and to ensure that the gene expressed is produced. Only those vectors that pass the QC are allowed to continue to be used 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

We propose to use rats, genetically altered rats and mice and normal mice in order to achieve the objectives of this proposal. Rats and mice have been proposed to be used in this study as they are the lowest suitable species that can be used.

In order to study chronic kidney disease an animal model has to be used as both processes involve several cell types and an inflammatory response which we are unable to replicate in cell culture. We believe that non-coding RNA are involved in CKD and renal/cardiac fibrosis and over expression/knockout of these non-coding

RNA should be beneficial therefore use of GA animals should minimise animal suffering.

The protocols selected which induce renal damage have been carefully chosen and have clear outputs and humane end-points to maximise animal welfare. The refinements we have introduced to maximise animal welfare include careful monitoring of the animals using special scoring systems to inform on the animals condition. This includes information on the animal's blood pressure, weight and urine. When severe models of renal disease are induced pain assessments will be made through careful monitoring of the animals and analgesia given prior to surgery, 24hrs post-surgery and again when necessary. All surgery is carried out using aseptic techniques which limits any infections and animal suffering as a result.

PROJECT 121

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.

Word limit; 1000 words

Project Title	Epigenetic inheritance of acquired traits
Key Words	Disease risk, stress, environment x gene
Expected duration of the project	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):

It is known that many complex diseases, such as depression, run in families. However, it has been difficult to pin point single genes that could explain the high heritability of these diseases. We believe that our ancestor´s environment played an important role in the likelihood of becoming ill. It is known from plants and lower animals such as worms that the ancestor´s environment can have effects on the offspring. We also know that in mice the experience of stress can change molecules in the sperm independent of genes. Such altered sperm can then signal changes to the offspring and sometimes this can lead to disease. We aim at understanding this process in detail, so that in the future we can block such inheritance and prevent disease.

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

To better understand the heritability of psychiatric disorders, scientists have been studying the genetic basis of disease heritability, but our understanding is still limited. We hope to identify both genes and their chemical modifications - so called epimutations - that alter disease risk across generations. We hope to learn how much different experiences can influence this. The genes and their chemical modifications uncovered by this project could help us better understand human disease susceptibility and it´s heredity. This will (A) facilitate the development of future research paths, to foster new treatments and therapies for psychiatric disorders and (B) help the design for novel strategies to prevent the inheritance of increased disease risk. In the long run our results may contribute to the minimization of the incidence of mental diseases and thereby mitigate the financial burden of the public health system.

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

Over the next 5 years, we will use up to 2000 mice (including genetically-modified) for breeding and up to 1700 mice for experimental procedures. 1420 of the 1700 mice used in experimental procedures stem from the mice generated in the breedings. Hence, we will approximately need a total of 2280 mice during the next 5 years.

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

We use a combination of environmental exposure and genetically modified animals to determine how changes in disease risk are inherited. To do so we expose wild type and genetically modified animals to one stressor, such as swimming or injection of stress hormones, and then breed them. At the same time we sacrifice some of these animals to obtain their sperm for in vitro fertilization. Then we do a range of behavioral and/ or metabolic tests on the resulting offspring and investigate whether a given stressor or genetic modification has heritable effects with implications for disease risk. Such tests typically include placing an animal in a box and observing where and how it moves around, placing an animal on an elevated plus maze with 2 closed and 2 open arms and again observe where and how it moves, allowing a mouse to swim and/or float in a tank of water, injecting an animal with insulin and taking small blood samples to measure glucose level just like in diabetic humans or injecting with glucose and taking small blood samples. We also try to track molecules, called RNA, that respond to the environment and that might be carrying information about the environment from one generation to the next. For this we also need to inject labelling substances into the body cavity of wild type and genetically modified animals during which animals are restrained by hand. The genetically modified mice are not expected to have any adverse effects. Many mice used in these experiments will experience enriched environments, for example when exposed to novel environments during behavioral studies, which could result in mild and transient anxiety when first experienced. Animals may be exposed to moderately stressful environments such as water, a restraint tube or substances with a slight sedative side effect for example a brightly lid field, but only for a short period of time. At the end of the studies, the mice will be killed using humane methods. Blood and tissues may be collected from them for measuring the body’s response to the environment.

Application of the 3Rs

Replacement

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

Replacement

Mental disorders are reflected by a wide range of (mis)behaviors. Behaviour is a complex process, involving many different parts of the brain interacting with the rest of the body. It also requires sensory organs responding to a stimulating environment. Further, mammals undergo a very complex developmental program that, to the best of our knowledge, is designed to reset parental potentially disease predisposing changes. The environment in the womb during gestation and the maternal behavior in response to health-compromised males can also substantially influence disease risk heritability.

We employ a thorough literature search and build on our knowledge gained in our previous work to identify potential alternatives to mouse experimentation.

We have considered the use of plants, since they can inherit information about the environment from their ancestors, however they do not show any behavior, and can therefore not model diseases such as depression. Further, their way of producing offspring is substantially different to the one used in mammals and mechanisms found to transfer environmental information to the offspring in plants do not exist as such in mammals.

We have also considered the use of in vitro technologies that use cells as a model. Only cells that can mature into sperm cells could adequately mimic the molecular processes necessary to encode environmental information and transmit it to the embryo. However, currently there is no cell model for growing mature sperm cells. Hence, current cell experiments cannot adequately reproduce the developmental complexity needed for the intended studies.

Another possibility to animal studies is computer simulation. However, the current state of this technology is not advanced enough and does not comprise enough data yet to adequately predict heritable changes of disease risk induced by the environment.

Therefore it is necessary to use live animals for studying the impact of environmental factors on offspring behavior and disease risk.

Despite efforts within our laboratory to undertake such studies in worms called C. elegans, a less developed animal, it has proven insufficient to model the complexity of human behavioral conditions and in recapitulating mammalian inheritance of environmental information due to them not reproducing wholly by sex between males and females. Worms can be male and female at the same time within the same animal and therefore can produce offspring without mating. Therefore the biological processes taking place during their reproduction do not reflect those happening in mammals. Nevertheless our studies in worms have gained valuable knowledge about certain genes to be involved in the transmission of environmental information, that are similar between worms, mice and humans.

Reduction

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

Reduction

We review the literature and analyze data from tissue collected from dead animals to identify those genes most relevant to study. Whenever these genes have similar relatives in worms we conduct preliminary studies in worms. Experiments will be well-planned and detailed protocols written before the start. The scientists conducting the mouse experiments will not know what treatment the mouse has had before so to ensure unbiased data collection. Another scientist will assign the order of animals to be tested, taking care that an equal number of treated animals and control animals is used each time.

We use statistics to estimate how many animals are needed to achieve our research goal. These mathematical calculations use existing data to determine how many mice are likely to obtain a meaningful answer to our question on the environmental impact on disease heritability. When no such preexisting data are available we base our estimations on our extensive experience with mouse behavioral and metabolic studies. The establishment employs specialized statisticians that can be consulted to ensure the accuracy of the estimations. This enables us to use the minimal number of mice necessary for each experiment.

After each experiment data are analyzed using state of the art mathematical methods. To this end specialists can be consulted.

Whenever possible we intend to use existing mouse colonies from national and international repositories rather than creating them ourselves. When different colonies are needed, we intend to use a REDACTED at our establishment that has specialist expertise to help reduce the number of animals used for generating these different mice. New mouse lines and our data will be made available to other researchers around the world.

Refinement

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

Refinemen

The mouse has been selected for this work since, mice have similar brains and sensory systems to humans and display many of the same behaviours. Mice also have very similar genomes to humans, so findings about heritable disease risk factors can be translated to humans. Due to their long history as a model organism the best tools are available to study their genes and the biological processes of their

inheritance. Mice also breed very quickly, so they are an ideal model for studying behaviour across generations in a reasonable amount of time.

We will use genetically modified mice that are produced by a team of highly specialized individuals. They are very experienced and have refined their working methods to obtain genetically modified animals. Our selection of genes to study is based on a thorough literature search and our data collected in preliminary studies in worms.

Many of the procedures employed in this project license are enriching for animals (such as providing additional physical exercise). If procedures cause transient harm, the discomfort will be kept to the shortest period possible, as to still obtain valuable data. Appropriate anesthesia and painkillers will be applied for any surgical procedure adhering to best practice guidelines from LASA to minimize animal suffering. If any animals have to undergo multiple tests the mildest tests will be done first, and the most invasive will be done at the end. We preset decision points, so that when data from milder procedures have been collected decisions can be made quickly on the continuation so to prevent unnecessary more invasive procedures. Whenever substances have to be injected we use standard operating procedures adhering to best practice recommendations.

We track all procedures carried out on a given mouse via a state of the art database. Decisions on mouse welfare can be made promptly, with access to the appropriate data for the mouse. We are continuously monitoring the literature to evaluate whether in vitro models become available to, at least in part, substitute our animal experiments

PROJECT 122

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.

Word limit; 1000 words

Project Title	Therapy resistance in blood cancer
Key Words	Therapy resistance, leukaemia, Cancer
Expected duration of the project	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;
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):

In this project we want to define mechanisms of resistance in patients with leukaemia by answering the following questions:

1.) Which are the malignant cells within the leukaemia which do not respond to therapy and why?

2.) Does the therapy itself play a role for the induction of resistance by selecting the few cells which can escape the effects of therapy through a process called clonal evolution.

3.) How can we eliminate these resistant clones by additional and novel therapy approaches in order to avoid further clonal evolution.

4.) Is it possible to predict the therapeutic potential of a certain compound regarding its capacity to suppress leukaemia-maintaining cells and to avoid clonal selection

What 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 findings will increase the knowledge on the mechanisms of why leukaemia develops and will have important implications for understanding mechanisms of resistance in leukaemia. Furthermore it will contribute to answer the question of why in some groups of leukaemia patients the therapy does not have the desired curative effects seen instead in other patients. The long term aim of this project is to create the basis for a future design of therapy regimens which are more precisely adapted to the individual patient.

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

For this project we plan to use only mice. We will need about 8300 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?

All measure will be taken to reduce adverse effects to a minimum. The worse expected adverse side effect is that the animals develop a picture of leukaemia which may reach a moderate level of severity. Otherwise all treatments and interventions will result in no more than transient discomfort and no lasting harm.

Application of the 3Rs

Replacement

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

Replacement

Leukaemia stem cells, which are considered responsible for the initiation/maintenance of the tumour are considered very rare (1:10^5 bone marrow cells). Such rare cells can't be detected by technologies available and thus can be defined only functionally by their capacity to engraft in animals.

It is not possible to recapitulate leukaemia development and therapy driven clonal evolution *in vitro*. Thus, in order to understand the function of these cells in the context of a complex organism, it is essential that these experiments are performed *in vivo* using an animal model.

Reduction

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

Reduction

Our experience with the proposed models allows to reduce optimisation of regimes required, keeping the number of animal required for pilot experiments very low.

We are basing our experiments on data that has been obtained as far as possible *in vitro*, further reducing mouse numbers needed.

Use of imaging systems (e.g Kodak Xenogen, positron emission tomography-computer tomography - PET-CT) to scan individual mice should significantly improve the accuracy of tumour burden measurements allowing conclusions to be drawn on smaller number of animals. Furthermore use of these techniques will facilitate serial

longitudinal analyses of tumour growth in individual mice, contributing significantly to reducing the number of animals required to achieve the objectives of this study.

For the syngeneic leukaemia models there are leukaemia samples in the liquid N_2 which engraft for 100% as shown from previous work . These samples can be used for the controlled induction of a secondary acute leukaemia.

Animal numbers are also minimised by the use of good statistical tests in order to obtain statistically significant results with minimum group 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 provides an excellent model in which to study the immune system. Mice are well characterised immunologically, and their immune systems closely resemble those of humans. In addition, a very large number of studies have previously utilised mice to study interactions between tumours and the mouse immune system. Thus, a large number of optimised reagents exist to facilitate designs of robust and reproducible experiments.

The use of special techniques such as *in vivo* imaging, or and the possibility to distinguish between human and mouse cells by using specific antibodies will help to avoid, where possible, that the animals experience the outbreak of leukaemia. The use of subcutaneous tumour growth for the cell line models allows to reduce animal suffering. We previously optimised irradiation protocols by splitting higher irradiation dosages. Because of the accumulation of radiation, the final effect is the same, but potential AE are reduced to a minimum.

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.

PROJECT 123

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.

Word limit; 1000 words

Project Title	Pluripotent stem cell derived hepatocytes for treatment of liver failure (PUSH for LiFe)
Key Words	Stem cells, liver injury, transplantation
Expected duration of the project	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:
Yes	(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):

Stem cells afford us the exciting possibility of being able to cure patients with liver failure by transplanting in cells where otherwise the only proven treatment modality is a whole organ transplant. Before such cells can be used in patients we must first demonstrate their (i) efficacy and (ii) safety in pre-clinical models of human disease. The aims of this project are therefore:

a) to test the efficacy of stem cell products by transplanting them into mice whose livers have been injured in such a way as to mimick as closely as possible human liver injury and assessing whether the cell therapy aids recovery

b) to test the safety of stem cell products by transplanting them into mice whose livers have been injured in such a way as to mimick as closely as possible human liver injury and assessing whether the cell therapy causes any undesired side effects such as cancer formation

Results from the above studies will allow us to expedite our overall objective of translating advances in stem cell science into novel therapies for patients.

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

The project will explore the potential of novel therapeutic strategies in the area of liver disease (Hepatology), including stem cell-based therapy to address the massively increasing burden of human liver failure. More specifically we aim as a result of this project to generate a clearer understanding of the limitations the current iteration of stem cell therapies have and define what parameters must be overcome in order to make them a successful therapeutic in future.

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

Mice will be used throughout this study. The minimum number of mice will be used to achieve meaningful data. Disease models will range from 1 to 90 days duration. A maximum number of 5,000 animals 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?

Animals under this license will undergo liver injury. Advice from the Veterinary surgeon will ensure that the welfare of the animals is maintained at the highest possible level for their condition. The severity of the protocols is expected to be mild or moderate. Any animals that show unexpected side effects of the liver injury or of treatments will be killed to avoid unnecessary suffering.

Application of the 3Rs

Replacement

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

Replacement

We have used *in vitro* assays as much as possible to replace *in vivo* experiments. However, they cannot adequately model the complete array of environmental factors that transplant material will be exposed to *in vivo.* Currently there are no alternative techniques that could replace the use of animals in our *in vivo* experiments.

Reduction

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

Reduction

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 via power analysis. We aim to further refine our techniques through high success rate of surgical procedures and good practises. Furthermore, we will continue reducing the use of animals as much as possible in this licence, in collaboration with the NVS.

Refinement

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

Refinement

Immunodeficient mice have been chosen as the experimental animals of choice as the fundamental features (cellular/subcellular/histological) of their livers are remarkably similar to other mammalian species and of comparable complexity to the human.

The protocols proposed in this application utilise well-established and tried techniques that have been refined to involve a minimum of suffering. Anaesthesia and analgesia will be administered to minimise discomfort and the animals will be assessed daily for any signs of distress. In all the proposed in vivo models, if animals display signs of distress, advice will be sought from the NVS and, if distress cannot be alleviated, the animals will be culled by a Schedule 1 method.

PROJECT 124

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.

Word limit; 1000 words

Project Title	Mouse models of human genetic disease
Key Words	Mouse, Genetic disease,, Development, Mutation
Expected duration of the project	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;
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 project aims to breed and study mice with altered genes in order to understand how genes function in human biology and disease. We are particularly in interested in understanding the biology that drives early mammalian development. Increasingly the mutations we will study will not be the protein coding regions of genes but will be changes in the sequences that lead to those genes being expressed in the right tissue and at the right time. These changes are more difficult to identify than coding mutations and it is harder to show what effect they have on the organism, so making mouse models becomes especially important.

What 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 diseases, this project will aid understanding the fundamental biological processes that drive development of the early embryo and later phenotypes. 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. All of the new models we characterize will be shared with other interested scientists to help their research.

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

Within the lifetime of this licence up to 20,200 genetically altered mice will be born. The great majority of these will be examined with mild, non-invasive techniques and have no procedures done to them before their death. A few hundred may be given

injection of substances that alter the way their genetic alteration behaves or may be given substances in their drinking water. A few hundred will have blood samples taken and all will ultimately be humanely killed.

In the context of what you propose to do to 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 disorder, they will be bred to have one genetically altered copy of a gene and one normal copy, and therefore the mice usually have normal biology. Within this licence, the vast majority of the genetically altered mice that do have altered biology have only mild symptoms that do not lead to pain of suffering. If any are bred that result in moderate adverse effects where possible these will be studied as embryos and therefore will not be born or will be kept for the minimum for time and humanely culled before the onset of severe symptoms.

Application of the 3Rs

Replacement

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

Replacement

The similarity of mouse and human at a genetic level makes mouse an ideal candidate to study gene function and to translate any findings in mouse biology to human biology, leading to a better understanding of gene function in health and disease. Where possible we grow cells in culture and have established cerebral organoids (cell-derived mini brains) to examine 3D tissue organisation but ultimately all findings need to be validated in the animal

Reduction

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

Reduction

We have staff with alot of experience in maintaining mouse colonies who only breed what is absolutely necessary for experiments and we freeze down lines that are not going to be used for a while. This also makes it easier to send lines to other researchers so they do not have to recreate lines we already have. Much of the work we do can utilize young embryos before they become protected by the Act and we can make embryos entirely from ES cells, which we have made to carry a mutation in which we are interested, so we do not have to breed and keep an entire line for a few samples.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 animals are kept in modern well maintained and resourced facilities and are looked after by experienced staff who care about their health and welfare and use the current best practice to ensure that this is maintained

Mice are examined constantly for signs of ill-health.

The observed phenotype of any mutation may change with the background strain so we are careful to keep this as similar as possible and use crosses where the control and the experimental animals both come from the same litter.

PROJECT 125

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.

Word limit; 1000 words

Project Title	An Education in Biomedical Research Methods
Key Words	
Expected duration of the project	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.

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):

The project covered by this licence will allow small groups of selected undergraduate and graduate students to receive an in-depth and highly supervised education in the use of animals in biomedical research. An advance in our understanding of, and ability to treat, disease depends on the continued production of scientists educated to the highest level. Over recent years, there has been a gradual decline in scientists educated as experts in the use of animals in biomedical research (experts in integrative biomedicine). Thus, it is the aim of this project to produce a cohort of highly-educated scientists who have the necessary knowledge and understanding to undertake research into integrative biomedicine in a professional and considerate manner.

The courses covered by this project will provide students who have received an in-depth and comprehensive education in the use of animals in biomedical research, with minimal animal suffering. This will benefit the students themselves in terms of allowing them to base their career path on highly relevant experience, to academic and non-academic research in providing highly qualified researchers in this area of acknowledged need, and to the general public in terms of maintaining and enhancing the research effort into the treatment of diseases of both animals and man.

What 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 licence is that it will lead to students (at both Bachelors, Masters and PhD level) graduating with the education considered essential by university teachers, the British Pharmacological Society, the Physiological Society, and the Association of British Pharmaceutical Industry. This is necessary for the continued

provision of highly-trained biomedical researchers, particularly for the pharmaceutical industry, but also for academic research.

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

The educational courses covered by this Licence will be targeted at small groups of students chosen for their academic ability, commitment, and career aspirations, which will reduce the number of animals used. The number of animals used will also be reduced by pooling data between groups in the same year and previous years to allow meaningful analysis of the results. Over the 5 years of the proposed project, the animals used will be rats (maximum of 710), mice (maximum of 1350) and guinea pigs (maximum of 100). These animals have been selected as the lowest species that could possibly be used to achieve the required experimental outcome.

In the context of what you propose to do to 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 anaesthetised throughout several of the protocols and so adverse events are not expected for these experiments. In the remaining protocols, if signs of adverse effects are detected (such as pain or discomfort at the site of injection) the procedure will be terminated, although no adverse effects are likely. Indeed, all of the procedures carried out on conscious animals are mild. All 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

Various alternatives to the use of animals in both biomedical teaching and research have been introduced and have proved useful. Indeed, they remain an integral part of our teaching in relation to this licence. However, animal experimentation will remain a vital component of research projects studying the causes of disease and attempts to find cures. The education of scientists involved in such projects must therefore reflect this. Despite their usefulness in increasing awareness of animal experimentation, computer simulations and videos are really no substitute for a ‘hands-on’ component. In this way, the scientists learn about the need to plan experiments to ensure the minimal use of animals, the proper monitoring and treatment of animals, and how the results from whole animal experiments may not always reflect what might be expected from previous ‘test-tube’ studies. However, we have produced a series of videos and on-line workshops to complement our

'hands-on' teaching that will be used to complement our *in vivo* teaching and replace animals wherever possible.

Reduction

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

Reduction

The educational courses covered by this Licence will be targeted at small groups of students chosen for their academic ability, commitment, and career aspirations, which will reduce the number of animals used. The number of animals used will also be reduced by pooling data between groups in the same year and previous years to allow meaningful analysis of the results. Where the welfare of the animal will not be affected, we will use the same animal rather than separate animals for multiple treatments, wherever 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

Although mice are most frequently used within this project licence, rats are used within some protocols rather than mice due to the impracticalities of physical size with regards to working with very small blood vessels. The guinea pig is used in one protocol because it is widely accepted as a good model for the investigation of the pharmacology of allergic asthma. Anaesthesia is used during protocols whenever possible. Organs are frequently harvested from animals to be used in other forms of undergraduate teaching.

PROJECT 126

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.

Word limit; 1000 words

Project Title	Tandem Core vaccine development
Key Words	vaccines, influenza, virus-like particles
Expected duration of the project	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;
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 main objective of this project is the assessment of experimental vaccine candidates towards the discovery and development of improved influenza vaccines. Current influenza vaccines have to be re-formulated each year and require yearly immunisation, this is due to high degree of mutation and variation in influenza viruses. Additionally, current vaccines often have low efficacy because the virus strains included in the vaccine do not match the circulating virus strains in the population, this occurs because the vaccine strains are predicted ahead of time using data driven educated guesses but it's often incorrect. We aim to produce a universal influenza vaccine which will provide protective immunity against all influenza virus from a single immunisation course, without the need of constant re-formulation or yearly immunisation.

What 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 towards improved influenza vaccines brings the possibility of a universal influenza vaccine closer. The benefits of improved influenza vaccines with universal coverage potential include decreased influenza associated illness and death, lower economic impact of influenza associated work absence and healthcare costs, and better control and containment of influenza epidemics and pandemics. This project aims to identify potential vaccine candidates which show promising results in a mouse model of influenza infection for future development of human vaccines.

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

We approximate the use of 3800 mice over a period of 5 years. The number has been calculated using a statistical power calculator and is consistent with previous experience working on similar types of project. Mice are the most widely used model of influenza infection due to their small size, availability of multiple strains, as well as broad accessibility of research tools to study their immune system.

In the context of what you propose to do to 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 develop improved influenza vaccines. Accordingly animals are vaccinated with the most promising vaccine candidates and then infected with influenza virus to test whether the vaccines are able to prevent or limit infection. The animals area kept in individually ventilated cages in typically in groups of 4-5 animals per cage. Vaccination is generally performed by injecting a low volume of vaccine substance under the skin with a small gauge syringe. In half of the cases mice will show temporary (up to 48hr) inflammation at the injection site characterised by redness or swelling. In some cases (approx. 20%) they will have a strong immune response to the vaccine which will itself produce flu like symptoms (for up to 48Hrs). One or two weeks after the final vaccination mice are infected with influenza virus to test whether the vaccine is working. The adverse effects in effects of influenza infection in animals mice closely resemble the symptoms experienced during human influenza infection by humans including reduced activity levels, fever and weight loss, typically lasting between 3-10 days. Humane end-point limits are in place to minimise animal suffering. At the end of the experiment animals are terminated and the serum and tissues are analysed for antibodies, pathology and cellular immune responses.

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 to vaccines is complex with interactions which require maturation and

interplay of different cell types, chemical signals, growth factors and immune proteins. Currently there are no in-vitro or non-mammalian models which can account for the complete extent and complexity of a fully functional mammalian immune system. The immune response to viral infection is equally complex, whereby immune protection is mediated by several innate and adaptive immune responses and often though a combination of multiple mechanisms (for example; antibody, ADCC, T-cell killing, NK cells, Macrophage phagocytosis, immune complement, and others).

Reduction

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

Reduction

In the experiments proposed, the number of animals used per experiment is just large enough to ensure the statistical validity of results. This is calculated using the size of the group required to reach a particular level of statistical significance with a 5% probability of falsely reaching the wrong conclusion. The average survival time and variability are calculated from real data gathered from experiments carried out under a previous licence, which shows 83% survival in vaccinated groups and only 33% in controls. Sham or placebo controls are needed due to the natural variability of infection and immune responses but also due to changes of the virus over time. In experiments where immune responses are measured it is our policy to ensure that the maximum amount of data is gathered from each in vivo experiment. To achieve this, several protocols are used, all of which are designed to reduce animal numbers and increase the quality of the data generated. For example we use protocol stacking to determine, antibody levels, levels of virus infection, cellular immune response and clinical signs of disease all from a single 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

The well validated animal models of influenza infection which are relevant for human disease include pigs, ferrets, non-human primates and mice. For this drug discovery project which involves testing multiple vaccine candidates in large enough numbers to generate statistically significant data we chose the mouse model due to their small size, availability of multiple strains, as well as broad accessibility of research tools to study their immune system. Furthermore the robustness and validity of the influenza infection mouse model has been cemented by over 30 years of influenza research and we have previous experience with this model. Our protocols contain well defined severity categories and humane end points aimed at minimising the welfare costs to the animals. Our testing of vaccine candidates programme has an ongoing refinement built into the research strategy; by including optimisation studies we will aim to find the lowest efficacious dose, the adjuvant and delivery route with least side effects. Thus procedures will become increasingly refined as the project goes on.

PROJECT 127

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.

Word limit; 1000 words

Project Title	Transmission and pathogenesis of influenza virus
Key Words	influenza, virus, respiratory, transmission
Expected duration of the project	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;
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):

We aim to understand how influenza viruses transmit and cause disease.

Influenza viruses cause respiratory disease every winter. Every few decades a new influenza virus emerges from animals and causes a pandemic. We don't understand why some animal influenza viruses can cross into humans whereas most don't, but we hypothesize that mutations in the virus genome sometimes adapt it for increased replication and transmission in humans. In addition the outcome of infection with influenza virus in people varies widely. Some people hardly get sick whereas others go to hospital or even die. We know that genetic variation in the virus strain causing the outbreak is one factor that controls this, but we don't understand the details of how.

We will infect animals with defined strains of influenza virus and monitor the outcome in terms of clinical signs, replication of virus in the respiratory tract, immune response and then transmission of the virus onto other animals. Then we will test novel interventions to see which best alleviate infection, transmission or clinical signs.

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

Having a better understanding of the pathogenesis of the influenza virus, how it evolves and is transmitted, will aid early identification of potentially dangerous viruses as they emerge. This will inform public health planners about how they should prepare for, and deal with, influenza outbreaks in order to minimise spread and also better predict which strains of virus are most likely to emerge each year, thereby improving accuracy of vaccine strain selection. Knowledge gained through

our research will make a major contribution towards helping control pandemics, reducing hospital admissions and saving lives of people who suffer severe influenza infections and may also contribute to a better understanding of other viral or bacterial respiratory infections.

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

Over a period of 5 years we plan to use maximum of 400 ferrets, 120 guinea pigs and 2250 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 expect that the animals will likely show mild to moderate clinical signs such as lethargy, weight loss, transient fever and sneezing. Animals will be regularly monitored for signs of potential distress and will either be given appropriate treatment under veterinary direction if this is likely to be effective or, if showing signs of distress, will be killed without delay using a humane method. Analgesia and anaesthesia will be used where appropriate in order to minimise pain. Once experiments have ended 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

We need to use some animals for our work because the processes of transmission and pathogenesis depend on the architecture of the respiratory tract, with a complex mixture of cell types and interplay with immune cells, which it is not possible to mimic in vitro.

Reduction

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

Reduction

We use the best available airway cell culture systems in vitro before our animal experiments to make sure that we don’t waste animals in experiments that would never work. The cell cultures give added information and increase certainty about interpreting the results of the in vivo work.

We use the minimum number of animals that will give us reliable data and information. Depending on the type of experiments conducted, we typically use

group sizes of 4-8 animals based both on our experience and on the advice from professional 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

We use ferrets because they are the only animal that can be infected with real clinical strains of virus and recapitulate the clinical signs seen in humans. Where appropriate we use smaller animal models, guinea pigs because they can transmit human strains of influenza and mice because they give a readout of replication and immune response in vivo. We can’t replace the ferrets with mice because mice do not transmit influenza and nor do they show the same array of clinical signs as humans after infection.

We use the least dose of virus and infect the animals in such a way that their infections are mild and limited to the upper respiratory tract rather than invading the lung and causing pneumonia.

PROJECT 128

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.

Word limit; 1000 words

Project Title	Immune regulation of metabolic pathways during neonatal and adult infection
Key Words	Immune, Neonate, Metabolism, Infection
Expected duration of the project	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):

We are interested in new ways to identify infections especially in the very young and very old as well as whether our metabolism impacts the body's response to infection and if this might reveal new treatments for infections.

Our proposed studies are focussed on two viruses, known as DNA herpes viruses - cytomegalovirus (CMV) and gammaherpesvirus (MHV68). These are significant because they commonly infect healthy people from childhood causing a range of issues but often without causing clinical disease at the time. They can have devastating and often fatal consequences for people including babies where the immune system is not working properly. Or in people later in life where their immune system is compromised, for example in chemotherapy for cancer or in liver or kidney transplantation. The viruses remain in the body cells for life even in someone with a good immune system.

At present there is no effective therapy against acute and any hidden infection and accordingly vaccine approaches have so far been unsuccessful. We are interested in identifying the relationship between the immune and metabolic pathways which control the viral infection and in particular understanding how our own metabolic genes together with viral genes switch "on" and "off" this virus. This application aims to uncover aspects of immune regulation by determining how and what triggers the "off" switch for the virus life cycle. Greater understanding of the interplay between the immune system, metabolism and the virus allows the possibility of development of novel diagnostic and anti-viral 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 projects links with our studies in examining human infections in early life (pre-term, neonates, infants and children) where we wish to test new approaches to diagnosis and new treatments based on detecting infection possibly involving manipulation of metabolism as well as the immune system. We hope that increasing our understanding of this control switch may lead to the design of drugs that will aid in slowing or stopping the destructive powers of the hidden virus as well as perhaps help improve vaccine and gene therapy approaches.

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

A significant proportion of our work is done without using animals (approx. 70% in vitro). To undertake these studies we plan to infect animals with genetically modified virus (no more than 400 animals over the course of these studies) and study the effect of the virus on the immune and metabolic systems. We will then investigate possible new treatments which will involve an additional 400 animals. In some of our experiments we will also use a small number (no more than 200 animals over the course of the project) to investigate what happens if the infection occurs during pregnancy as this is the commonest time humans are infected. Lastly for breeding and maintenance of genetically modified animals we will plan to use 600 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?

These infections are largely of mild to moderate severity. All animals will be humanely terminated at the end of the study. The majority of animals that are infected with virus will have mild clinical signs, all animals will be closely monitored and where signs are detected that indicate a more severe infection these animals will be humanely killed. Staff are highly trained in the identification of these signs.

Application of the 3Rs

Replacement

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

Replacement

We will undertake a significant amount of work without animals using an established in vitro macrophage infection model. Infected macrophages mount an effective innate-immune response that also recapitulate aspects of metabolic changes and therefore can provide to a limited extent a closer predictive model for in vivo infections. The macrophage model accounts for an isolated immune-cell virus interaction and is thus inherently limited.

For this reason this project work cannot be done without using the live animal because we cannot recreate the complicated links between all aspects of the virus, immune and metabolic systems in a laboratory. For the purpose of studying infections in a whole intact immune physiological system we will use CMV and MHV68 infections of its “natural” host- in this case mice, as a model for human disease.

It is very difficult to study these infections directly in humans however there is clear evidence of close similarities between what is seen in mice infections and what is seen in humans. This makes this a robust model to study for these diseases.

Reduction

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

Reduction

We will apply a statistical test (power calculation) to ensure the minimal numbers of animals are used for each of the planned experiments whilst still giving robust results. For this purpose these experiments will be independently performed using at least three different cohorts to ensure reproducibility of 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

MCMV and MHV68 are quite species specific and infection of mice has proven to be excellent animal model systems for studying immunity and progress of disease relevant to human disease. Animals will be closely monitored. In the case of clinically apparent infections, monitoring frequency will be increased and animals sacrificed before severe or prolonged clinical signs occur. A score sheet is in place and used for monitoring purposes and determination of endpoints. Animals are housed in facilities according to the Code of Practice for Housing and care of animals bred, supplied or used for scientific purpose. Technical and veterinary staff are familiar with our models and experienced at identifying clinical signs on the occasions those occur. Clinical signs are mild for the vast majority of our work.

PROJECT 129

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.

Word limit; 1000 words

Project Title	Rodent models to assess dependence of nicotine and related psychoactive substances
Key Words	Addiction, Conditioning
Expected duration of the project	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;
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 overall aim is to increase understanding of the dependence-producing properties of nicotine using rodent models of addiction. The aim is to advance our understanding on the processes underlying the nicotine reinforcement in order to formulate new strategies for treating nicotine dependence. Novel compounds that may be effective in treating in tobacco dependence may also have potential for other drugs such as cocaine addiction, which represents one of the biggest problems in society. The potential discovery of novel targets for treating addictions may be further evaluated using clinically-derived behavioural endpoints, such as relapse behaviours. Finally, by utilising electrophysiological processes along with brain stimulation techniques, the neural circuits relevant in mediating the dependence-producing effects can be targeted using novel techniques such as optogenetics and designer receptors. Identification of these substrates will help to formulate better medications for drug addictions.

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

It is anticipated that these approaches will lead to identification of those aspects of nicotine dependence that are important in the process of quitting, and with better insights, lead the way for the development of more effective medications in reducing relapse rates in smokers. The benefits from this project will come from further understanding how nicotine acts in the brain to mediate the satisfaction/pleasurable effects from tobacco products and e-cigarettes. Overall, this work will assist with the development of new aids for smoking cessation and thus, will reduce the burden that tobacco smoking places on society.

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

Male hooded Lister rats will be used in all experiments and the numbers requested (150 rats per year over the 5 year duration) are derived from previous experience with the behavioural techniques and anticipated continued research funds over this period. This ensures that the work is completed with an adequate subject population that may be published in an International peer-reviewed journal.

In the context of what you propose to do to 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 examine the effects of drugs on nicotine self-administration and to clarify the role of different neurotransmitter systems by comparing to other abused substances such as cocaine. In 1 hr daily sessions, rats will be free to move within a chamber to voluntarily self-administer drugs of abuse intravenously. Rats will be closely monitored during recovery from surgical implantation with an intravenous catheter. Tethered via a swivel, the rat learn to self-administer the drug, the number of lever press responses will be increased. Once stable levels of drug intake are exhibited, then the effects of compounds will be evaluated. Some subjects will receive the treatment via microinjectors, directly into the brain. For experiments involving electrical recordings, microwire electrodes will be implanted during the surgery and recordings made at various stages of the behavioural experiment. In tests focussing on drug-seeking responses, the behaviour will be reduced by eliminating cues and drugs. The persistence to respond on the lever previously associated with drug injections following a priming dose of the drug will be used as a measure of the drug-seeking response. Treatments will examine the ability of compounds to reduce this reinstatement effect. These experiments can last as long as 6 months depending largely on the patency of the catheter. Once the experiment is finished, all animals will be killed either by a schedule 1 method, whilst under non-recovery anaesthesia or by decapitation where the brains are required for analysis that have not been damaged or exposed to anaesthetic gases (20%). Given the intravenous surgeries, the severity of the procedures is classified as moderate. Regarding welfare, animals giving cause for concern will be removed from study until they recover. The animals adapt well to the task and they maintain their body weight and stay in good health. Doses of drugs to be tested will be chosen so as not to have adverse effects. Repeated exposure to compounds repeatedly produces little adverse effect. A few animals may develop an infection where catheters are implanted, which is a rare event and veterinary advice will be sought on these occasions. On a daily basis, animals will be checked by technical and/or the scientific staff. Body weights will be recorded at least twice each week.

Application of the 3Rs

Replacement

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

Replacement

The rat is the most appropriate species for this work. A large body of literature already exists on the neurobiology of drug-seeking and drug-taking behaviour in rodents.

There is no other approach to examine dependence-producing effects of psychoactive drugs – which is based upon a behavioural endpoint. The available alternatives are similar models that have been described for primates and dogs, but these species are not required for the work proposed under the proposed license. It simply cannot be done in a test tube. While computational network modelling is being introduced within the cognitive behavioural field, there really is no appropriate alternative to examine the neurobiology of drug dependence.

Reduction

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

Reduction

Experiments will be designed carefully to minimize on the subject numbers while retaining statistical power, the design of the proposed experiments have incorporated within subject tests – each animal serves as its own control. All tests with pharmacological treatments will be tested in a multi-factorial randomized sequence design. Also, pilot tests will be able to inform the number of subjects required to inform on a significant observation with confidence.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 considered to be one of the better alternative species to study reinforcing properties of drugs. In the past, non-human primates were used for this purpose and over the last 30 years significant advancements have been made in understanding the optimal conditions under which rodents can be trained and tested with psychoactive substances and also ensuring prolonged catheter patency. There is no other approach to examine dependence-producing effects of psychoactive drugs – which is based upon a behavioural endpoint. The available alternatives are similar models that have been described for primates and dogs, but these species are not required for the work proposed under the proposed license. While computational network modelling is being introduced within the cognitive behavioural field, there

really is no appropriate alternative to examine the neurobiology of drug dependence.

In terms of maintaining welfare and minimising adverse effects, most of the rodents will suffer no more than mild stress. The animals adapt to the task readily and they maintain their body weight and stay in good health. Doses of drugs to be tested will be chosen so as not to have adverse effects. Repeated exposure to compounds produces little adverse effect. A few animals may develop an infection where catheters are implanted, which is a rare event and veterinary advice will be sought on these occasions. On a daily basis, animals will be checked by technical and/or the scientific staff and body weights will be recorded frequently. As part of the experiment, the animals will also be handled and thus any adverse effects of drugs will be detected.

PROJECT 130

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.

Word limit; 1000 words

Project Title	Heterotopic Ossification in blast injured tissues
Key Words	Blast injury, tissue damage & mitigation
Expected duration of the project	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;
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):

After severe injuries such as blast, the patient's body sometimes forms bone within the soft tissues (muscles and ligaments), in places where bone is not normally found. This is called Heterotopic Ossification (HO). This causes pain and soft tissue damage, and is particularly difficult for amputees who often rely on soft-tissue bearing sockets to support their prostheses. Heterotopic Ossification formation is poorly understood, and so it is the aim of this project to develop a realistic *in vivo* model of blast induced heterotopic ossification (HO) and study the mechanical influences (blast, fracture, amputation) on HO 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)?

If a good model of blast induced HO is developed, it will be possible to further develop treatments and mitigations for this debilitating condition.

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

We will use approximately 288 rats over the duration of the licence (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?

Possible adverse effects are • The loss of a limb in this way may lead to unbalanced gait and loss of balance. Animals normally cope quite well as long as they are not in pain. Analgesia will be administered to ensure no distress is caused by this pain. • Localised bruising and oedema at the most severe blast exposures may occur, which is part of the healthy healing response to fracture and tissue damage. This will

probably dissipate as healing progresses. • Some animals may experience Phantom Limb Pain syndrome, which can cause them to stare at the amputation site, or even autotomy (see next clause) • In the presence of a damaged limb (especially loss of sensation) some animals may bite at the site and remove tissue (Autotomy). If either of these signs are reported, appropriate local anaesthesia on the amputation site will be applied, and analgesia may be resumed/instituted after NVS or NACWO consultation. If the symptoms persist unabated, the animal will be euthanized by a Schedule 1 method.. 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

Primary blast injury is followed by a “secondary injury” that develops hours to days later, so these injuries need to be studied and modelled *in vivo*. Likewise, evaluation of therapeutic interventions to prevent or limit secondary injury need *in vivo* studies. Computational and cadaveric models are entirely unvalidated for this work.

Blast-induced HO relies on many complex factors (as yet not understood) that can only be studied in an *in vivo* model for a realistic scientific approach. *In vitro* studies cannot reproduce the interactions that occur in the macro-environment of the blast-amputated limb. Computer modelling can provide only supporting and limited data for the formation of HO *in vivo* since the contributing factors involved in HO formation are not yet known.

Reduction

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

Reduction

Previous studies in our group have determined which characteristics of shock wave result cause blast injury. Using these known blast characteristics reduces the number of animals needed to obtain sufficient data for statistical validity. Non-invasive optical imaging, such as micro-CT or NIR, allows the visualisation of HO progression without the need to sacrifice the animals at each time point, reducing the numbers required.

These lines of investigation follow the most relevant and up-to-date models, which address the principle research question as discussed and refined with our clinical colleagues. The findings should definitely be publishable, in reputable and high

impact journals which also expect rigorous statistical reasoning and analysis; which is consistent with conforming with the NC3Rs ARRIVE Guidelines.

Our continued approach to research is to attempt to identify all possible confounding factors before undertaking the work, to analyse preliminary data as soon as it is gathered to check for unexpected confounding factors (and hence to adapt the experiment to account for these as early as possible), and to maintain our communication with colleagues and peers working in similar areas or models (to hear of developing lines of research and understanding as soon 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

When developing new models the mouse is often the first considered since genetically modified animals and probes are available which enable examination of cellular or genetic mechanisms or entities. However applying blast causing HO in a limb without causing death would be technically difficult in mice, but more practical in rats due to their size.

Bone formation and turnover in rats is closer to that of humans than in mice, which may undermine the mechanism of HO formation. However, the rat is sufficiently realistic that it is possible to avoid using larger animals with greater ability to experience pain, suffering and distress or lasting harm.

All animals will be anaesthetised during blast imposition, and provided appropriate analgesia as or when required or indicated.

The rats will be housed in groups to minimise stress or distress.

It is expected that most animal deaths during the development of the model will occur immediately after the blast or surgery, whilst the animal is still under general anaesthesia. In that case there will be no suffering to the animal concerned.

PROJECT 131

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.

Word limit; 1000 words

Project Title	Central nervous system repair by cell transplants
Key Words	spinal cord, regeneration, cell therapy
Expected duration of the project	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;
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):

This project aims to investigate potential therapies for central nervous system repair. Rodent injury models will be developed to optimise repair with transplantation of cells into focal lesions. The extent of repair and functional improvement can then be observed. This will provide direct models for repair of human spinal cord injuries, brachial plexus and other spinal root injuries, and glaucoma 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 work carried out under this project will build on previous research which is of direct relevance to the development of therapies for patients with brain, spinal cord and nerve injuries. Findings from these studies will also be beneficial to animals with similar injuries.

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

Around 10 rats a week are used: they are humanely terminated after 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 project involves surgical and behavioural procedures to study functional repair of the nervous system. In the first few days the animals may be less active than pre-surgery. It would not be unexpected to see slight weight loss (5% or less) in the first week following surgery. They may not groom as frequently or thoroughly in the few days following injury. Serious adverse effects as a result of these procedures are not expected but might include infection, abnormal limb function, or wound problems.

Animals that have received glaucoma surgery can be hard to distinguish from normal rats. There may be some discolouring of the eye or the animal may be slightly less easy to handle due to previous day surgery –these signs would be mild. In the case of unexpected adverse effects, animals will be culled to prevent suffering. At the end of all experiments all animals are killed using approved 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

To determine safety and functional efficacy of therapeutic cells for transplantation it is essential that they are first tested in an appropriate model of disease. The complexity of the central nervous system means that the use of in vitro models is insufficient to ultimately determine whether transplants have an impact on cell growth and connectivity in a living system, and generate improvements in an injury model.

Reduction

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

Reduction

Cell culture studies will be used before and in parallel with animal models to develop and optimise therapies, and small pilot experiments on animals will be used to minimise the use of animals. We aim to reduce animal numbers by developing imaging techniques to allow tracking of the transplant 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 have been selected for these studies as they are the lowest vertebrate in which these injuries can be reliably modelled and functionally assessed, for which techniques have been well optimised. All animals are housed in licenced facilities with optimised care and welfare from trained staff. For surgical procedures, optimised aseptic techniques will be used with anaesthesia and analgesia, and animals observed continuously and procedures terminated if signs of suffering are observed.

PROJECT 132

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.

Word limit; 1000 words

Project Title	Genetic manipulation of rodent Plasmodium spp.
Key Words	malaria, Plasmodium, Transmission, Sexual development, Antimalarial drug discovery
Expected duration of the project	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;
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 aim of this project is to improve the fundamental understanding of the biology of the malaria causing parasites *Plasmodium* using the rodent infecting *Plasmodium berghei* as a model for further research into the 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)?

The study of the rodent infecting malaria parasite, P. berghei, gives access to all life cycle stages (in mammalian liver and blood, and in mosquito midgut and salivary glands), which cannot be achieved in the study of the human species. This study will focus on disruption or modification of genes known to, or expected to, control the sexual stages of the parasite, as it is these stages which become transmissible, and through this transmission allow progress of the parasite from mammalian host to insect vector and ultimately via a mosquito bite, back into another mammalian host. As malaria kills more than 600,000 people a year from the more than 200,000,000 infected and resistance to current antimalarials is so widespread, a long-term programme of control is desperately needed. A further potential benefit could be that the identification of proteins involved in the transmission of the parasite may be targets for vaccines. Effective control of transmission is one of the most effective ways to control the spread of the disease. Our system can be used to test potential vaccines, or other drugs and antimalarial compounds, in vivo before their use in clinical trials.

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

We have on average REDACTED members working on the rodent malaria model. We use approximately 2400 mice, and 56 rats per year. The rodent malaria parasite, Plasmodium berghei, cannot be maintained in culture throughout its entire life cycle, and so we depend on animals to fully investigate the entire life cycle of this malaria model. Approximately 100 mice/year are used to maintain our mosquito colony. We require animals to grow and replenish our stocks of modified parasites, in order that we can continue to study the genetic modifications. The rats are predominantly used to expand our existing parasite lines because we can achieve a greater number of vials from a single rat as opposed to the use of multiple mice. Although new methods are being reported in order to isolate specific populations of genetically modified parasites, these methods are not routinely used as yet. The most common method still requires 10 mice for every modified parasite. As the newer methods become more established we will utilise them, and reduce our animal numbers. In order to state experimental results with assurance we also need to ensure our data has statistical significance. This therefore requires experiments to be repeated.

In the context of what you propose to do to 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 are typically infected with the rodent infecting malaria parasite P. berghei to study a phenomenon known as experimental cerebral malaria (ECM), which is thought to be analogous to cerebral malaria experienced by many humans infected with P. falciparum. Mice infected with the parasite will develop ECM at day 7-9 after infection and die after convulsions unless treated or euthanized. However, since ECM is not currently a topic of our research we avoid the use of mouse strains that are susceptible to it. Animals will be infected with malaria parasites by injection, either into the abdomen, or into the tail vein. The infection is then assessed on a daily basis by smearing a small amount of the animals' blood, which is obtained by pinpricking the animals' tail. Infections are not permitted to get high enough to manifest as disease in the animal. If any animal shows sign of illness, they are euthanized immediately. Once parasites are detected in the blood, and reach a desirable level, the animal is heavily sedated, the blood removed, and the animal culled via either snipping of the heart, or neck break. If an animal is not infected following injection of parasites, then they will be maintained for use in one further procedure. Outwith the discomfort of injection, the degree of suffering to the animal is minimal as we do not allow infection to develop to the stage of physical symptoms of experimental cerebral malaria, and procedures that would cause pain are performed under sedation. This includes the procedure whereby parasites are transmitted from an infected animal to mosquito or from mosquito to uninfected animal via a blood meal. In this instance the animals are under general anaesthesia, and are placed on top of mesh mosquito cage, their paws, tail and faces protected from the insects. The mosquitoes (up to 100 in number from a cage of 200 only 50% of which will be females) are allowed to feed on two mice for up to 20 minutes. The mice then recover and assessed on a daily basis for infection. A number of mice will

be used to maintain the mosquito colony as a blood meal. In this instance the animals are under general anaesthesia, and are placed on top of mesh mosquito cage, their paws, tail and faces protected from the insects. The mosquitoes (up to 1000 in number from a cage of 2000 only 50% of which will be females) are allowed to feed on two mice for up to 15 minutes. The mice are then culled via neck break. The protocols are designed to involve minimal stress to the animals. Most routinely used protocols require no or minimal anaesthesia. Our protocols are further designed to incorporate administration of drugs via drinking water wherever possible and the minimum numbers of mosquitoes used for transmissions. Furthermore, we use strains of mice that are not susceptible to ECM (experimental cerebral malaria.)

Application of the 3Rs

Replacement

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

Replacement

The rodent malaria model allows access to all stages of the malaria parasites lifecycle for research. However, it is not possible to culture *Plasmodium berghei* long term in culture. Access to all stages of the life cycle requires the use of rodents as a host for the parasite, and mosquitoes as the vector. This comprehensive coverage of the parasite life cycle is not available for research in human species of *Plasmodium.*

Reduction

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

Reduction

Experiments are planned very carefully to ensure that the minimum number of animals are used, and whenever possible animals will be shared between lab members. A number of procedures have undergone refinement, which has also decreased animal usage. For example, a method by which we select a specific parasite population in an infected mouse now requires the use of one mouse in comparison to four mice by the application of a drug in their drinking water, rather than drug application by injection. Likewise in certain cases we can isolate a population of parasites by a method called FACS that uses one mouse, rather than the ten mice used previously with another method. We also use a strain of mouse from which we can obtain a greater blood volume in comparison to other strains.

If an animal does not become infected with the parasite following injection, then the animal can be used for one further attempt at infection , further reducing the number of animals required.

Plasmodium berghei readily infects both mice and rats. Rats are used infrequently when a large yield of blood from a single host is required in order to minimise mouse numbers and potential variability.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 mouse model to study malaria as it allows access to all stages of the life cycle of the parasite. Mice are typically infected with *P. berghei* to study a phenomenon known as experimental cerebral malaria (ECM), which is thought to be analogous to cerebral malaria experienced by many humans infected with *P. falciparum*. Mice infected with the parasite will develop ECM at day 7-9 after infection and die after convulsions unless treated or euthanized. However, since ECM is not currently a topic of our research we avoid the use of mouse strains that are susceptible to it.

To prevent suffering of animals, parasite numbers in the blood are monitored daily. If an animal has a low number of parasites, but looks unhealthy, we humanely kill the animal.

We have recently made advances in the identification of a gene, ap2-g, which when over-expressed dramatically increases the production of a specific parasite life cycle stage, the gametocyte. Previously we had to allow a much higher infection rate in the animal in order to obtain high numbers of gametocytes. Research is continuing into ap-2g to ensure that the gametocytes produced are like those found naturally. If this is the case, then significantly fewer animals will need to be used for gametocyte studies in the future.

Alternative methods of drug treatment have and will continue to be researched. If drugs can be administered orally via drinking water and similar results achieved as with injection into the abdomen or the tail vein, then procedures will be modified to incorporate these.

PROJECT 133

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.

Word limit; 1000 words

Project Title	Production of Immune Cells and Sera
Key Words	Monoclonal antibodies
Expected duration of the project	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;
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):

Monoclonal antibodies derive from a natural immune defence mechanism whereby foreign proteins, for example on invading bacteria, are specifically identified and neutralised. These properties have led to a wealth of applications in the pharmaceutical industry. The aim of this project is to produce monoclonal antibodies to support drug discovery activities with applications ranging from novel therapeutics, tools for drug target discovery and validation and development of assays.

What 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 supports a number of drug discovery programmes that are directed at patients with severe disease and high unmet medical need.

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

The project utilises 3 species, rabbits, rats and mice that have all been shown to elicit good antibody responses in the past. Per annum we expect to use approximately 60 rabbits, 50 rats and 150 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 expected level of severity is mild with transient pain and discomfort associated with the injections used to immunise the animals. Anaesthesia will be used to reduce discomfort where appropriate. At the end of the immunisations animals will be killed and immune cells harvested to screen for cells producing antibodies of interest.

Application of the 3Rs

Replacement

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

Replacement

Where appropriate we will use an in vitro method of antibody discovery. However this method is often poor in finding rare antibodies with the desired functional characteristics. In these circumstances the quality of a natural immune response directed at the target protein is more likely to succeed.

Reduction

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

Reduction

Where appropriate proteins will be combined to allow immunisation against more than one protein in a single animal. We will instigate any immunisation with low numbers of animals and if unsuccessful try a different approach again in low numbers. This should reduce umbers from a single immunisation strategy directed at a large 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 animals have environmental enrichment and our rabbits are socially housed unless there is a specific need not to.

PROJECT 134

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.

Word limit; 1000 words

Project Title	Studying the pathobiology of enteric bacterial pathogens in rabbits
Key Words	Food-borne pathogens, Diarrhoea, Therapeutic, Bacterial virulence
Expected duration of the project	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;
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 aim of the work is to better understand and control infections caused by food- and water-borne bacterial pathogens. We will:

1) identify factors that enable bacterial attachment to the intestine

2) characterise how these factors impact on the development of disease,

3) explore the effectiveness of new treatments in controlling disease, and

4) find factors that play a role in transmission between 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)?

This study will result in new knowledge that can be used develop novel intervention or control strategies. This could benefit society by reducing the number of people that are infected during disease outbreaks.

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

This project uses New Zealand White rabbits and we plan to use approximately 220 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?

The animals will be infected with different types of bacteria which may cause diarrhoea. Depending on the bacterial species, diarrhoea may appear 'watery' and

cause marked fluid loss in the animals over time or be evident as loose stools, which is more likely to be linked to intestinal damage. Extensive fluid loss can cause dehydration in the animals despite the animals feeding normally. This type of diarrhoea is usually considered as moderate severity because fluid loss can affect animal posture, temperature and levels of activity. The onset of loose stools can cause mild to moderate severity depending on the extent of intestinal damage. The health of the animals will be carefully monitored to ensure rabbits do not suffer prolonged periods of diarrhoea and the endpoint criteria are specific to each type of bacteria under study. Based on our actual severity data over the past few years, about 75% of rabbits showed mild severity after infection and this level of severity was sufficient for our studies. All animals are euthanased at the end of the study, in accordance with experimental and humane endpoints.

Application of the 3Rs

Replacement

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

Replacement

Diarrhoea disease can only be seen in a living host, with a functioning intestine. Performing studies in humans would be unethical due to the lack of treatment options for some of the infectious agents and challenging due to the difficulty of controlling for variations in diet, lifestyle etc. Non-protected animal alternatives such as embryonic forms of mammals, are not deemed suitable because their immune and developmental systems are not fully developed.

A range of *in vitro* assays will also be used to characterise the bacterial strains/mutants. This will include the use of immortalised cell lines to study bacterial adhesion to the epithelium or to assess effects on host cell processes; assays to measure biofilm formation, motility and chemotactic response; assays to measure protein secretion in response to host conditions (e.g. pH, temperature) as well as the use of insect larvae models (e.g. Galleria mellonella).

Reduction

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

Reduction

The number of animals used in this study has been derived using actual past data and following the advice of experts in experimental design and statistics. In addition, modern molecular techniques based on DNA sequencing will be used to study the impact of different factors in a small 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

Rabbits are used in this project because oral infection of these animals results in disease that is most similar to that seen in humans. The pathogens do not stably colonise laboratory mice and rats, and these animals do not develop disease. To minimise distress in the rabbits, we group house the adults prior to use in the experiments. After infection, we employ a timely monitoring and observation schedule with a custom-made clinical score system that best reflects the disease seen in the animals to effectively capture experimental and humane endpoints.

PROJECT 135

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.

Word limit; 1000 words

Project Title	PATHOGENESIS AND TREATMENT OF BACTERIAL PATHOGENS IN FISH
Key Words	FISH, BACTERIA, DISEASE, AQUACULTURE, TREATMENT
Expected duration of the project	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;
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 overall aim is to reduce fish losses in aquaculture systems from bacterial infections by producing improved understanding of disease outbreaks, leading to the development of better prevention / control/treatment regimes to support the health and welfare of farmed fish species. Disease outbreaks in fish farms significantly impact on the sustainable development of this growing aquatic food sector. The need for improved disease diagnosis, prevention and control strategies is recognised by academic and industry alike.

What 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 is the advancement in our ability to control infectious diseases in aquaculture. This is beneficial for the fish populations as it will improve animal health and welfare. It also directly improves our aquatic environment by reducing the amount of chemicals used during treatments as we aim to produce better health management tools. Improvements in fish health and welfare will lead to higher quality and quantity of fish available for global consumers.

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

The programme of research will use a maximum of 26,100 animals over the 5 year period, representing 10 species of fish. These are fish species of economic

importance in global aquaculture, primarily food fish for human consumption but also include cleaner fish used as biological controls in aquaculture.

In the context of what you propose to do to 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 fish species suffer from a wide range of bacterial diseases naturally and display a diverse array of clinical signs therefore the infectivity work will be considered severe, whereas the studies on medication and vaccination are considered relatively mild to moderate procedures. All fish are closely monitored during the studies for any signs of distress or clinical signs of disease similar to those reported during natural infections and any fish displaying these changes will be killed humanely using a Schedule 1 method. At the end of each study all animals will be sacrificed except for those used for blood sampling which can go back into stock tanks.

Application of the 3Rs

Replacement

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

Replacement

Currently there are very few validated alternatives to *in vivo* studies for diseases in fish. While there are fish cell cultures available these do not represent the complexity of host pathogen interactions which are required for a disease outbreak to become established. Alternatives to whole animal testing in fish are being developed, often for a specific purpose such as testing chemical toxicity from water bodies, but they remain limited in their application for infection work. The issues in the existing alternative models is that they are unable to replicate the interactions required between the fish and the bacteria that must happen before a disease outbreak can occur and so they are limited in their ability to replace completely whole animal testing. In terrestrial farmed species, computer generated models can be used but at present these are not available for fish diseases. However, during this programme of work should alternatives become available these will be fully explored and applied where appropriate to replace whole animals.

Reduction

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

Reduction

The need for effective disease control, including better medicines and vaccines is required to ensure the health and welfare of farmed fish species. Given the lack of alternative tests available, fish disease models are required to provide robust data

and deliver improved disease control data that can then be used to support the health and welfare of our farmed fish globally. Where possible, laboratory based tests will be used first before any animal studies, which means that only diseases of clinical significance will be investigated allowing the lowest numbers of animals to be used in the fish disease models. Many factors can contribute towards the occurrence of disease in a fish farm where not all causes are due to an infection. Therefore, by using data sourced from natural disease outbreaks at the farm, combined with laboratory testing of the bacterial isolates using routine laboratory based tests all studies will be designed with a minimum number of animals using the most robust methods 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

The fish species are those of economic importance within the aquaculture industry and while these species are important and information on disease control is available for some of the diseases there are still many challenges to be addressed including increasing development of antibiotic resistance. For some bacterial diseases there are no other options than antibiotics and so we need to find better alternatives to control disease outbreaks in our farmed fish species as well as improve our ability to accurately diagnose the infection, particularly from emerging disease outbreaks. Animal models play an important role in helping tackle natural disease outbreaks and there are a range of models available for the top 5 global aquaculture species produced. Each study is closely monitored numerous times daily and humane end points rapidly applied should any animal show signs of definitive disease. We have highly trained staff available to provide guidance and support and all individuals receive competency training. These factors help prevent unnecessary suffering and deliver robust and reliable data.

PROJECT 136

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.

Word limit; 1000 words

Project Title	Pharmacokinetic evaluation of novel therapeutic agents
Key Words	drug delivery, pharmacokinetics, PK, PKPD, therapeutics
Expected duration of the project	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;
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):

For new medicines to be effective, we need to ensure that enough of the medicine will reach the right place in the body to have a positive therapeutic outcome. Pharmacokinetic (PK) studies enable us to test potential new medicines and determine how long they last in the body, and which organs they reach.

What 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 enable the discovery and development of new medicines across a wide range of diseases that affect human patients. This could include: cancer; lung diseases such as asthma and chronic obstructive pulmonary disease (COPD); cardiovascular and metabolic diseases including kidney fibrosis and diabetes. This project will also enable us to investigate new ways of delivering medicines to patients in the future, that are more convenient or safe, such as inhalation or delivery of DNA to cells so that they can make the medicine directly inside the body.

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

We will use adult rats and mice, and we estimate that over the 5 year period of the licence up to 3200 mice and 1300 rats will be used. The majority of these will be in pharmacokinetic studies where animals are dosed with a potential new therapeutic agent and then have blood samples taken over a period of hours to days, typically less than 1 week.

In the context of what you propose to do to 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 typical experiment would be to dose the animals with a substance and then take blood samples over a period of days to weeks and measure the substance in the blood over that time. Blood samples are usually of a small volume and are taken from a vein in the tail. A smaller number of studies could involve more complex designs, for example giving an animal an additional dose of a substance that will stimulate the immune system so we can measure how levels of a potential new medicine can change the inflammatory response. Other studies may look at technologies that will enable to us to deliver medicines more effectively in the future, which could require injections directly into muscles or skin under anaesthesia. The majority of studies performed are expected to be of mild or lower severity, but some studies using surgical techniques to inject into muscle cells r skin, or using animals with surgically implanted tubes for sampling blood or bile will be in the moderate band. No studies will be in the severe category. At the end of the studies 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 and computer modelling, living animals are still needed for some studies because isolated cells and organs do not reproduce the complex nature of in vivo biology. For example, the interactions between immune cells, nerve cells, complex hormonal systems and organ-specific cell changes cannot be recreated outside of living animals or simulated using computers.

Reduction

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

Reduction

Studies on this licence will generally use serial sampling techniques, where a small blood sample is taken from each animal on multiple occasions. Usually only three replicates are needed at each sample point. These practices reduce the total number of animals used. We use statistical methods to ensure that the correct number of animals are used in studies where the objective is to test the effect of a new substance in modulating a biological system, which reduces the need for studies to be repeated in the future. Other measures such as random assignment of animals to treatment groups and elimination of observer bias also increase the robustness of 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

Rats and mice are the most appropriate animals for these studies as they are mammalian species with many features in common with humans. They have been studied for many years and there is good understanding of their biology and how this relates to humans. Animals are kept in high quality facilities, free from pathogens and with access to food, water and environmental enrichments.

PROJECT 137

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.

Word limit; 1000 words

Project Title	Maintenance of barrier immunity in health and disease
Key Words	Lung, Inflammation, Chronic disease, Treatment
Expected duration of the project	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;
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):

The overall burden of respiratory disease is growing – it now costs the NHS £6.6 billion per year. Millions of 'bed days' every year are taken up by respiratory patients and the most commonly reported illnesses in babies and children are lung-related. One in five people are dying from respiratory disease in the UK.

Respiratory disease also causes secondary problems in the skin and kidney. They also increase the risk of lung cancer, but the reasons for this are not known. In this license our aim is to determine how inflammation of the lung causes these secondary problems and to develop treatments for them. This area is under-researched and yet extremely important.

We will therefore examine:

Why lung inflammation alters the severity of subsequent lung inflammation,

Why lung inflammation predisposes to debilitating conditions in the skin and kidney and,

Whether prior lung infections make the lung a supportive site for tumour cell growth

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

Short term benefits include a greater understanding of why these secondary complications occur and ultimately the discovery of new medicines to prevent them. Our approach is different: we want to return the lung to its healthy state, not stop a disease out of control. Scientifically this is important and for the pharmaceutical

industry it would open up new developmental pipelines for medicines. Based on our track record we would expect to have 5 new lines of enquiry at the end of 5 years.

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

Short term benefits include a greater understanding of why these secondary complications occur and ultimately the discovery of new medicines to prevent them. Our approach is different: we want to return the lung to its healthy state, not stop a disease out of control. Scientifically this is important and for the pharmaceutical industry it would open up new developmental pipelines for medicines. Based on our track record we would expect to have 5 new lines of enquiry at the end of 5 years. The conditions we study affect patients of all ages and particularly young children. This license will therefore use adult mice and occasionally neonatal mice. This project licence merges the interests of a number of groups interested in lung, skin and kidney that encompass approximately REDACTED for 5 years. The number of mice quoted is the maximum scenario. Mice will be bred only when required to provide offspring for experiments. These offspring and those bought from designated sources will be used in the other protocols (a maximum of 17,000 mice). This represents approximately 226 per year per person. This relatively low number reflects that the work has now predominantly moved into human 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?

This project license examines lung, kidney and skin inflammation. It also addresses why some get lung cancer when others do not. All are linked, as we have evidence they are all caused by lung inflammation. We have carefully considered the effects on the mice and have refined the experiment so that only mild/moderate effects are experienced. Any suffering can be gauged firstly by weight loss caused by inflammation of the lung, a reduction in feeding or movement and by their general appearance, but we have not noted obvious signs of respiratory distress. All mice, recover their original weight within approximately 10 days. The license also contains a kidney inflammation mouse model which displays no overt signs of clinical disease except raised protein and blood in urine by protein levels in the blood. In the skin inflammation models skin thickening, sloughing of cells and typical features of a wound appear. Mice appear otherwise unaffected. In some experiments we will administer tumour cells to mice and examine how lung inflammation causes them to migrate to that site. We are only concerned at the earliest stages of lung cancer and so mice will not develop overt tumours. At the end of the experiment mice are euthanised using an injected anaesthetic

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 conduct our research on human tissues and have funds to collect airway washes from patients and to store lung tissue from patients undergoing surgery for suspected lung cancer. However, this tissue lacks a gaseous or blood supply, it does not remain live for long in the laboratory and it does not allow us to study disease across different organs. Less sentient animals, such as zebrafish or drosophila do not possess all the immune cells present in mammals, nor structures that resemble human skin, kidney or the cancers that develop.

Reduction

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

Reduction

Animal reduction will be facilitated by group members sharing tissue and employing rigorous experiment design developed by our statisticians to calculate the least number of mice needed to address our questions. Where an experimental type is used more than once we will assess performance over time to ensure that it is continuing to perform well.

The laboratory has archived specimens that can be used by subsequent researchers. Unfortunately storage processes can affect our results and so these archived tissues have limited use. As proof of principle however, they are adequate.

Refinement

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

Refinement

Minimisation of animal suffering critically depends on close monitoring of mouse condition (body weight, mobility, the quality of mouse fur etc). Staff perform this inspection daily, or twice daily if concerned. We try to avoid intraperitoneal injections where possible, using intradermal where the alternative exists. The latter, we feel is less intrusive than intraperitoneal.

All of our procedures are performed under anaesthetic that allows recovery within less than a minute. This prevents mice from losing body temperature whilst immobile.

For the purposes of our experimental questions, this project license looks at the impact of lung infection on common complications that occur in other sites that often require the patient to be hospitalised. These include the development of skin complications, kidney complications, other infections and the spread of cancer. To study these life-limiting events we have chosen the least disruptive method to answer the question. For example, we only need to look at the early stages of cancer spread, and not established disease, which would cause more animal distress.

PROJECT 138

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.

Word limit; 1000 words

Project Title	Mechanisms of Carcinogenesis
Key Words	cancer, carcinogen, environmental, metabolism, DNA
Expected duration of the project	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;
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 work of this project seeks to understand the mechanism of action of chemicals that cause cancer. The benefit of this knowledge is that it may help to identify new carcinogens, discover the unknown causes of existing cancers and to lead to the development of effective strategies for preventing cancer

It is clear from many scientific studies and clinical observations that environmental factors are important factors in cancer causation, but in many cases the causative agents remain unknown. We can learn a great deal about the cancer process by studying the effects of known carcinogens, e.g. those present in polluted air or food, and from this knowledge we may gain new insights into as-yet-unidentified causes of cancer, knowledge which is essential for devising effective strategies for cancer prevention.

Many carcinogens are thought to exert their effects by binding to DNA in cells, thus making it vulnerable to copying errors. A building block of DNA to which a carcinogen is bound is called an adduct. Most carcinogens only form adducts after the metabolic machinery of cells (the enzymes) has converted them to a reactive derivative. At the same time, other metabolic processes may act to detoxify the carcinogen (convert it to an inactive form).

By studying the formation of carcinogen-DNA adducts, we can learn a great deal about how carcinogens are activated, and what processes may be protective against such activation. The latter will help to develop strategies for cancer prevention. We can also learn the characteristics of tumour formation by a particular agent and understand the underlying mechanism(s) leading to cancer development in humans.

Thus, while we may suspect certain chemicals as being causative of certain cancers, humans are never exposed to single carcinogens in isolation. In order to gain more definitive proof of causation, examining the molecular events surrounding administration of a known carcinogen to an experimental animal can be compared with the events observed 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)?

The pursuit of the aims of this project will lead, it is anticipated, to a better understanding of the mechanisms of cancer causation of known carcinogens and the assessment of the carcinogenic potential of suspected carcinogens. The project will also lead to better understanding of the cellular processes critical to cancer development and has the potential to unveil strategies for intervention whereby the carcinogenic process can be inhibited or prevented.

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

Mice are the lowest vertebrate group in which the enzyme complexes for carcinogens have been studied and are comparable to those in humans. In addition, the inclusion of human genes in mice makes them a very relevant surrogate for human tissue. The methods of analysis that will be used are sensitive enough to permit use of minimal numbers of animals, the size of groups (3-5 animals) being dictated by requirements for reproducibility and statistical significance, rather that requirement for sufficient animal tissue. It is estimated that up to 3400 mice may be required for the proposed studies 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?

Animals will be administered carcinogenic, or potentially carcinogenic, doses of chemicals of interest because of known or potential human exposure to them. In most experiments the short-term effects of exposure (relevant to early stages in the carcinogenic process) will be studied. Thus animal suffering and distress will be minimal. Isolation of DNA from tissues and investigation of mutations in key genes will be used to define the molecular fingerprint of the carcinogens and to enable the predictability of short-term, non-animal tests for mutagenesis to be validated.

Application of the 3Rs

Replacement

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

Replacement

While many cell or cell-free culture systems can partially reproduce the effects in live animals, they rarely mimic accurately the balance between activation and detoxification pathways for carcinogens. This balance is crucial in determining exactly where and when a carcinogen will exert its harmful effects. While a great deal of useful information can be obtained from non-animal experiments, it is an essential for a full understanding of carcinogen mechanism for some experiments to be conducted in whole animals, in order to determine how processes in one organ or tissue affect the fate and effects of the carcinogen in another tissue, as well as determining which are the organs where tumours may develop and where they will not. Nevertheless, our research strategy is one in which we seek to use alternatives that do not involve animals (e.g. mammalian cells in culture) wherever possible and the symbiotic knowledge gained by a variety of experimental approaches means that animal use is kept to a minimum.

The work initially involves the detection and characterisation of interactions between carcinogens and DNA in non-animal experimental systems (cells in culture) . Extrapolation from these data to in-animal interactions of carcinogens and DNA requires additional factors such as route of administration, absorption and tissue-specific expression of enzymes that metabolise carcinogens to be considered which can only be mimicked in animal models. Thus limited animal studies are conducted subsequently in addition to cell culture studies and chemical studies to synthesis and compare the structures and properties of the synthetic products with those formed in animal tissues. Where human exposure is known or suspected, examination of human tissues (e.g. tumour and adjacent normal tissue from patients) can be compared. Thus a comparison of effect in animals and animal cells in culture, and human cells in culture, will lead to a better prediction of effects in humans.

Reduction

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

Reduction

All genetically modified mouse lines used in the proposal have been engineered to investigate specific mechanisms relevant to study carcinogen metabolism, thereby significantly reducing the number of animals required. Similarly, some mouse lines have been humanised for the expression of genes which makes the model more relevant for human risk assessment, thereby reducing the overall number of animals required for investigation.

The majority of animal experiments follow the same study design. Most treatment regimens will follow established methodologies thereby considerably reducing the number of animals required for the proposed experiments. The number of animals required for each experiment has been kept as low as possible to obtain statistically valid and biological meaningful results, i.e. usually 3-5 animals/group based on

previous studies. By coordinating different administrations where possible, fewer control groups will be required. The majority of the proposed animal experiments are designed in such way that they will allow multiple analyses simultaneously in the same animals, thereby minimising the number of animals required for 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

Mice are the lowest vertebrate groups in which the enzyme complexes for carcinogens have been studied and are comparable to those in humans. Animal suffering will be minimised by treating with sub-acute doses and employing short-term follow-up. The proposed experiments will focus on early cellular responses/alterations important in the carcinogenic process. Compared to previous experiments this does not involve animal studies that involve tumour development and thus presents a significant refinement of the previous methodologies used.

PROJECT 139

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.

Word limit; 1000 words

Project Title	Molecular analyses of caspases in infection and immunity
Key Words	infection, inflammation, caspases, IL-1beta, inflammasomes
Expected duration of the project	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):

The immune system protects us against infections but, if left unchecked, it can also cause damaging inflammation. Many molecules are involved in regulating anti-infection responses and inflammation. We will study enzymes called caspases which are molecular scissors that cut other molecules. In addition, we will study the molecules that caspases cut. In my laboratory, through the use of complementary computational, biochemical and genetic experiments, we have discovered several new molecules that regulate immunity & inflammation. However, the functions of many of these molecules remain unknown. In this project, we want to investigate the roles of caspase enzymes and related molecules during infection and inflammation. Our work on mouse-models of human diseases will inform and be informed by our laboratory-based work.

The main aims of this project are:

1. Breed and maintain genetically unaltered and altered mice

2. Evaluate immune responses to microbial infection

3. Evaluate immune responses to inflammatory 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)?

Our immune systems naturally protect us against many pathogenic microbes, including bacteria, fungi, viruses and parasites. Therefore, a better understanding of the basic biology of these processes will help tackle the scourge of infectious diseases which are a major human-health burden worldwide. Deregulation of immune responses and caspases is linked to hereditary fever syndromes in humans

characterised by skin inflammation, rashes and fever that can be triggered by cold temperatures (called cryopyrinopathies), and related inflammatory syndromes. Further, many non-communicable diseases such as diabetes, atherosclerosis, types of arthritis, colitis and cancer are also linked to excessive inflammation. Blocking inflammation with drugs improves clinical symptoms in many of the above conditions, and medications are approved in >70 countries. However, as they have side-effects, there is a need to improve existing drugs and develop new ones. Importantly, initial studies that led to the development of new drugs were carried out successfully in mice, which points to their usefulness in infection and inflammation research. In addition, transgenic mice that mimic human hereditary diseases have helped understand these diseases better. Similarly, we will use established mouse-models of human diseases for our studies described here. In summary, this project should provide new insights into the mammalian immune response in infectious and inflammatory scenarios, and help in the development of new therapeutic interventions in the future

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

~5000 mice/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?

Infections in mice are often self-limiting in normal mice, but could lead to mild to moderate disease in genetically altered animals. Disease may manifest as weight loss, changes in diet, posture and movement. The highest severity in our studies will be moderate disease and humane end-points will be used to avoid unnecessary pain and distress to experimental animals. At all stages, trained staff will monitor animal welfare and ensure that severity does not exceed Moderate levels. Genetic alterations may result in unanticipated responses, in which case advice from the NVS/NACWO will be sought. Experiments will typically end at the peak of infection or inflammation or earlier. Animals will be killed using humane methods

Application of the 3Rs

Replacement

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

Replacement

The response to infection is a complex multi-organ one and involves many different types of tissues. While some of this can be studied by cell-culture methods, currently available cell-line models do not faithfully reflect the behaviour of cells within the

natural environment of tissues. Similarly, newer organoid-based systems have drawbacks such as their inability to capture multi cell-type interactions.

Furthermore, complex situations, such as response to infection in the intestine in the context of normal gut-microorganisms or whole-body inflammation that affects different organs differently, cannot be studied *in vitro*. Use of animals for these studies is therefore scientifically justified.

Reduction

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

Reduction

Wherever possible we will use cell lines or recombinant proteins for our studies, and avoid obtaining cells/proteins from mice i.e. we will avoid using animals only to provide material for routine work. Colony management will ensure breeding on demand to avoid surplus animals from over-breeding.

Relying on principles of the 3Rs, the minimum number of total animals required to obtain statistically significant inferences will be used. While comparing treatment-groups, we will carry out as many conditions as possible in a single experiment to avoid repeated use of untreated/vehicle-treated/uninfected control groups.

Wherever possible we will use light-emitting reporters that allow non-invasive optical imaging of the same animal throughout the experiment. This avoids the need for additional animals that would have otherwise been required at various stages of 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

The mouse has a proven track-record of providing valuable insights on mammalian, including human, immune responses. Furthermore, there is a large collection of reagents and genetically altered animals in the field which will help our studies.

We will keep ourselves updated with latest literature on optimisations and refinements of reagents, treatments, handling of mice etc. Experimental end points will be clearly defined. Animals will be housed in groups where possible, with appropriate environmental enrichment and fed according to current institutional best practice. Animals may be killed by humane methods that allow harvesting of blood and tissue for studies.

PROJECT 140

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.

Word limit; 1000 words

Project Title	Production and maintenance of genetically modified zebrafish
Key Words	Zebrafish breeding husbandry genetically modified
Expected duration of the project	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):

Zebrafish are a commonly used animal system to study gene function and how these genes are altered in human disease. As zebrafish genes, organs and tissues share many similarities with humans, what we learn in zebrafish helps our understanding of how the genes work in humans. Many of the changes to genes which cause thousands of different human disorders in adults and in children are currently poorly understood. By studying similar genetic changes in zebrafish we can begin to answer which genes are important or not in human disorders. This licence allows experienced animal technicians to raise and look after different types of zebrafish which are used for research into genetic 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)?

REDACTED The benefits of this licence will be supporting research into the fundamental mechanisms of human biology and disease. This is worthwhile because it provides the basis for our understanding of human and animal life, as well as for the development of therapeutic potential to manage and treat human disease. Specific examples include, but are not limited to, identifying genes which cause skin cancer, identifying genes responsible for abnormal development of the eye, and understanding mutations that result in failure of brain development in children.

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

Zebrafish. Up to 42500 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 fish will not experience any adverse effects from the changes made to their genes. As some of the genes changed are related to human disorders, a minority of fish may have problems which relate to the function of the gene. This depends on the gene in question e.g. the fish with the genetic changes related to skin cancer will have a higher rate of developing skin cancer, while fish with genetic changes related to human eye conditions may have a higher rate of eye problems. Most fish will experience no adverse effects. A minority will experience minor, temporary discomfort, such as cutting off a small piece of a fin under anaesthetic (which then grows back naturally). A very small minority (likely to be less than 1%) will experience medium adverse effects, such as developing skin cancer. Any zebrafish which is approaching the limit of allowed severity will be humanely euthanised before the limit is exceeded. All zebrafish will be humanely euthanised at the end of the experiment if deemed to be suffering or when they reach their maximum healthy lifespan.

Application of the 3Rs

Replacement

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

Replacement

Animals are required because there is no non-animal system that can provide us with the insight into how these genes work within the context of a living vertebrate system.

We try to only use zebrafish after first learning as much as we can using computer models, cells in dishes and simple animals like worms.

Most of the experiments will be carried out on zebrafish larvae that are less than 5 days old and not considered developed enough to be protected animals.

Reduction

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

Reduction

Before we make a new genetic line of zebrafish we make sure it does not already exist somewhere in the world.

We breed only the animals we will need to use or to increase or replace our breeding stocks.

We keep a database to keep track of all the fish.

If a genetic line of zebrafish is not being used, we will freeze the sperm and keep it until the line is 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

Unlike most other animals used in the laboratory, zebrafish embryos are externally fertilised and are transparent during early development. This allows for imaging of cell and tissue development in a living animal without harming it.

Pain will be controlled by using anaesthesia.

We will not keep old or sick fish.

We have a highly skilled staff that is focused on improving their skill set to refine experimental procedures and handling to reduce stress to the fish.

We will stay up to date and well informed on all animal welfare developments relevant to zebrafish and will communicate this information to everyone working with the fish.

PROJECT 141

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.

Word limit; 1000 words

Project Title	XENOPUS EMBRYONIC DEVELOPMENT
Key Words	Xenopus, Embryo, Development, Egg, Sperm
Expected duration of the project	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):

Summary: This project proposes to harvest eggs and sperm from adult frogs in order to examine the formation of frog embryos in frog spawn and discover processes and mechanisms that are used in frogs, but are generally conserved in vertebrates and therefore also informative and relevant for understanding normal formation and the repair of tissues and organs in humans.

Background and scientific question: The tissues and organs in our bodies are formed in the embryo in processes that are regulated by our genes, which are the keepers of our hereditary information. Some genes have already been identified as having some role in regulating the formation of the embryo; however, we know much less about what their precise role is at different steps in the process and how these genes interact with each other to coordinate the formation of tissues and organs. We find equivalent genes both in humans, frogs and many other animals. Where we have discovered in the past more details about the mechanisms of how some of these genes function in frog embryos, some of these mechanisms were later found to be similar if not identical in humans. Furthermore, these mechanisms are found to be not only important for the initial formation of the tissues and organs in the embryo, but also later in the maintenance and repair of these tissues in the adult. We can therefore be confident that what we discover in frog spawn is likely to be useful for understanding human embryos and maintenance and repair of human tissues.

Reasons for studying animal embryos: The interaction of genes in the embryo is complicated because of the three-dimensional complex structure of the embryo itself. In order to understand the complicated interactions of genes they have to be studied in this three-dimensional landscape of the embryo. Some aspects of the function of these genes can be studied in isolated tissues, which allows a reduction of whole-

animal experiments, however, discoveries from artificially isolated tissues will always have to be verified in the natural three-dimensional setting of the embryo, before they can be relied upon.

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

Reasons for studying frog embryos: We want to study general processes, which are expected to be conserved from frogs to humans. By studying these processes in frog embryos we minimise potential animal suffering, because frog embryos develop in frogspawn completely apart from adult frogs, as they do in ponds in the wild. The adult frogs are therefore hardly disturbed. Embryos of animals that are much closer relatives of humans, such as mice, develop inside their mother. Studying mouse embryos would therefore be more intrusive and more distressing for the adult mouse mother. Potential benefits for humans: The interaction of genes that regulate the formation of tissues and organs in embryos is to a large extent very similar in frogs and humans. The same processes also tend to be used in adults to repair and maintain tissues in our organs. If the genes are defective or if the processes that they normally control are interfered with, an illness can develop. If these defects occur in the embryo, birth defects develop; if however the defects only become apparent in the adult, they can lead to diseases such as cancer. Studying and understanding the function of genes in frog embryos will therefore ultimately help understand human diseases and contribute towards finding future treatments for these human diseases.

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

REDACTED 250 normal adult females and 50 males over five years and up to 1000 genetically altered tadpoles.

In the context of what you propose to do to 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 administer fertility hormone treatment to adult frogs to increase egg and sperm production to produce developing fertilised eggs for observation and experimental study. The fertilised eggs (embryos) do not have the capacity to undergo pain, suffering or distress before developing a functional nervous system (at around stage 46, after which they may experience mild discomfort if their DNA has been experimentally altered). There are no expected adverse effects from the hormones which mimic the process in nature – only producing an increased number of eggs. We will use some genetically altered frogs which have undergone minor changes to their DNA. The fertilised eggs/offspring are not expected to show anything other than a mild level of discomfort. Small amounts of tissue may be taken for DNA analysis under anaesthesia. At the end of the study the animals will be humanely killed and some will undergo tissue analysis.

Application of the 3Rs

Replacement

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

Replacement

Investigating general principles of embryology and developmental biology of backboned animals on frogs represents replacement of more invasive and potentially harmful procedures on animals that are more related to humans but who like us, bear their embryos internally. Cultured stem cell experiments can only replace some whole-embryo experiments in research and are technically much more difficult to manipulate.

Reduction

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

Reduction

We are reducing the overall use of frogs by coordinating collaborations and arranging sharing of produced eggs and sperm among different research and teaching requirements. We also accustom our frogs carefully to the hormone treatment so that many of them can undergo this minor treatment several times, thereby reducing the overall number of animals needed in total.

Refinement

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

Refinement

Studying frog embryo development will provide information which is likely to apply to humans. The frogs are housed according the Home Office Code of Practice and are provided with tubes and artificial lily pads for a sheltered, comfortable and stimulating living space.

There are strict criteria for the re-injecting of frogs which have been agreed with the vet and a maximum number of times the frogs may be injected over their entire lifetime.

This contributes to their increased welfare, fertility and quality of the produced eggs and sperm.

PROJECT 142

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.

Word limit; 1000 words

Project Title	Tissue and tumour adaptations to suboptimal environments
Key Words	Early-life, brain, growth, tumour, metabolism
Expected duration of the project	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):

Growing tissues are able to adapt to stresses in suboptimal environments, such as limited amounts of nutrients. This type of stress adaptation is critical for tissues to grow and function healthily from embryo to adult. Unfortunately, similar adaptations are also used by tumours to promote their uncontrolled growth during cancer. It is not yet clear which genetic and metabolic changes are most important for tissues to adapt to stress, nor how similar the adaptations are between normal and cancer tissues. The overall aim of this project is to discover the genetic and metabolic adaptations that are important for the growth of normal and cancerous brain tissue in the presence of only limited nutrients or a poor blood supply. We will compare adaptations between brains, brain tumours and several other organs in order to pinpoint their different vulnerabilities to stress. In the context of brain tumours, drugs targeting stress adaptations will be tested as potential anti-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)?

There are three potential benefits likely to derive from this project. First, advances in fundamental knowledge via the identification of genes that regulate the growth of normal tissues and tumours. This will help to pinpoint the vulnerabilities of different growing organs to metabolic disease and cancer. Second, the development of improved experimental methods for studying metabolic disease and cancer, which can then be shared with other researchers. And third, the identification of drugs or combinations of drugs that, in the longer term, may be developed for treating human cancer and metabolic disease.

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

Mice. 2000 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?

The expected adverse effects on the wildtype and genetically altered mice in this project are a moderate loss of body weight and the formation of small tumours under the skin. Genetically altered mice may also develop other adverse outcomes, which will depend upon which gene is being altered. Many of these adverse effects will present at embryonic and fetal stages, and are not usually compatible with continued life. The maximum expected level of severity for any procedure conducted within this project is moderate and follows strict guidelines in accordance with the Home Office. At the end of procedures, animals 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

It is necessary to use animals to study the growth of organs and tumours because this process is subject to regulation inside the body from numerous other tissues and circulating hormones. It is not yet possible to recreate this level of biological complexity in a test tube or petri dish. Therefore, studies in the context of the whole intact animal are needed to identify metabolic and anti-cancer therapies that will ultimately be meaningful for human clinical studies. The proposed project also makes extensive use of non-protected animal alternatives such as the insect *Drosophila* and mammalian cells grown in a Petri dish.

Reduction

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

Reduction

Wherever possible, we will always use non-protected animal alternatives. Alternatives that we use extensively include mammalian cells grown in a Petri dish and also the insect *Drosophila.* The numbers of mice needed to be bred for this project will be reduced by sharing mice with other researchers. Experimental designs will use the minimal number of mice required to obtain statistically significant data. We will maximise the amount of data obtained from each mouse by studying multiple tissues, by analysing them using several different methods. We review our breeding

strategies regularity and cryopreserve any strains which are not under current investigation.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 share similar genetics, metabolism and physiology with humans and so are an appropriate mammal for providing insights into human diseases. They are not a primate or an endangered species. Mice also have well-established laboratory procedures and advanced genetics, which both help to expedite research progress. 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.

PROJECT 143

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.

Word limit; 1000 words

Project Title	ENVIRONMENTAL CHALLENGES DURING DEVELOPMENT IMPACT ON POSTNATAL HEALTH AND BEHAVIOUR IN RODENTS
Key Words	
Expected duration of the project	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):

The health status of both parents, mother and father, can influence lifelong health of future generations. The period around conception, a time when pregnancy is not yet recognised, is critical not only for establishing a pregnancy but also for optimising offspring health. This is of high clinical relevance given that 1 in 7 couples experience difficulties to conceive and seek fertility treatment requiring manipulations around conception. The overall aim of this study is to understand better how development is shaped by external cues experienced around conception to inform clinical scientists and advice given to patients. This new knowledge will also inspire us to develop and test safe and effective strategies to protect lifelong health of future generations. Collectively, our aims fit well with the most recent NICE recommendations for fertility 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)?

Previous work has confirmed that most, if not all, bodily systems are affected by different challenges experienced around conception, a time before pregnancy is recognised. This project will clarify further how the environment the very early embryo finds itself in can shape its future health. For example, understanding which molecular factors induce embryonic responses and how they interact will lead to improved culture conditions for human ART to ensure the health of IVF children. It will also provide clearer information about the importance of parental health and diet when trying to conceive, and the implications of becoming pregnant if sick with an infection. To develop safe and effective ways to protect the health of future generations we need to establish how the parents' physiology responds to challenges around conception and how these responses are communicated to the

developing embryo. This knowledge will benefit farm animal production scientists and inform clinical scientists for health purposes and advice given to couples trying to conceive.

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

9650 mice and 1030 rats of all ages 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 are purposely avoiding severe challenges to the animals and do not expect serious, long-lasting adverse effects. The level of severity of our proposed procedures is mostly mild with a few exception at moderate level such as short-term immune modulation and minor surgery which may result in a brief period of mild discomfort. All the animals will be killed at the end of the study and tissues will be sampled for analysis.

Application of the 3Rs

Replacement

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

Replacement

Ethical constraints forbid invasive human experimentation targeting the periconceptional period in healthy parents and/or fetal sampling during pregnancy. It is also unethical to assess directly intervention strategies without first finding out how these treatments may be affecting the developing conceptus in humans. In humans, it would take years to find out how periconceptional challenges affect the health and disease susceptibility of the offspring in later life. Using mice with their short generation interval will allow to assess the long term effect of the treatments within a short time frame. Our rodent studies may inform and influence advice given to parents to be and help develop measure to prevent adverse health outcomes for offspring as a consequence of poor periconceptional environment.

Reduction

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

Reduction

We use statistical power calculations based on our past studies to determine the ideal number of animals to use in our experiments. The experimental design that we will use will allow publication of the results according to the ARRIVE (Animal

Research: Reporting of *In Vivo* Experiments) guidelines. This will maximise information published and minimising unnecessary duplication of 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

We have chosen the rodent model (mainly mouse) as a well-established model for perinconceptional environmental programming and consistent with human observational data. In addition to ethical constraints prohibitive of human experimentation the rodent models' short lifespan facilitates a lifecourse approach as well as transgenerational assessments. In addition, a large variety of genetically modified mice are available to study human disease that is still lacking in other animal models.

PROJECT 144

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.

Word limit; 1000 words

Project Title	Optical imaging of cardiac electrophysiology
Key Words	
Expected duration of the project	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;
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):

Sudden cardiac death is a major cause of premature mortality worldwide and is commonly caused by abnormally fast heart rhythms known as ‘ventricular arrhythmias’. For some people these dangerous rhythms can be the first presentation of heart disease, often with devastating consequences. These rhythms occur when the electrical signal which governs the heart beat becomes disrupted between cells in different areas of the heart. How and why this happens remains unclear, meaning that treatment options and methods to identify those at risk are extremely limited.

Research studies in isolated heart cells has led to a clear understanding of their electrical function (electrophysiology), but ventricular arrhythmias only occur in the whole heart in which understanding electrical function is much more complex. In this study, we will use state-of-the-art imaging techniques with carefully selected genetically-modified animal models to address some fundamental issues in cardiac electrophysiology:

1. How does the electrical behaviour of a single heart cell differ when it is connected to other cells within the heart?
2. How do other cells within the heart influence it’s electrical behaviour?
3. How do nerves supplying the heart influence it’s electrical behaviour?

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

The goal of this research is to understand how electrical imbalances arise to produce life-threatening fast heart rhythms in the whole heart. By understanding these mechanisms, we aim to develop new strategies to identify which patients are at risk

of these fast heart rhythms, as well as identifying novel targets for treatments which will prevent these rhythms from occurring, thus reducing the impact of sudden cardiac death.

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

In this study, we expect to use 6000 mice over a 5 year timescale.

In the context of what you propose to do to 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 expect very few adverse effects to be experienced by the animals included in this study. The genetic manipulations required are designed to optimise the experimental output (rather than inducing disease) and have not been reported to have any adverse consequences for the animals. Both normal and transgenic animals will be cared for in our animal unit. Some animals may experience mild distress during blood or tissue sampling required for genetic characterisation. All animals will be humanely killed and their hearts will be removed for use in experiments.

Application of the 3Rs

Replacement

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

Replacement

Studies of whole-heart arrhythmia mechanisms using human tissue are extremely difficult for several reasons. It is not possible to induce life-threatening arrhythmias *in vivo* for research purposes and so these studies must be performed *ex vivo*. The only source of potentially viable *ex vivo* human myocardium is that from hearts explanted at the time of cardiac transplantation. In order to work towards future replacement, and to ensure optimal translation of our lab's work, the applicant is leading a local initiative to secure myocardial tissue from explanted human hearts for use in research. This project is currently in a pilot phase, and we have been successful in securing myocardial tissue from 5 explanted hearts, but viability for experimentation has been variable. We are currently working to optimise this process. Even once optimised, cardiac transplants are relatively infrequent, not all patients consent and this approach only yields end-stage failing hearts, without providing any normal hearts to serve as experimental controls. Even accepting these limitations, there are then considerable challenges in interpretation of any results given the marked variation in age, medication, underlying pathology and comorbidity of any obtainable human heart tissue. The applicant is committed to developing this tissue source in future, but at present it should be regarded as an important way to

confirm some selected experimental results in human tissue, rather than a realistic option for replacement of animal use in this type of experiment.

The vast differences in structure and physiology of the cardiovascular system in invertebrates mean that use of non-protected animals is not an option for this work. Thus, in the absence of any suitable alternative, vertebrate animal models will be used to study ventricular arrhythmia mechanisms.

Reduction

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

Reduction

The minimum numbers of animals possible will be used in each of the sub-projects involved in this study. We have appropriate expertise in experimental design and statistical analysis, and for all planned experiments power calculations have been made to establish the minimum number of animals required to obtain statistical significance between variables. In cases where the predicted number is high (e.g. >10 animals / group) we would redesign or exclude the experiment. In all cases the minimum number of animals will be used to achieve statistical relevance and meaningful results. In this study we have also included mice with genetic manipulations which will reduce the overall number required for experiments.

To minimise the number of animals and the severity of the procedure while maximising the information from each experiment, heart function will be examined *in vitro* after terminal anaesthetic. Standardisation of the conditions used *in vitro* reduces inter-measurement variation and therefore keeps numbers as low as possible. We will continue to seek, review and incorporate alternatives where possible 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

We have selected the most appropriate experimental models based on our knowledge and extensive experience of the field. Mammalian hearts are required to give any relevant insight into human cardiac electrophysiology. Mice are suitable for the study of cardiac conduction, with broadly similar cardiac activation as seen in humans. They are also suitable for generation of transgenic models. In this study we have included mice with genetic manipulations which will increase the yield from our

experiments and thereby represent a refinement in comparison with the use of hearts from normal animals.

The protocols included in this study are either mild or non-recovery and we are guided by the principle that the protocol with the least severity should always be used. We will work to minimise and mitigate any possible adverse effects wherever possible. This can be done by ensuring all animal handling and procedures are only carried out by experienced staff, that appropriate anaesthesia and analgesia are used, and that appropriate injection volumes are selected and administered by the least painful route possible.

Our staff are highly experienced and they will oversee the day to day maintenance of animals on this licence. The NACWO/NVS will be contacted if any animals are seen to be in distress and an appropriate treatment will be decided. Animals in distress will be examined and treated as soon as possible, included euthanasia if appropriate.

The animals will be housed in a modern, well equipped facility. There will be adequate housing in well ventilated, humidity controlled rooms with circadian rhythm programmed lighting. The NACWO/NVS will be consulted to ensure a continuing high health status and in continuing improvement of housing and environmental enrichment. Health status and most recent screening results will be sent before the animals are imported to assess their housing needs. After arriving, the NVS will assess their health and regular examinations and health screening will be carried out. Standard barrier facilities and protocols will minimise risks of infection from environmental factors. Where appropriate, animals will be kept in filter top caging.

PROJECT 145

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.

Word limit; 1000 words

Project Title	Macrophage gene regulation in vascular disease, its prevention and resolution
Key Words	Macrophage, inflammation, cardiovascular, haemorrhage, resolution
Expected duration of the project	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;
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.

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):

Coronary heart disease, the major cause of heart attacks, is contributed to by tiny bleeds within the walls of vessels supplying the heart. We have already made significant progress in identifying protective genes that limit this damage. (1) We want to understand the roles of these genes in vascular disease in live animals. (2) We want to understand the roles of these genes in clearing up after inflammation or bruise in live 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 will know whether a common anti-diabetic treatment is likely to reduce cardiovascular disease by acting directly on blood vessels, rather than only via glucose. We will know which white blood cell genes regulate clear-up and recovery after stroke, heart attack and other forms of bleed and inflammation, e.g. surgery.

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

Mouse. 200 per year 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?

Transport to a specific cull room and euthanize using a Schedule 1 method (i.e. free of pain and distress). General anaesthesia, details as advised by vet, will be used to cover subcutaneous injections and implants. One of the models use subcutaneous injections in the femoral triangle, superficial to and not into the femoral artery and vein. This is an anatomical model of the subcutaneous femoral hematoma that

occurs after femoral arterial access under anticoagulation. The leukocytes are injected into the same anatomical site for comparison. Preliminary experiments under a matrigel license indicates this is well tolerated, as a refinement of the matrigel model, which was carried out at exactly this site. The site has been discussed with vet and NACWO. All other sites of injections and implants will be as initially indicated by vet and/or NACWO: the site is not material to the experiment. As a matter of further reiterative reduction, we may transfer the 'femoral' subcutaneous space injections to subcut injections at other sites. This would have the advantage of facilitating a larger number of datapoints per mouse (up to 24, Appendix). we would wish to be sure that no local side-effects derive from the injections before scaling up to large numbers per mouse though. The case for general anaesthesia during implant insertion is unarguable. For injections, it is arguable. However, light inhalational anaesthesia will be preferred for comfort of both mouse and operator during what are fairly complicated and precise injections. In addition to it allowing more accurate anatomical placing of injections; and eventually possibly also larger number of injections whilst maintaining anatomical accuracy (eg regular spacing). It does also assist operator health and safety by reducing the risk of mouse bite. Bone marrow transplantation, which involves: Change in conditions (acidified and antibiotic treated water); transport to irradiator and back; effects of irradiation (primarily transient bone marrow loss and immunocompromise, but not bleeding, nor gastrointestinal symptoms, nor hair loss). Mice are observed daily, and weighed weekly; tail vein injection. Not more than one tenth of a millilitre is injected, by one person trained and validated, and mice are observed for symptoms. Genetically harmful strains (knockouts). We have not seen baseline clinical symptoms with the knockouts that we study. The model of human high-blood cholesterol and heart disease becomes symptomatic on high fat diet and we restrict to low fat diet. Mice are observed weekly. The expected severity is mild for the majority of the mice. The endpoints are: Animals which fail to recover fully from an anaesthetic will be promptly culled. Any animal showing any one of the following signs of distress will be promptly culled: -Intermittent hunched posture -Marked staring coat -Intermittent abnormal pattern of respiration -Intermittent wetting of fur under chin -Intermittent tremors -Little peer interaction -Subdued provoked behaviour -Chronic weight loss of 20% (over any time period), or less severe weight loss (10%) over 1 week. Any animal judged to be in distress that is more than mild, for any other reason, will be culled. Mice are normally culled if there are any detectable clinical symptoms before these worsen, unless recovery is thought likely by the NACWO or NVS.

Application of the 3Rs

Replacement

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

Replacement

We have already used up the non-animal alternatives and now have some specific predictions about what our findings mean for whole animal physiology.

We have a serious ongoing programme of in vitro work. This falls into a number of overlapping modalities.

In our major limb, we investigate inflammation based on experiments with leukocytes from normal human volunteers. We spend a day each experiments simply isolating and purifying the relevant white blood cells (leukocytes). These are the precursors of inflammatory cells. We will then experimentally treat them and determine the effects on function. Typically, we inhibit one gene in a highly precise and targeted manner (using si-RNA or pharmacological reagents or both), and then measure levels of other genes. This reveals the interconnections between genes in inflammation.

The second platform comprises cell lines. We use human cell lines for measurements that require large quantities of material, eg to study low abundance proteins, weak or infrequent interactions, or when using insensitive measurements. With human cell lines we also make permanent gene edits to obtain total removal of a gene. We also carry out experiments in parallel with mouse cell lines. These have advantages largely parallelling those of human cell lines, and also allow human-mouse comparisons. Cell lines have serious drawbacks however. They typically originate in cancers, leukemias in the case of leukocyte cell lines. They are often more representative of cancer than of inflammation. Indeed, our cell lines have serious perturbations of the signalling pathway that we study.

The third platform is the use of bred mice, but without any experiment during life. The genetically modified mice have a gene missing, so-called knockout. These can be used to more precisely define the quantitative role of any given gene. This can make a significant difference. We have previously inferred a second pathway independent of our gene of interest, and now understand that this second pathway is essentially absent, and the data reflect only incomplete inhibition. That inference was only achievable with study of cells from complete knockout.

With this, the knockout and control mice are simply bred, live and are then culled by Schedule 1. Their inflammatory cells are then grown and compared (with and without gene). The inflammatory cell precursors are extracted from the bone marrow and representative inflammatory cells are grown. This is a far more robust method of determining the roles of genes than the methods with human blood inflammatory cells. This approach also allows an estimation of mouse-human similarity. It therefore reports on the likelihood of any in vivo experiments being translatable to patients, before they are carried out.

Only the fourth platform involves any work on live mice. This is highly integrated with the other three and the size of experiments is kept as low as possible by using this aspect for confirmation of in vivo relevance of key findings.

Reduction

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

Reduction

We avoid any procedural losses and have performed power calculations based on our published data. The models are refined so that scientific strength is optimal with low numbers and minimal clinical severity. We exclusively use in-house inbred mice. Where genetically modified, we use littermate controls, or at most the F1 generation from littermates. This matches the genetic background precisely. The controls are moreover matched as closely as possible to time, diet, caging conditions, operator. Where procedures are carried out each test mouse (or cage of test mice) is matched to controls. There is use of F1 generation from littermates, because this minimises recombination and genetic drift, but at the same time avoids breeding a large number of excess heterozygous mice that would add to total numbers culled. Typically, analyses are blinded where practically achievable. With the resolution experiments, the contralateral side is used as an 'internal control' or very tightly matched control limb. Where in vitro experiments are conducted using primary isolated cells, each test well is matched to an immediately adjacent control well. Vehicle controls are used throughout, or non-targeting siRNA or empty plasmid where applicable. Within the overall project, each finding is cross-validated using a number of alternative and independent methods. Taken together, these approaches minimise confounders and also save on 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

Mice are the most appropriate species because:

1. they are the neurologically simplest organism that is complex enough to model the pathophysiology
2. they are the only organism that supports routine gene knockouts

The models have been refined as follows:

Hematoma and leukocyte resolution – small injection volume (in line with LASA guidelines), swift procedure, non-irritant material, well tolerated to date.

Atherosclerosis – early cull point and low fat diet. This has been used before to obtain technically equivalent data to different questions.

Bone marrow transplantation – fractionated and lowered radiation dose, antibiotic cover, older age of procedure (12-14 weeks), prompt bone marrow injection, injection of large number of bone marrow precursors. We have used this protocol to obtain interesting data (manuscript in preparation).

Unless there are exceptional circumstances, animals will not be housed singly.

All animals will be housed in groups where possible, with appropriate environmental enrichment and fed according to current institutional best practices.

Animals will be bred in an ultraclean specific pathogen free facility and all experiments will be carried out in a specific pathogen free facility.

In addition to the daily monitoring by animal care staff, animals are routinely monitored weekly by clinical inspection and any animals at high risk (1 week post-procedure) are monitored daily by clinical inspection

PROJECT 146

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.

Word limit; 1000 words

Project Title	Trypanosomiasis; pathogenesis, diagnosis and treatment
Key Words	Trypanosome, pathogenesis, chemotherapy, virulence, blood-brain barrier
Expected duration of the project	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;
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):

Trypanosomiasis is a parasitic infection affecting both humans and animals. The disease causes significant social and economic impact through mortality, morbidity and loss of livestock. Although trypanosomiasis has been recognised for centuries, many aspects regarding the development of the infections and the factors defining the virulence of the parasite remain unknown. Furthermore, only a handful of drugs are currently available for treatment of trypanosome infections, all of which must be given by injection and most are associated with adverse side effects. The primary goals of this research are to investigate the development of trypanosome infections, discover potential drug targets and develop improved therapeutic strategies to combat the disease using a mouse model of the 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)?

Reaching the goals of this project will not only increase the current knowledge available regarding trypanosome infections but may also provide information applicable to other infection or conditions that affect the brain. In the longer term the findings could lead to improved chemotherapy or disease interventions. This would greatly benefit, both socially and economically, the developing countries where this disease is endemic.

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

A well-established mouse model will be used to closely mirror the various stages of trypanosome infection. During the 5-year licence period requested it is estimated that a maximum of 8000 mice will 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?

This project involves infecting mice with trypanosomes and monitoring how the infection progresses in the animal and the effects of drug intervention this may lead to signs of illness in the mice. In the majority of cases the procedures may induce mild adverse effects such as the transient pain associate with injection or periodic mild clinical effects of the infection, such as the development of a rough / stary coat or lethargy. In some cases the mice could develop more moderate clinical signs including an altered gait or flaccid paralysis of one hind limb. In a few cases more severe reactions including complete flaccid paralysis of the hind limbs may be induced. Although this severe clinical sign may develop the majority of the animals exhibiting this complete hind-limb paralysis remain bright and inquisitive and show normal social behavioural interactions. However, if this occurs the animals will be maintained for a maximum of 72 hours and food and water provided in easy reach. If no signs of clinical improvement are noted during this period the animals will be humanely killed. It is also possible that a few animals may become moribund, if this does occur the animals will be humanely killed. Extensive experience of this infection model and a familiarity with the techniques used, allows us to recognise quickly any unexpected adverse signs. All mice will be closely monitored throughout the procedures and endpoints implemented as required All animals will be sacrificed on completion 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

Trypanosomes can be maintained in tissue culture and this technique will be used to create genetically modified parasite lines to study disease virulence and in initial drug investigations. However, trypanosome infection cause changes in multiple interconnected systems and organs within the host and neither the development of the disease nor the ultimate success or failure of potentially useful treatment strategies can be investigated fully in the isolation of an *in vitro* system. For example, when the effects of trypanosomes were examined using a tissue culture model of the blood-brain barrier no lasting harm to the integrity of the barrier was detected. When this was investigated this using a mouse model of trypanosome infection a progressive increase in barrier impairment was associated with disease development. This clearly illustrates a disparity between the mechanisms at play in tissue culture models and animal models of this disease.

Reduction

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

Reduction

Statistical support is easily accessible and advice will be acquired when designing new experimental approaches. If appropriate factorial designs will be followed to ensure that the greatest amount of information using the lowest number of animals is achieved. In addition, where possible, experimental groups of animals will be used to fulfil two objectives, *e.g.* pathology or gene expression studies can be performed using material harvested from animals assigned to *in vivo* imaging 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 most suitable laboratory animal to use in this system as trypanosome infections can be manipulated to reproduce each of the disease phases that are essentially similar to those found in both human and animal trypanosomiasis. In addition, the mouse provides a standardised animal with a wide range of analysis reagents and genetically altered research lines available.

Extensive experience in the use of this mouse model provides familiarity of handling and maintaining infections and in the consistent induction of the various stages of the disease. This also results in a reduction in the severity of the procedures performed and in the number of animals required to achieve the goals of the experiment. To minimise the impact to welfare all animals will be closely monitored throughout the procedures and supportive treatments or interventions implemented as required.

PROJECT 147

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.

Word limit; 1000 words

Project Title	Tumour-bone interactions in skeletal malignancy
Key Words	Cancer, bone, multiple myeloma, prostate cancer
Expected duration of the project	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;
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):

Cancer-associated bone disease is a major cause of morbidity and mortality for those patients with multiple myeloma, or with malignancies that commonly metastasise to the skeleton, including prostate cancer. Once tumours are present in bone, they are largely incurable and treatment is purely palliative. Interactions within the specialised bone marrow microenvironment promote both tumour growth, and the development of the bone disease. It is only by increasing our understanding of these complex mechanisms that we will identify new therapeutic targets for the treatment of these devastating malignancies.

The overall objective of this project is to increase our understanding of the mechanisms involved in tumour growth within bone and the associated bone disease, in order to develop new therapeutic approaches. We have identified a number of factors that may play a role in tumour growth within bone, or the associated bone disease, including novel roles for obesity and fat cells, which we have found to increase tumour growth within bone We propose to use murine models of cancer-bone disease to investigate (i) tumour-derived factors, (ii) host-derived factors and (iii) novel therapeutic approaches.

What 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 two major short term benefits. Firstly, it will increase our understanding of how tumours grow within bone and how the destructive bone disease develops. Secondly it will test new approaches to reduce tumour burden and/or bone disease in multiple myeloma or prostate cancer bone metastases. In the long-term, following clinical evaluation, this project has the potential to result in the

development of therapeutic approaches that can be tested in man. In addition, our research into obesity has the potential to lead to advances in effective dietary interventions or lifestyle changes. Those that will benefit from this include patients with multiple myeloma or prostate cancer, scientists interested in tumour and/or bone biology, the pharmaceutical industry and clinicians, in particular oncologists and bone biologists

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

We propose to use approximately 8,500 mice over a five year period.

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

We propose to inoculate mice with tumour cells, creating models where tumour growth within bone and the associated bone disease closely replicate human disease. In order to study the specific role of the bone, it is necessary to also inoculate mice with tumours at non-bone sites, including under the skin and in the prostate. Together, these models allow us to investigate how the disease develops, and to evaluate new approaches to intervene. In order to either understand the mechanisms increasing tumour growth, or to block tumour growth, we propose to treat tumour-bearing or control animals with drugs, administered orally, intravenously or by injection under the skin or through the abdominal cavity. We also propose to alter the diet or restrict the food intake of animals in order to understand how fat cells and obesity promote disease development. The adverse effects are mainly related to the tumour growth, and may include pain. We monitor tumour growth very closely and as such, tumour growth will be kept to a minimum. Restricting food intake for limited time periods is not expected to have any adverse effects other than hunger. The likely levels of severity are mild to moderate, and all animals will be culled at the end.

Application of the 3Rs

Replacement

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

Replacement

The reason that tumour growth within bone and the associated bone disease is so rapid and aggressive is due to the complicated interactions between tumour cells and host cells within the bone that promote both tumour growth and survival and bone disease. These interactions are impossible to accurately replicate in non-animal means at the present time, and there are no in vitro models available that can model effects on both tumour growth and bone disease. For the same reason, it is

no possible to use non-protected animals since the crosstalk between the tumour cells and bone cells is not adequately represented. We do test isolated components in tissue culture, but in order to fully investigate disease development, animal models are necessary.

Reduction

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

Reduction

Animal numbers used in experiments will be kept to the minimum required to test the hypotheses outlined in the plan, based on previous experience in doing these types of studies and on statistical calculations to determine the number of animals required. We have considerable familiarity with most of these experimental approaches and will perform pilot studies in those cases where we do not, to gain understanding of the timing of the tumour development and response to therapy and of the statistical variability to ensure that the smallest numbers that can yield statistical significance are used. Where possible, we pool data from repeat experiments for statistical analysis which allows us to reduce the number of mice used in each repeat. Where possible, we use one control group for multiple experimental groups to reduce the number of mice required. Breeding is usually managed by support staff specially trained in maintenance and breeding of genetically altered mouse colonies. This allows us to keep the number of surplus animals 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 use mice because they are the most characterized model for tumour response to therapy and have the widest scope for genetic manipulations. The models we use are the models that most closely replicate human disease, with tumour growth within bone and development of an associated bone disease.

We take a number of steps to minimise welfare costs to the animals. Where needed, animals will receive pain relief and/or will be under general or local anaesthesia. the protocols use anaesthesia and/or analgesia. Tumour growth and animal behaviour is closely monitored.

The models that we use are well characterised and standard in the field, and allow the study of tumour growth within bone and the associated bone disease. We use

the most refined model that can answer the scientific question. Typically, this is a model that requires tumour growth within bone, but where possible we use a less-invasive subcutaneous model. Aseptic techniques are used during surgery to minimise any risk of infection. When our experimental design requires single-housing of animals, where possible, we will rehouse animals after this period to minimise stress. Where it is necessary to fast animals, we will use the minimum amount of time necessary to achieve accurate measurements of the factors under investigation. For long-term or continuous drug treatments, osmotic minipumps may be implanted to reduce the frequency of drug administration. Taken together, these steps will minimise the welfare costs to the animals, while enabling us to achieve our scientific objectives.

PROJECT 148

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.

Word limit; 1000 words

Project Title	New treatments for polycystic ovary syndrome
Key Words	Obesity, PCOS, Ovary, Programming, Metabolism
Expected duration of the project	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):

Polycystic ovary syndrome (PCOS) is common and effects around 8% of women. It is associated with ovarian, hormonal and metabolic problems that mean that women frequently present to the health service. Treatment is symptomatic and largely unsatisfactory and there is an unmet need for new ways to treat the condition. There is evidence that exposure of a female fetus before birth to an increased concentration of male hormones results in offspring with all the features of PCOS. This model allows us to investigate why PCOS might happen, how it progresses and how it can be changed, as well as the ability to test new treatments. One key problem in PCOS is the presence of obesity. In some countries two thirds of women with PCOS are obese. Weight loss helps the condition but they find weight loss difficult. We have discovered that both women with PCOS and the PCOS animal model are less able to burn calories after eating and this contributes to obesity and difficulty losing weight. In addition, women with PCOS don’t grow and release eggs regularly and this is associate with the ovary making more male type hormone that makes the metabolism worse which further deteriorates ovarian function.

We aim to develop new treatments for PCOS. The first aim is to use new strategies to make the burning of calories after eating normal. The second aim is to study how this works at a whole body and tissue level. The third aim is to use a new strategy targeting the ovary to make the ovary work normally in the longer-term to break the hormone and metabolism vicious cycle. The fourth aim is to understand how this works at the ovarian level. In addition to this the studies will also allow us to understand the effects of the prenatal environment on males, as the brothers of women with PCOS have some hormonal and metabolic problems too. As we look at

the ovarian blood supply in these studies we aim to provide data that can help quantify the number or small blood vessels in a tissue.

What 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 provide the proof of concept that these techniques work and will allow rapid translation into clinical studies in women. As well as women with PCOS, the clinical and academic community will also benefit by the increase in knowledge to tackle various clinical problems and how the prenatal environment affect lifelong health.

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

We use sheep as, unlike rodents for example, their metabolism and ovaries work similarly to women and, as their organs are a similar size to women, we can use drugs, doses and investigations (such as scanning and blood tests) like those used in the clinic. The numbers of sheep used in total in these experiments is small (on average less than 50 a year) and we look after them in excellent premises with dedicated, caring and experienced staff.

In the context of what you propose to do to 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 this is a long-term experiment the studies last several years and the sheep have extended periods where no interventions are undertaken. They will undergo a range of procedures including CT scanning as well as abdominal surgery to monitor ovarian function. These are all performed under general anaesthesia and with appropriate analgesia and in our experience are well tolerated and are not expected to have any significant adverse effects on the sheep. Very occasionally surgical complications can occur as they do in women and veterinary surgeons will advise where needed. At the end of the experiments the sheep are humanely sacrificed and tissues collected for additional laboratory experiments by multiple research teams to ensure that no opportunity to benefit human or animal health is missed.

Application of the 3Rs

Replacement

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

Replacement

A clinically realistic model of PCOS is vital to allow us to understand how it develops and progresses, discover new ways to treat and test new treatments. We are studying a very important condition that involves changes and interaction of many

mammalian body systems. While using cells can help refine parts of the project we need to study animals where all these systems interact in complex ways.

Reduction

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

Reduction

It is important that we use just enough animals to allow us to get accurate results with minimal numbers. We look at our experience and the results from other researchers in the UK and internationally to ensure that we accurately calculate the numbers required to show changes that are clinically important. These calculations are fully displayed and reviewed during funding applications. We ensure that all the tissues are carefully labelled and stored at the end of the study so that ourselves and others can design new laboratory studies with already collected tissue to ensure that experiments do not need to be repeated. As some of our experiments are of minimal severity with no long term effects we are able to re-use animals in similar and related studies 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

It is important that we use just enough animals to allow us to get accurate results with minimal numbers. We look at our experience and the results from other researchers in the UK and internationally to ensure that we accurately calculate the numbers required to show changes that are clinically important. These calculations are fully displayed and reviewed during funding applications. We ensure that all the tissues are carefully labelled and stored at the end of the study so that ourselves and others can design new laboratory studies with already collected tissue to ensure that experiments do not need to be repeated. As some of our experiments are of minimal severity with no long term effects we are able to re-use animals in similar and related studies to minimise the number of animals used.

PROJECT 149

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.

Word limit; 1000 words

Project Title	Generation and breeding of genetically altered rodents
Key Words	breeding, genetically altered, mice
Expected duration of the project	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;
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):

Generation, rederivation, maintenance and supply of genetically altered (GA) mice for use in biomedical 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)?

Genetically altered (GA) mice enable us to study human diseases in many ways, including the use of gene knock-out (KO) technology to understand the role of a particular gene or gene product (protein) in human diseases such as cancer, heart and lung disease. Other GA mice can be used to model certain types of human cancer, increasing our ability to understand how new treatments might help patients. In addition, GA mice can be made which carry the human versions of genes, which helps us to better understand human diseases and how medicines designed for use in humans might work in patients.

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

We will keep colonies of live GA mice and breed them to supply animals for experimental work and to maintain the lines. We estimate that up to 41,550 animals may be used during the lifetime 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 overwhelming majority (>>95%) of animals reported to the Home Office against this Licence are expected to experience no greater than Mild severity. The genetic alterations are not expected to produce any overt disease or clinical signs while they

are held under this Licence. Animals may have a skin sample taken from the ear under anaesthetic so we can perform genetic tests. There is a protocol to allow up to Moderate severity but this will only be used in restricted circumstances following discussion with a veterinarian. Animals under this Protocol may for example have a spontaneous intestinal pathology that results in Moderate severity weight loss and change in condition. GA mice that are used in experimental studies are transferred off this Licence and reported to the Home Office separately.

Application of the 3Rs

Replacement

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

Replacement

Research projects use many non-animal techniques such as growing animal and human cells artificially before any testing on animals. Animals are still needed because these systems do not reflect the full complexity of *in vivo* biology. For example the repertoire of interactions between immune cells, nerve cells, complex hormonal systems and organ specific cell changes cannot be recreated outside of living animals.

Reduction

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

Reduction

Animal breeding will be tightly controlled to match the supply of GA mice to the planned demand for use in experimental studies. Due to genetics, some animals produced during breeding cannot be used for experiments, and we will optimise our plans to reduce this 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 mouse is the most appropriate animal for these studies as they are a mammalian species with many features in common with humans. They have been studied for many years and there are a large number of GA mice already available for research studies. Animals are kept in high quality facilities, free from pathogens and with access to food, water and environmental enrichments.

PROJECT 150

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.

Word limit; 1000 words

Project Title	Ornamental fish nutrition and health
Key Words	Fish, Nutrition, Health
Expected duration of the project	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):

This project is focussed on enhancing the quality of nutrition and care given to ornamental fish, to result in an improvement in the health and well-being of these animals. Nutritional studies conducted with fish have primarily used commercially farmed fish species due to their economic value. Consequently there is a paucity of data on the nutritional requirements of ornamental species. This area warrants further investigation particularly when the life style, feeding strategy and physiology can be vastly different when compared to farmed species.

There are three primary objectives of the programme: 1) the evaluation of food stuffs and ingredients through parameters such as feed acceptance, feed conversion, digestibility, growth rate, pigmentation, feeding behaviour and impact on water quality, and the effect of product on markers of fish health such as plasma biochemistry, haematology, immunology, longevity and reproductive output; 2) to aid development of new food stuffs and water treatment products that are supplemented with functional ingredients; and 3) to develop novel measures of nutritional status and health in ornamental fish.

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

Findings from this project will advance the scientific understanding of the nutritional requirements, health and care of ornamental fish. This will result in the development of suitable food stuffs and water treatment products. The knowledge will be shared through scientific publications, scientific congresses and in the general aquarists literature so making it available to as wide an audience of scientists and fish keepers as possible.

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

Ornamental fish species (multiple) 4000 over 5 years Fish will not be endangered / critically threatened species.

In the context of what you propose to do to 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 planned trials are considered to be mild in severity. Procedures involve manipulation of the external environment e.g. the addition of water additives, limited cessation of the water supply and diet manipulation. Others include mild stressors (e.g. netting, aerial exposure) and some, using light anaesthesia for restraint purposes only, include blood sampling, faecal collection, scale and fin sampling, colour measurement, and mucus collection. Further information regarding the precision of adverse effects is given in each protocol. Briefly, general adverse effects manifest as malformations, behavioural problems, fin clamping, increased respiration, reduced movement and/or obvious injury. Animals are observed several times per day to detect any adverse effects at an early stage. It is planned that no animals will be euthanised as a result of this project and fish will be homed. However, if exceptional circumstances dictate humane killing would be employed as a control measure.

Application of the 3Rs

Replacement

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

Replacement

We have available a number of alternatives, i.e. tissue cell lines, including epithelial and fin fibroblast cells to using live fish and use these where appropriate and review the literature to keep abreast of developments. However, the majority of measures apply to whole body systems that are difficult to replicate in an *in vitro* situation. This is particularly true where the aquatic environment in which the fish live can exert a significant influence on health.

Reduction

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

Reduction

Statistical power calculations are used in the design of all experimental trials to ensure that the appropriate number of animals are utilised; variance data will be used where available from historical studies and applicable 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

Since the term ‘fish’ represents a highly diverse number of species, it is important to conduct the studies with the specific species of interest. The knowledge outcomes of these studies will be applied to the species studied and so the research is in direct service of the species. The procedures employed are considered primarily in the context of achieving the objectives while not exceeding the mild severity threshold. An Animal Welfare and Ethical Review Body and appropriate staff training will ensure that every effort is taken to minimise potential pain, distress or lasting harm.

PROJECT 151

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.

Word limit; 1000 words

Project Title	Pathogenesis, diagnosis and control of mycobacterial infections
Key Words	Mycobacteria, Pathogenesis, Diagnosis, Vaccine
Expected duration of the project	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;
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 aim of this programme of work is to improve our understanding of host-pathogen interactions and pathogenesis and to develop improved diagnostic tests and novel vaccines for the effective control of mycobacterial diseases.

Current control programmes for mycobacterial infections of livestock rely on detect and cull policies and are ineffective largely due to the poor specificity and sensitivity of the current diagnostic tests for detecting all stages of the disease. Vaccination would be a suitable alternative but reliable effective vaccines are not available. A better understanding of the biology of disease, particularly of pathogenesis and the complex host-pathogen interactions is required to develop improved diagnostic tools and novel vaccines. This programme seeks to address this by 1) characterising the immune responses and pathological events early in infection to understand the disease process 2) identify biomarkers that can be utilised for diagnostics or vaccine development 3) evaluate potential candidates for vaccines or drug therapy in model systems and the natural host.

What 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 help us define the immune responses that determine if a mycobacterial infection is cleared, if it persists or whether it progresses to clinical disease. This information is needed to develop new effective vaccines or treatments. We will also identify bacterial components and host biomarkers that can be employed in specific diagnostic tests, which in the longer term can be utilised in disease control programmes and may also reduce the numbers of animals unnecessarily slaughtered due to false positive results in animals with multiple

mycobacterial infections. Additionally, we will provide preliminary data on the potential of live attenuated and sub-unit vaccines for controlling mycobacterial infections in model systems. Ultimately, better diagnostic tests and effective vaccines for the management and control of mycobacterial diseases will improve animal health and welfare and may possibly benefit humans in the case of zoonoses.

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

Over the five year span of this licence we will use cattle (up to 1000) on commercial farms where they will be blood sampled for evaluating immune responses and diagnostic tests. We will use sheep (up to 100), which will be clinical or suspected cases donated by farmers to provide clinical samples. Cattle (up to 100) and sheep (up to 50) may be used for experimental infections to study the early immune and pathogenic responses to infection and vaccination. Up to 800 mice will be used to assess virulence of live vaccines and for vaccination 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?

Small volumes of blood and faeces samples will be taken from cattle and sheep, which will cause minimum stress or discomfort to the animals and will be done by trained individuals. Similarly, the administration of bacteria or vaccine orally or by injection will cause minimum stress. Infections by Mycobacterium avium subsp. paratuberculosis and Mycobacterium bovis are fatal for ruminants and therefore ruminants used in this work will be killed by an approved method before the onset of moderate clinical signs (diarrhoea and a loss of body condition). Clinical effects in rodents are unknown but not expected. Animals will be killed by an approved method at the end of the experiments and blood, faeces and tissues will be taken for further studies. Animals sampled on farms will continue to be treated as commercial livestock.

Application of the 3Rs

Replacement

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

Replacement

We use *in vitro* models for preliminary studies wherever possible but we have to use live animals for investigating the early stages of pathogenesis and immune responses and for evaluating diagnostic tests and potential vaccines.

Reduction

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

Reduction

All experiments are scrutinised and approved by an internal Experiments and Ethical Review Committee and are designed in conjunction with statistical advice to ensure that as few animals as possible are used to generate statistically robust 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

We avoid experimental infection wherever possible by utilising naturally infected animals from affected farms, for example to evaluate a new diagnostic test. For *in vivo* experiments evaluating virulence and potential vaccine candidates, small laboratory animals will be used for the primary screen and further assessment in the target (ruminant) species will be carried out only if the results of the primary screen are favourable. Any substances administered to the animals will be done using procedures that cause the least possible pain and animals will be monitored regularly.

PROJECT 152

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.

Word limit; 1000 words

Project Title	Understanding pathogen behaviour in relation to the immunity, vaccines and antibiotic treatment
Key Words	Bacteria, infection, vaccine, antibiotic
Expected duration of the project	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;
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 overall aim of our research is to understand how infections caused by bacteria can be defeated or prevented by making better vaccines and antibiotics. Bacterial infections are a big problem because they cause about 6 million deaths in the whole world. Many bacteria are becoming resistant to antibiotics and many of the vaccines that we use are not sufficiently good. Therefore we do not have optimal weapons to fight infections. We primarily work on bacterial diseases that affect and kill children in poor areas of the world. For example, one of these diseases, invasive non-typhoidal salmonelloses (iNTS) causes about 680,000 deaths every year, 68% of which are in children less than 5 years old in Africa and South East Asia. Currently there are no vaccines against iNTS and an increasing number of iNTS bacteria are becoming resistant to the antibiotics that doctors use to fight them. Furthermore, we do not understand how these bacteria spread in the environment and how they infect people. Therefore better vaccines and antibiotics remain the main weapons to fight these infections in poor countries.

Our research will study how and where the bacteria hide in the body to resist to vaccines and antibiotics. This will enable us to produce new vaccines and antibiotics that can reach the bacteria in the locations where they hide and persist and kill them more efficiently.

What 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 create scientific knowledge that will guide a better use of the vaccines and antibiotics that are currently available to us and will make it easier to produce new and better antibiotics and vaccines. Doctors and patients will benefit from this

research that will improve the treatment of sick people, especially children and will also reduce the spread of diseases in the community. These benefits will be stronger especially in developing countries where there are many conditions that weaken the immune system especially in young children (for example, viral infections, gut parasites, malaria, malnutrition). In fact a weak immune system makes vaccination and treatment of an infection a lot harder to accomplish. In the long term, better use of antibiotics and vaccines will reduce the disease burden and slow down or stop the emergence of bacteria that are resistant to antibiotics. Our work will also impact on disease prevention in the veterinary field and in food-animals where vaccines and antimicrobials are widely used often with suboptimal results.

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

Approximately 10000 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 animals will be infected with live bacteria via several possible parenteral routes, intranasally or via oral gavage and then may be treated with antibiotics or molecules that affect the immune system. In some experiments, new vaccines will be tested, selected and optimized by immunisation schedules followed by reinfection with pathogenic bacteria and monitoring of the immune responses. In the majority (> 90%) of experiments no animals will show signs of infection. However, it is possible that very occasionally a small number of animal show clinical signs. These animals will be closely monitored and assisted via careful and skilful husbandry that is typical of the culture of care present at our establishment. If signs persisted for more than a few hours the animals would be killed to avoid further suffering.

Application of the 3Rs

Replacement

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

Replacement

We perform many preliminary experiments in systems that do not involve animals. However, these systems cannot reproduce the complexity of the body of a whole animal where the blood transports the bacteria between different sites and each organ influences the functioning of other organs. Therefore to ensure that our research has a real future impact on human health, it is necessary also to study infections in a whole animal where we can capture the impact of medical treatments and new vaccines on the behaviour of bacteria in an environment that closely resembles the human body.

Reduction

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

Reduction

We greatly strive to reduce the numbers of animals that we use in our experiments. Whenever possible we perform preliminary studies in systems that do not require animal experimentation so that we can improve our protocols and use smaller numbers of animals only for the final validation of our results. We combine several experiments in one so that, for example, we can compare the effect different vaccines or treatments using just one untreated (control) experimental group. We use the smallest possible experimental number of animals for each experiment being very careful that this does not affect the accuracy of our results. To determine the smallest number of animals that we can use in our work we use calculations bases on advanced statistics and mathematics. Statisticians and mathematicians have become an important part of our research group.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 infections in adult mice. This model captures the essential traits of many bacterial infections of humans and other animals. Mouse models are a reliable tool to study vaccines and antibiotics before these are used in humans and domestic animals. The availability of genetically altered mice allows us to mimic model human conditions and immune-deficiencies such as malaria, AIDS, congenital absence of components of the immune system that predispose to infection. The model therefore enables refined studies on the interaction between bacteria and the immune system in the course of vaccination or medical treatments.

Most animals do not show any signs of infection during our experiments. We minimise suffering via careful and skilful handling of the animals, use of the smallest possible size of needles, minimal numbers and frequency of repeated procedures and light anaesthesia for some procedures. Whenever possible we use less infectious bacterial strains for our studies to minimize the signs of infection that may occur. We progressively refine our protocols to ensure that the smallest possible doses of bacteria are administered to the animals and we perform observations at time points before the occurrence of signs of infection. To achieve this we are making use of new technology to increase the sensitivity of our assays that detect bacteria, bacterial genes/proteins, or immune parameters triggered by low numbers of bacteria in the body of the infected animal. This has also the

scientific advantage of looking at infections when bacterial numbers are relatively low and more closely related to what happens in the human infections that we model.

PROJECT 153

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.

Word limit; 1000 words

Project Title	Neuroprotection and neurorepair of the immature brain
Key Words	Cerebral palsy, neuroprotection, neurorepair, immature brain
Expected duration of the project	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;
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):

This project will focus on the development of new therapeutic strategies to provide neuroprotection and also support repair of the damaged central nervous system in cerebral palsy (CP). This condition is a consequence of injury to the human brain in the period of development occurring before birth or immediately after birth. Perinatal brain injury has life-long consequences and at present there is no treatment which can significantly attenuate the tremendous burden of disability of CP sufferers.

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

Injury of the immature brain leads to a complex range of disorders described generically with the term cerebral palsy (CP).There are around 17 million sufferers of CP worldwide. CP sufferers have major impairments of their motor function and their memory and cognitive abilities; many of them cannot walk, cannot talk, have poor vision, epilepsy or chronic pain. There are no treatments for these patients and CP is associated with lifelong disability. Treatments that would be successful in producing neuroprotection in the acute phase after the injury occurs, or that could repair damaged circuits in the chronic phase, could reduce the disability burden associated with CP. There would be huge personal benefits for patients and carers and there would also be a very significant impact in terms of public health costs.

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

We propose to use mice and rats, which are species used in the established models of immature brain injury used worldwide. We expect we would use maximum 800

mice per year and 440 rats per year. Over the 5 year duration of the licence we would use a maximum of 4000 mice and 2200 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?

All the experiments carried out are considered to have a moderate severity, and animals can show some temporary decrease in their motor and cognitive function, which may sometimes impair their suckling and eating/drinking functions, but it is unlikely to critically affect their vital functions. Animals will be closely monitored to ensure their well-being, with temporary supportive feeding if needed, and supportive nesting bedding, to enhance maternal care and support pup growth. Intensive monitoring and care will be implemented, in order to prevent and rapidly manage any maternal rejection response and animal suffering. Humane endpoints have been specifically addressed to ensure that there is minimal suffering of neonate and pregnant/lactating animals. At the end of the experiments, animals will be humanely killed and their tissue used 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 aim of this work is to assess the efficacy of interventions which protect the immature brain against a variety of injuries which can happen around the time of birth. The injury leads to very complex changes in the brain: several different types of cells in the brain are simultaneously destroyed and the barrier between the brain and the rest of the body becomes leaky, and this increases the risk of toxic substances easily getting access to the brain. There is swelling of the brain, generalised inflammation and a lot of oxidation. We are not aware of any alternatives to the use of whole animals, in which all these complex parallel aspects of the real life injury of the immature brain can be modelled effectively.

The main neurological deficits seen in consequences of injury to the immature brain, such as in cerebral palsy (CP) patients, include significant motor problems (e.g. development of spasticity), general problems with cognition, and memory and learning deficits. These deficits can only be modelled in whole animal models of the injury and there is no alternative in which such complex integrated functional outcomes can be assessed.

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. 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 immature brain injury worldwide. Both rodent species reproduce many of the relevant deficits seen in the human newborn.

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 immature brain injury will be moderate and will not be allowed to progress beyond the minimum required to achieve the scientific objectives of the project.

PROJECT 154

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.

Word limit; 1000 words

Project Title	Pre-clinical evaluation of cancer vaccines
Key Words	Cancer, Immunogenicity, Vaccine, Efficacy
Expected duration of the project	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:
Yes	(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 primary aim of this research program is the development a novel immunotherapy against cancer. Despite significant efforts to advance the field, the clinical efficacy of experimental cancer vaccines has been very modest. This is due to the fact that usually cancer vaccines target self-proteins that are normally not recognised by the immune system. Our approach to designing effective vaccination regimes is the use of weakened viruses (i.e. vectors) whereby a protein from the tumour of interest is expressed from the virus. We have now shown in other research that these types of vaccines are superior to other vectors in that they induce unprecedentedly strong cell and antibody based immunity against infectious diseases in both animals and humans. Within the scope of this project we will extend this approach to cancer settings with the aim to develop a novel highly immunogenic vaccine that would be effective in protection against tumour development and progression in mice. These will be proof of concept studies that will provide rationale for further clinical development of viral vectored based vaccines against various cancer types including prostate, lung and kidney tumours.

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

The likely benefit of this project is a novel cancer vaccine available for early clinical development. We will assess the vaccine for efficacy both when tumours present and to cease development. This novel vaccine is likely to be not only therapeutic but potentially may be able to protect against the tumour onset and development. Also, in the process of developing new vaccine candidates, this work will expand our understanding of the immune response generated by vaccination and required for protection against cancer. We expect our discoveries made in animal models to be

rapidly translated into human clinical trials. The vaccine approach developed here should be broadly applicable to the design and development of vaccines against a wide range of cancer types.

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

We will use mouse models for these vaccine studies as they are the best immunologically studied animal species and have proved to be excellent indicators of immunogenicity in terms of identifying regimes with improved immunogenicity. Mice will be immunised with a vaccine preparation. We shall attempt to improve the immune response through the use of adjuvants and optimising vaccines and vaccination regimes. With these multiple approaches, we estimate up to 2000 mice will be used to test immunogenicity of our vaccines and vaccination approaches over the next 5 years. To test the efficacy of our candidate vaccines, we will perform efficacy studies on vaccinated mice by inoculating them with tumour-derived material. Also, we will evaluate our vaccines in spontaneous tumour models which has been well characterized in terms of stages of the disease and tumour progression. We will seek to minimise the numbers of animals used while using adequate numbers to allow statistically significant data to be obtained, maximising the amount of data from each animal. We estimate the use of up to 3000 mice over 5 years for these efficacy 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 expected adverse effects will be mainly attributed to autoimmunity, withdrawal of blood, inoculation of tumour material and development of spontaneous tumours. In the unlikely event that the severity of any procedure threatens to exceed the “moderate” level which means a minor impact on animal after which it rapidly returns to normal behaviour, the animals at risk will be humanely killed. At the end of the experiments or when animal displaying overt signs of disease, they will be sacrificed immediately 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

To study the immunogenicity and protective efficacy of human vaccines there is no adequate alternative but to use animals. The immune response to vaccination involves complex interaction of body systems which cannot be replicated in tissue culture. Cancer development and tumour response to treatment are also complex

processes involving interactions that take place between a tumour and the host, therefore, they cannot be reproduced in tissue culture either. Importantly, it's required by law that any new vaccine should be tested in animals before it is given to humans.

Reduction

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

Reduction

We will carefully design our experiments and seek advice from a statistician to ensure the smallest number of animals sufficient to obtain scientifically valid data is used.

We will maximize the data obtained from an individual animal by taking sequential samples from the same animal across one experiment.

We will continue to develop and deploy new methods that allow more information to be obtained per experiment and thus reduce the number of animals studied, e.g. imaging of tumours over time.

Whenever it is possible without compromising scientific validity of the data, we will be testing as many conditions per experiment as possible in order to use just one control group.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 for these studies since they are the most characterised small animals in terms of the immune responses. Also, laboratory mice are widely used in cancer vaccine research because of large number of established tumour models that closely mirror the development of cancerous tumours in humans.

Based on previous experience, we will minimize a number of sampling time points to collect the most informative ones.

Our models will involve the injection of tumour cells to induce cancer or the spontaneous development of primary tumours. Although mouse tumour models that we use is the standard in the field certain tumour cell lines are not as well characterized. So, small scale pilot experiments will be undertaken to establish the

dose of tumour cells required to achieve the development of palpable tumours within the reasonable short period of time.

Mice with tumours will be monitored regularly and as disease progresses, the frequency of monitoring will increase to ensure undue suffering does not occur. We will not allow subcutaneous tumours grow above 11 mm in any dimension. Alternatively, an experiment will be terminated as soon as a valid scientific outcome is reached.

PROJECT 155

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.

Word limit; 1000 words

Project Title	Investigating the role of a novel compound in healing of intestinal anastomoses
Key Words	Intestinal anastomosis healing
Expected duration of the project	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;
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 environment within the bowel is complex and wound healing here is still poorly understood. Our aim is understand how the immune response and the resident bacteria might interact to influence wound healing within the bowel.

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

Bowel operations are one of the most frequently performed emergency and elective operations. But wounds within the bowel sometimes fail to heal. In humans, this can have devastating long-term consequences or even death. With increased understanding of wound healing in the bowel, this project has the potential to help eradicate this complication.

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

Approximately 500 adult mice 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?

All animals will undergo a bowel operation where the gut is transection and surgically re-joined under appropriate anaesthesia. Due to the nature of this operation, it is classed as a severe. However, all the mice are expected to recover, and will be given optimal analgesia and post-operative care. All animals will be carefully monitored for wellness using validated and sensitive scoring systems, and 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

The complexity of a fully developed immune system and its relationship to the bacterial ecosystem within the bowel cannot yet be fully simulated in lower vertebrates, cells, or with computer modelling. This is why these animals are currently needed. However, throughout the duration of the project, we will be actively seeking, developing and incorporating any alternatives that could replace, reduce or refine these procedures.

Reduction

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

Reduction

We have performed complex statistical modelling overseen by a qualified and experienced biostatistician for our experimental design. A limited number pilot study will be incorporated to ensure that the absolute minimum number of animals to achieve statistical significance will 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

The mouse models of bowel surgery are the best characterised thus far, and they possess an immune response and an intestinal bacterial ecosystem that is close to humans, ensuring that our results will be meaningful. All experiments are performed in a well-resourced and well-equipped facility by experienced teams. We will be using optimal peri-operative care at all times, and experiments are carried out in a limited time frame to minimise any harm to the animals. Sensitive scoring of wellness will be performed regularly to ensure establishment and maintenance of animal health, with regular involvement of our veterinary surgeon

PROJECT 156

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.

Word limit; 1000 words

Project Title	Investigating the regulation of energy homeostasis
Key Words	AMPK, cancer, diabetes, metabolism, obesity
Expected duration of the project	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):

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.

PROJECT 157

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.

Word limit; 1000 words

Project Title	Validating behavioural and cognitive indicators of animal affect and welfare
Key Words	behaviour, depression-like state, mice, rats, waking inactivity, cognitive bias
Expected duration of the project	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;
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):

Accurate measures of animal emotions are essential in order to correctly assess whether the ways we house and manage animals promote good well-being. However, existing behavioural and physiological indicators of animal emotions are still imperfect, and there is thus a need for new and well-validated measures.

This project aims to improve a promising indicator of emotions in rats and mice, based on how they judge ambiguous situations (a *cognitive* 'judgement bias' indicator). This cognitive '**judgement bias'** method is based on the fact that people reporting negative emotions (for instance being sad or anxious) are more pessimistic about ambiguous events than happier people (they are more prone to see the glass 'half-empty'). Being pessimistic (for instance judging that a rustle in a bush might signal a dangerous animal hiding there) can promote survival in certain environments. Hence these emotion-related judgment biases are likely to occur not only in people, but also in animals. Numerous studies have used a judgement bias test to show that animals in negative emotional states judge ambiguous situations more pessimistically than animals in more positive emotional states. The test however requires further improvement to make it easier to use. Because it can take a long time to train the test, there is also a need for quicker cognitive bias tasks. One example is a test of '**sensitivity to positive or negative stimuli'. This idea comes from the fact** that happier people more readily detect positive stimuli, while sad or anxious people more readily detect negative/frightening stimuli. We will therefore also develop this type of task in rodents.

Finally, we will also develop and validate a new *behavioural* indicator of negative emotion in mice. This **behavioural indicator** is 'waking inactivity in the home cage', namely remaining motionless but awake (with eyes open) in the home cage, as if the

animal is ‘doing nothing’. Our preliminary studies suggest that this may be useful new measure of a *depression-like* state in mice. To evaluate whether this is so, we will investigate whether waking inactivity is triggered in mice by the same risk factors that cause depression in humans; whether it co-varies in mice with a range of symptoms similar to those of depressed people (including cognitive biases tested using the methods we will develop); and whether it is alleviated by antidepressant treatments that cure depression in humans or by housing the mice in enriched conditions. If confirmed, our results will provide new and refined measures of animal emotions and welfare.

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

The work will continue ongoing research into the use of cognitive bias tests as good indicators of emotions and welfare in animals. It will also provide new information about the expression of depression-like states in a widely used laboratory animal, the mouse. This is important for animal welfare and for the wider scientific community. Cognitive bias tests that are faster to train should result in more research, and consequently more knowledge, about animal emotion and welfare. Waking inactivity may be a new and simple-to-use welfare indicator that can be monitored in the home cage without disturbing the animals. If so, future work will explore whether measuring waking inactivity in the home cage can be done automatically (e.g. using computer vision methods rather than requiring researchers to watch the mice), and hence provide an easy method for monitoring animal welfare. Validated methods for identifying negative emotions are essential to maximize the welfare of laboratory animals. Being able to detect depression-like states is also vital because such states may potentially invalidate research where stress and depression are not of interest. For instance if a mouse used in Alzheimer research is actually ‘depressed’ because of the way it is housed, there is a risk that the depression-like state interferes with the effects of the drugs researchers are testing, and hence invalidate their results. Validating measures of animal emotion in rodents could also help research on emotions and welfare in other animals. Our results will thus be important for researchers interested in better understanding animal emotions, and ensuring that inadvertently induced depression-like states do not influence the outcomes of scientific studies. It will also be of value to laboratory managers & scientists seeking to further improve the housing and use of their laboratory rodents in order to enhance animal welfare.

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

Mice ≈ 500 over the 5 years of the PPL; Rats ≈ 240 over the 5 years of the PPL

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

These are behavioural studies, and our main procedures are mild or unlikely to cause any suffering: observing behaviour of the animals and training them in behavioural tasks that involve acquiring rewards such as food. We may also use procedures that include induction of short-term, reversible negative emotions (e.g. exposure to an air puff); exposure to temporary separation from the mother during early life; exposure to environmental enrichment; exposure to medications that have been previously shown to reduce stress and to induce a comparatively more positive affective state. These reversible treatments will be used to test whether our measures of animal emotion can reliably detect positive or negative emotional states. This is necessary before the measures can be used more widely to evaluate animal welfare. During these studies we will carefully and regularly check all animals for body condition, weight and the presence of abnormal behaviours, and immediately seek veterinary or care staff advice if any animal exhibits sign of illness or adverse reactions. At the end of the studies, animals will be humanely killed or re-homed.

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 improve and develop indicators of well being in animals, which can only be studied in living individuals.

Reduction

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

Reduction

Estimates of animal numbers are based on statistical methods (called ‘power analyses’) and sound experimental design, which ensures that the appropriate and minimum numbers of animals will be used in our research and validity to the data 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

We will study the laboratory rat because it has been most widely used in cognitive bias research, and the laboratory mouse because this species displays waking inactivity. Both are highly important laboratory animal species.

This is principally a behavioural study and most of our procedures are mild / unlikely to cause any suffering. Staff involved in the study will be trained prior to administrating any procedures. We will group-house both rats and mice, because they will be stressed (especially females) by social isolation otherwise. Some animals may be exposed to conditions designed to induce more negative or positive affective states in order to validate our measures. Such conditions will generally be short-lasting and, for mice, we create a contrast between housing conditions by making the positive housing much more positive than what it is normally, rather than by making the regular environment more negative to create the negative housing. We will use training methods in the behavioural tests relying on reward acquisition that do not require food deprivation. A few behavioural tests may also involve stimuli such as air puffs which animal can easily avoid. Animals will be habituated to handling (using techniques which have been shown to reduce stress), and will always be habituated to new testing environments, first together with their cage-mate. Some animals may receive medications and will be habituated to handling and monitored to ensure they are not developing any adverse side effects. All animals will be routinely monitored for body condition, weight and the presence of abnormal behaviours, in the home cage as well as during behavioural testing sessions by trained technicians and/or researchers.

PROJECT 158

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.

Word limit; 1000 words

Project Title	Epithelial stem cell homeostasis and disease
Key Words	tissue stem cells, epithelia, RNA methylation, cancer, tissue regeneration
Expected duration of the project	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;
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):

Most adult tissues and organs are maintained by resident stem cell populations, and the skin is one of the best-characterized tissue containing stem cells. The outermost layer of the skin is called epidermis, which forms a protective barrier to prevent water loss, infection and injury. Stem cells are established during foetal development and remain present throughout life to maintain and replenish tissues and organs. To replace damaged or dead cells, stem cells undergo cell divisions to maintain their population (self-renewal) and to generate progeny (differentiation). The progeny then form all functional cell types of the tissue. A tight control of the balance between self-renewal and differentiation is essential to maintain a healthy tissue and imbalances can lead to human diseases. An imbalance towards self-renewal increases the number of undifferentiated cells and may lead to tumour development. Too much differentiation may lead to the exhaustion of the stem cell pool and may lead to failure to regenerate the tissue after injury.

This project aims to identify novel key regulators of stem cell functions. We then aim to modulate these regulators to promote the regenerative potential of stem cells and to prevent or treat cancer. This is important because despite many progresses in treating cancer, it remains responsible for killing 40 per cent of all the men and women who die before the average life expectancy (between the ages of 25 and 74). Of all skin cancers, squamous cell carcinoma is the second most common cancer and its incidence has increased up to 200% over the past 30 years. Most skin cancers are caused by repeated and unprotected skin exposure to UV radiation. Cancer metastasis is the leading cause of cancer-related deaths and is often caused by development of drug therapy resistance.

Loss of a stem cell population or increased cell differentiation contributes to the progressive deterioration of a tissue and reduced regenerative capacities. Poor tissue regeneration after injury (wound healing after trauma, surgery, acute illness, and chronic disease) affects millions of people each year. Reduced regenerative capacities of tissue is at least in part the consequence of impaired cell repair responses that can include loss 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)?

This project will help to define the fundamental properties of stem cells in epithelial tissues. We will identify and characterize novel mechanisms that regulate stem cell functions and determine how their dysregulations leads to cancer or poor tissue regeneration after injury. By testing whether a new stem cell regulator increases or decreases tissue regeneration, we may discover new strategies for treating human diseases that are associated with poor tissue regeneration (e.g. chronic wounds). By validating how a new stem cell regulator contributes to tumour development, metastasis and chemotherapy resistance, we may identify novel drug targets to treat cancer. The transgenic animals developed will be valuable to other scientists interested tissue regeneration and cancer.

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

We are using mice as an animal model. We use mice with aberrant expression of stem cell regulators in skin and other epithelial cells that line the cavities and surfaces of blood vessels and organs throughout the body. Our mouse models may additionally be modified in such a way that we can visualize stem cells in the tissue under the microscope or using other detectors. This allows also us to see the specific cells even in alive mice using only minor and non-stressful procedures. We are using well-characterized mouse tumour models that carry mutations in the most prominent genes known to cause human cancers. Over the next five years, we expect to analyse four novel stem cell regulators using mouse models. The generation of the optimal mouse model carrying the modified stem cell regulator is by breeding (~14,000 mice over five years). The vast majority of these animals (>90%) will not experience any harm or distress. Tumour or injury experiments require 10-20 animals per experiment and overall less than 20% of experimental mice will experience some harm and distress. In total, we estimate to use ~10,850 experimental 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?

When we are testing the function of the novel stem cell regulators in skin and other epithelia, we are expecting to either see an increase in the number of cells or a defect in maintaining the structure of the tissue. The majority of animals are not

expected to experience harm or distress. In around 10-20% of animals, adverse effects may occur that include skin inflammation, skin flaking, hair loss, skin thickening, skin erosion, wounds or tumours. Should this cause the animal pain or distress then the animal will be humanely killed. When we are modelling tumour development, growth, metastasis and drug resistance, we are expecting all mice to develop tumours. The mice will show adverse effects as a result of developing the tumour that besides the tumour itself may include inflammation, bleeding and chronic wounds. Mice with tumours are regularly monitored and humanely killed if they become ill. Signs of illness are for example lack normal grooming and avoidance behaviours, being unable to eat or drink, or the tumor may hinder normal body movement. When we are testing the effect of commonly used chemotherapeutic agents and novel anti-cancer drugs, we are expecting adverse effects in around 10% of the treated animals, which is similar to human patients undergoing chemotherapy. Known side effects in humans are the reduction of the number of white blood cells which makes it more likely to get an infection (more than 1 in 10 patients will show these side effects). Should an animal experience signs of discomfort, pain and suffering from the side effects of the medication, then the animal 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

At present, there is no cell culture system available that enables us to generate skin outside an animal that contains all functional cell types of skin, such as hair follicle and sweat glands for example. This is because we cannot re-create the optimal environment for stem cells to generate all these skin lineages. Thus, stem cells in culture do not represent stem cells in their normal physiological condition in the whole animal. In consequence, it is still essential to test the potential, efficacy and side effects of a novel anti-cancer drugs on a whole tissue in an animal. We will refrain from animal experiments whenever possible and perform the experiments using alternative three-dimensional culture systems using normal and diseased human cells.

Reduction

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

Reduction

Before we start an experiment that includes animals, we estimate the number of animals needed to obtain a meaningful result by performing pilot experiments using

a small number of animals and by taking advice from the Bio-Statistician. The experiments are designed to identify biologically meaningful differences reliably using the smallest number of animals possible.

By using only well-established reagents and assays will reduce the number of animals. When we use a novel compound, such as a small inhibitor, the drug will be pre-screened in cells and pilot experiments will be performed to reveal the minimum dose that is likely to be effective. To maximise the information from a single animal, we will collect skin samples from multiple body sites post mortem, and provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. Other tissues than skin that might be harvested from the animal after death are brain, mammary gland, thymus, trachea, intestine, lung, testes amongst others.

We will reduce the number of animals by using suitable mouse lines that already exist instead of generating novel lines. The mouse lines will be kept and bred in such a way that the animals will not suffer from a harmful phenotype. When we perform an experiment on animals we will keep a strict record and detailed description of the methods used, so that each experiment is reproducible.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 genes we study are known to cause a wide range of human diseases that include neurological disorders, male infertility, diabetes, mitochondrial diseases, and cancer. The mouse is widely considered as the model organism of choice for studying these diseases. All methods will be under constant review to adapt or integrate novel approaches that reduce distress, harm and discomfort of an animal in an experiment.

We will constantly review our protocols and adapt novel state-of-the-art methods should they become available. This includes the route of application of drugs, the way of visualization methods of cells in the alive mouse, and the use of specific mouse lines that produce tumours.

Before we test novel anti-cancer drugs with an unknown toxicity on animals that develop tumours, we test the lowest dose on a minimal number of animals to establish experimental and humane endpoints.

By only using well established and extensively described methods and protocols we minimise the uncertainty of how compounds affect the wellbeing of an animal.

PROJECT 159

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.

Word limit; 1000 words

Project Title	Effects of testosterone on atherosclerosis
Key Words	Heart disease, Testosterone, Treatment, anti-inflammatory
Expected duration of the project	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;
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):

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, his 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. 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.

PROJECT 160

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.

Word limit; 1000 words

Project Title	Stem cell transplantation into models of inner ear disorders
Key Words	Hearing loss, Vertigo, Cochlear Implant, Stem Cell
Expected duration of the project	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;
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):

More than 3 million adults in the UK have a bilateral hearing impairment that is moderate to profound (larger than 45 dB HL). The numbers rise to more than 10 million if we include sufferers of mild impairments. This is important, since severity increases rapidly with aging (presbycusis). Together the cochlea responsible for hearing, the inner ear also harbours the vestibular organ that controls balance. The impact of vestibular failure on a patient's mobility and functional independence can be devastating. Patients with bilateral vestibular failure rarely recover. Traditional vestibular rehabilitation benefits a limited number of patients and has no significant effect on number of falls. Impaired vestibular function is a major risk factor for wrist and hip fractures from falls.

The current project has the overarching aim of developing a stem cell therapy for the treatment of inner ear conditions.

Previous work has concentrated on the hearing organ, the cochlea. Now we will expand on the cochlear studies, and further explore the applicability of this approach to the vestibular organ.

Specific objectives to be addressed by this program include exploring the ability of the transplanted cells to engraft within the host vestibular system and to functionally integrate with it. We will explore the use of biomaterials for the delivery of cells to the inner ear. We will also study the effect that genetic mutations acquired during the process of cell expansion in vitro, could have on the ability of cells to differentiate, engraft, and functionally integrate with the host as well as their general safety (i.e. production of tumors). We will assess the ability of transplanted cells to interact with

a cochlear implant, and will study different methods that can be used to purify them before 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)?

The initial benefit of this work will translate in the generation of new knowledge on how these cells integrate functionally and anatomically in a recipient. Data to be gathered will advance our understanding on how to repair the inner ear, but we believe that some fundamental principles could be beneficial to the neurosciences in general and to the field of regenerative medicine in particular. Besides the scientific benefit, this project could have a direct impact in the treatment of hearing loss and vestibular pathologies. Previous work has established that a functional recovery of hearing is possible by a stem cell intervention, but we do not know if we can restore vestibular sensory input. This work will assess the viability of using a cell based therapy for this patient group. Data regarding interaction with cochlear implants, genetic variants, cell purification and delivery would facilitate the translation of the work into clinical trials.

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

The maximum number of animals expected to be used in this project are 800 gerbils, 800 mice and 800 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?

We propose to implant these cells into gerbils, rats and mice that have two different types of deafness and vestibular problems. Gerbils are a good experimental model with a human-like hearing range, while mice and rats allow us to study genetic defects that impact on the inner ear. In the first, the main deficit is manifested by the degeneration of their cochlear or vestibular neurons, the second will have primarily a loss of cochlear of vestibular hair cells. These conditions resemble the more common mechanisms that produce deafness and vertigo in humans. In a third model, we will explore the interaction of stem cells with cochlear implants. Cell transplantation and implantation will be done through a surgical procedure under general anaesthesia. Animals are expected to be deaf, or to have vestibular problems. We anticipate that the vestibular problems will be detected with behavioural tests (e.g. walking on a beam) but should not substantially disrupt their daily routine. The severity of the procedures is considered moderated. At the end of the procedure, the animals will be humanely euthanized.

Application of the 3Rs

Replacement

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

Replacement

The cells to be applied have been extensively studied in culture, and we have evidence that they can differentiate into functional cells when treated with the appropriated conditions. We are performing more experiments in vitro, trying to understand their molecular and functional properties. However, interactions of cells with a live recipient are too complex to be modelled in the test tube. Before transplanting them into a human patient, we need to study them in an animal model to analyse the responses they may trigger, as well as their therapeutic effects.

Reduction

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

Reduction

Each experiment has a design subjected to statistical analysis to determine the ideal sample size, based on the predicted outcome. Cells to use are routinely monitored, to ensure their quality is appropriated for the experiments. Most experiments are performed as longitudinal studies, where an animal is monitored for a period of time, collecting information of different nature (e.g. hearing test thresholds, behavioural responses, etc.). On completion, tissue samples are collected for multiple analysis (histology, molecular profiling, etc.) maximizing the information gathered.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 initially selected the gerbil since their inner ear is very similar to the human one, primarily in the range of low frequencies they can detect. Rats and mice are also good models of hearing loss. Animals to be used are going to be closely supervised and all the interventions will be performed under anaesthesia and in sterile conditions. Analgesia will be administered during the post-operative phase and they will be monitored after the recovery from surgery in case of infections, which will be treated with antibiotics. Veterinary support is on site and their advice will be sought in any eventuality.

PROJECT 161

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.

Word limit; 1000 words

Project Title	Repairing the damaged brain after hydrocephalus
Key Words	hydrocephalus, axon regeneration, neuroprotection, scarring
Expected duration of the project	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;
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 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 hydrocaphalus 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: 2,000 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 of 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.

PROJECT 162

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.

Word limit; 1000 words

Project Title	The Breeding, Maintenance, Genotyping and Genetic Monitoring of both Genetically Altered & Wild Type Rodents
Key Words	Genetically Altered Rodent Breeding Maintenance
Expected duration of the project	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):

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 as 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 bought 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 an 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.

PROJECT 163

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.

Word limit; 1000 words

Project Title	Research on Bacterial Products used in Medicine
Key Words	Vaccine, Biological medicine, Safety, Potency
Expected duration of the project	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:
Yes	(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.

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 aims and objectives of the project are to be able to provide assurance that bacteriological products used in medicine, many of them paediatric vaccines, are safe and likely to be effective and also to allow the production of antisera necessary for the evaluation of these bacteriological 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)?

The principal benefit from this research is that the Division will have an improved understanding of whether a new vaccine or therapeutic product is likely to be safe and how it works. The knowledge gained will be used to inform regulatory process in the UK (MHRA), in Europe (EMA and EDQM) and worldwide (WHO). In addition, the data will be used for the development of an appropriate package of batch release tests. Typically the output from such research would be published in peer reviewed journals.

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

The project involves use of small animals only (mice, rats, hamsters, guinea pigs and rabbits). For the duration of the project (5 years) it is expected that up to 21,335 animals will be used (18020 mice, 1050 rats, 500 hamsters, 1,700 guinea pigs and 65 rabbits).

In the context of what you propose to do to 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 16 protocols, of which one is unclassified procedure, one is mild procedure, 10 are moderate procedures and 3 are severe procedures. The majority of animals are not expected to experience more than moderate adverse effects (for example irritation or inflammation at the site of injection, or signs of general toxicity such as loss of appetite, weight loss and reduced activity). Frequent monitoring and supportive husbandry measures are used to minimise the impact of these adverse effects. For the 3 protocols with a severe severity limit, 2 involving mice and one involving rats, adverse effects may include shock, convulsion, weight loss, trauma (as a result of brain injection), and loss of consciousness. In some cases death occurs for some of the test animals (up to 20% across these three protocols). At the end of all tests animals are humanely killed using a schedule 1 method. The impact of these adverse effects is mitigated as far as possible by rigorous observation of the animals by experienced staff and the application of recognised humane end points where necessary.

Application of the 3Rs

Replacement

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

Replacement

Protective potency and safety of biological medicines cannot be examined without the use of animal procedures, as multiple factors contribute to a protective immune response and the immune system itself is too complex to be modelled in vitro. Also in vitro biochemical models for safety are often product specific and in vivo assays are still required for new products or formulation as well as for product specific validation. The purpose of much of the research described in this licence application is to facilitate further reductions in the use of animals by validation of alternative procedures. However, multiple factors contribute to a protective immune response thus, in many instances there is no alternative but to generate data on vaccine safety and efficacy using existing animal models. The aim of this objective is to provide data to validate modifications, refinements and in vitro alternatives to widely accepted pharmacopoeial or other regulatory procedures. This can only be achieved by comparing the existing animal procedure with the novel assay designed to replace it.

Production of polyclonal antibodies by immunising animals with the required antigens is necessary to produce reagents needed for the evaluation of biologicals medicines and we continue to investigate the possibility of using of monoclonal antibodies produced in vitro (e.g. phage display) for suitability to replace the use of polyclonal antibodies.

Reduction

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

Reduction

The number of doses and group sizes used in the protocols described in this license are based on well established and validated methods that have been laid down in reference documents issued or endorsed by scientific advisory bodies such as the WHO, licensing authorities and national or European Pharmacopoeias.

In the absence of regulatory endorsed procedures, assays are designed based on the ARRIVE guidelines taking advice from biostatisticians, with an aim to use the minimum number of animals that is expected to provide information of required accuracy, precision and reproducibilty. Normally an experiment will consist of a sufficient number of experimental and control groups, each containing an appropriate number of animals, to obtain statistically significant 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

This work will employ animal species chosen from information published in literature or as required from regulatory guidance, documents or monographs on the basis of generating relevant effect and suitable dose responses. Such information will often be supported by collaborative studies taking into account suitability per product, generating information on multiple antigens and with the least severe end points where possible. Extensive validation studies have contributed a significant body of information on the use particular species or end point.

Most of the protocols are based on regulatory requirements and that some of these are severe due to the systemic nature of the infection/disease to be modelled. Efforts continue to refine these models and improve the welfare and monitoring of the animals during the studies.

In all probability the replacement or modification of an existing bioassay will ultimately result in the use of a less severe procedure for testing the potency or safety of bacteriological products. Thus, the existing pharmacopoeial protocol represents the “worst case” in terms of the severity of adverse reactions. To obtain the necessary comparative data for the validation of the new assay, it is necessary to perform the existing pharmacopoeial assay exactly as specified to obtain the definitive data. Prior to validation however, most, if not all, the development work leading to the establishment of the new assay can be carried out as standalone experiments without the need to include the existing assay for comparison.

Efforts continue to refine procedures with substantial or moderate severity limits. Challenge assays will only be used when there is no alternative. Protective animal models are considered more severe than serological models for the evaluation of vaccine potency. Where serological correlates of protection provide reliable indicators, serology is performed on a routine basis and protection models are used only occasionally to confirm potency of a new vaccine or therapy or to validate the serological assays, as protection is not a simple measure of antibody response but also depends on the affinity and the specificity of the induced response which could not be determined solely from the serological assay, unless it has been validated for the particular product under testing. The introduction of serology as alternative method to challenge means that the severity of the procedure is mild compared to the moderate limit assigned to the challenge models. The infant rat model is only used when a new vaccine/therapy is evaluated to demonstrate the correlation between in vitro and in vivo protection.

As part of routing ongoing animal welfare measures, animals are housed in groups in cages suitable for the species used, with a range of varied and appropriate enrichment to allow natural behaviour.

PROJECT 164

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.

Word limit; 1000 words

Project Title	Mechanisms underlying primary headaches
Key Words	Migraine, Headache, Pain, Brain
Expected duration of the project	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;
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):

Headache disorders are amongst the most disabling brain disorders in the world. They present as severe attacks of throbbing pain which may be accompanied with symptoms, such as visual disturbances, nausea, aversion to light and sound that render the sufferer unable to carry out normal daily functions. Given that approximately 50% of adults worldwide suffer from headaches annually and that migraine alone impacts approximately 16% of the world population there is a major unmet need to advance our understanding of this severe group of conditions from a scientific point.

Currently very few specific medications exist and the most effective acute treatments work only in approximately 30% of cases. The treatment of migraine is also confounded by the use of for example, anti-epileptic drugs which may have unwanted side effects and very few disease specific medications exists.

When you consider that there are approximately 1 billion sufferers of migraine alone, not to mention the more severe but rarer cluster headaches (females often describe the pain as worse than childbirth) the depth of the problem is clear.

A further major scientific and clinical need is to understand the underlying causes that lead to attack initiation as headache is only a small component of the disorder. As such, associated symptoms like fatigue and difficulty concentration can occur days before the headache and severely impact quality of life. In this programme of work we seek to generate new knowledge on what causes these associated symptoms and how this can lead to attack triggering with a view to developing targeted effective therapies with limited potential side effects.

Separate to the suffering highlighted above to sufferers, the economic cost of headaches is unacceptably high. It is estimated to cost the EU over €43.5 billion per year in healthcare and lost productivity costs (days off work/school etc.) and places an enormous burden on the NHS. Resulting in a large proportion of A&E admissions and 1 in 4 neurologist appointments. Thus a greater understanding and improved treatment of headache disorders would substantially benefit sufferers and help ease the burden on the NHS.

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

The first potential benefit from this project is an increased understanding of what causes headache and why different individuals such as females (females are up to 3 times more likely to suffer from migraine than men) are more prone to migraines for example. This will also include an improved understanding of associated symptoms such as the aversion to light and sound common in some people during attacks. A second potential benefit is an increased understanding of the role that headache plays in other disorders such as epilepsy. The information gained from this project will advance general scientific knowledge, but also strive to lighten the burden of sufferers and help reduce the financial burden of the disease. The project will benefit from close collaboration with clinical colleagues and industrial partners ensuring rapid sharing of appropriate results to help advance the development of novel treatment options.

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 number of animals with human studies or other methods used when possible. Only when essential for specific aims will rats and mice be used. We expect to use 1,900 rats and 3,500 mice over the five year duration of the project; however, every effort will be made to minimise this number.

In the context of what you propose to do to 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 types of surgeries that will be performed are known to lead to rapid and uneventful recovery and every effort is made to minimise the impact to the animals (rats and mice) including full general anaesthesia during surgery, good surgical technique, detailed aftercare monitoring/intervention plans and pain relief as appropriate. All such procedures are discussed and agreed in advance with our named veterinary surgeon. Surgical interventions are conducted under sterile conditions where appropriate and include for example surgeries to allow the placement of stimulating and recording devices into specific areas relevant to headache and the placement of a drug delivery system under the skin of the animals. Complications including infection are rare due to the above mentioned good

surgical techniques. Any animal that shows signs of ill health, for example excessive weight loss or infection will be removed from the study and humanely killed. In such circumstances the named veterinary surgeon will be informed. Rats and mice may be exposed to alterations in sleep-wake patterns and this results in only a minor jetlag like effect that has no lasting impact. Behavioural tests conducted (e.g. sensitivity to touch or mild non-damaging heat) commonly measure for example the natural behaviour of the animal or the threshold for the animal to withdraw from a stimuli such as gently touching their cheek with a plastic fibre that is flexible to prevent excessive force. Thus the above readouts expose the animals to only limited mild transient stress that is further minimised by training the animals to become familiar with the equipment used. Given that we will explore light-aversion in animals this will again be conducted in a device that allows the animal to withdraw from the light into a dark zone and as such the animal can freely walk into the dark zone providing us with a readout of light-sensitivity without excessive exposure to bright light. All animals will be carefully monitored to ensure good health and minimal stress responses, any animal that shows signs of distress during behavioural work (e.g. excessive movement/weight loss) will be immediately removed from the test and if the signs persist the animal will be removed from the study and humanely killed.

Application of the 3Rs

Replacement

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

Replacement

As we are investigating a complex condition which only affects species with a nervous system we have no alternative but to conduct aspects of this work in animals as we cannot replicate a functioning brain and nervous system in a dish in a laboratory. Where possible we will aim to work with clinical colleagues to use human models and any work that we do conduct in animals outlined in this project will be in rodents.

We will utilise a number of approaches to minimise animal use including using stored samples of rodent tissue from previous work or human post-mortem tissue where possible.

Reduction

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

Reduction

All work which will be carried out under this project is and will be carefully planned by experts to ensure the minimum number of animals are needed for each study. This

includes working with trained statisticians and our own expertise in this area which allows a pre-calculation of the number of animals needed to answer a specific scientific question. No study will be conducted unless the scientific goals are deemed to be of significant scientific impact and this will always be balanced against the impact and number of animals required.

We use a number of novel highly effective models related to different headache disorders and where possible we use internal control data to minimise the number of animals used.

We will further use novel genetically modified mice which carry human mutations which predispose individuals to forms of migraine. While these conditions are rare we can use animals to model the condition and to gain detailed information about more general headache disorders. As these mutations are known and controlled the number of animals needed for this type of work is often lower than for unaltered 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

We intend to use a number of models of primary headache disorders, the majority of which have undergone rigorous scientific review for their benefits and ability to detect meaningful clinical predictions.

Each of the models allows a different component of headache to be targeted. We and others have used these models for a number of years carefully characterising their benefits and refining the procedures in rodent models to minimise the use of higher species.

Aspects of the work are conducted under non-recovery anaesthesia to minimise the impact on the animals concerned. However, where appropriate animals may undergo chronic drug administration/interventions during their lifetime. We have fully optimised these interventions and where this has not been possible pilot studies will be conducted to ensure full optimisation of doses and techniques prior to large-scale studies.

We will provide post-operative pain relief where appropriate and when it does not interfere with the experimental aims, for example during certain surgical recovery experiments.

For genetically altered animals the adverse signs associated with the genetic alteration are not always known but for existing ones, used on this project, there are no known adverse health effects arising from the genetic alterations.

At all times during the project should any unexpected adverse events occur animals will be humanely culled and immediate advice requested from the named veterinary surgeon to ensure we understand what went wrong?

PROJECT 165

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.

Word limit; 1000 words

Project Title	The development and plasticity of synaptic circuits
Key Words	Brain, Synapse, Development, Plasticity
Expected duration of the project	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):

Nearly every neurological disorder is associated with abnormal connections between nerve cells, which are called synapses. Synapses are the major route by which nerve cells communicate with one another and therefore understanding how synapses are formed and maintained is crucial if we are to understand disease processes. To advance our understanding and identify new avenues for the treatment of neurological disorders such as epilepsy, autism and schizophrenia, we require a much greater understanding of how much of a neuron's synaptic connectivity is controlled by its genetic information (i.e. cell intrinsic information) and how much is influenced by ongoing signalling between cells as the brain is developing (i.e. cell extrinsic information). This project will examine how a neuron selects the other cells it will form synaptic connections with, the type of synapse it forms (e.g. an excitatory synapse versus an inhibitory synapse) and how the strength of each synaptic connection can be regulated (i.e. synaptic 'plasticity'). More specifically, we will investigate three areas of scientific unknowns: (i) We will examine how neuron's synaptic connectivity is influenced by the cell from which it is born during embryonic development. The genes that control this process represent candidate molecules that are implicated in neurodevelopmental disorders such as autism and schizophrenia. (ii) We will examine the effect of changes in the strength of inhibitory synapses, such as occur during normal brain development and our sleep-wake cycles, but also in pathological states such as epilepsy and chronic pain. (iii) We will examine how different types of nerve cell contribute to the mechanisms by which synapses are able to change their strength, which is thought to be crucial for normal brain development and recovery from injury.

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

The benefits will be the advancement of the scientific community's knowledge about the genetic and environmental factors that control how neural circuits are generated during development and how they are altered by ongoing neural activity within the nervous system. These benefits are worthwhile because abnormalities in how synaptic connections are formed are believed to underlie neurodevelopmental disorders, including epilepsy, schizophrenia and autism. Findings from the work will be published in academic journals and the resulting information will be of immediate interest to physiologists, neuroscientists, molecular biologists and clinicians, with an interest in the process of brain development at the cellular level. It will also be directly relevant to scientists examining how the nervous system can exhibit 'plastic' changes, such as occur in degenerative and regenerative diseases. A longer-term benefit relates to the application of the results of the work, which may be of value in the identification of molecular targets at which new pharmaceutical products could be aimed in order to improve the development of neural circuits in individuals with neurological disorders.

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

This proposed programme of research will use wildtype and transgenic mice, rats and Xenopus laevis. Over the lifetime of this licence all effort will be made to minimise the numbers of animals used. Our licence application estimates that we will not use more than 19,300 mice, 460 rats and 700 Xenopus laevis over the 5-year lifetime of the licence. More than half of these animals (>50%) are for breeding rather than experimentation.

In the context of what you propose to do to 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 the project will be classified as either 'mild' severity (86%) or 'moderate' severity (14%). There are no protocols that are categorised as 'severe' in this project. The vast majority of mouse breeding (>98%) uses a mild protocol as these transgenic animals are well characterized and will not exhibit any adverse effects. A small number of transgenic mice (<2%) will be bred under a moderate protocol as it is possible that these animals will exhibit adverse effects associated with their transgene. For example, one transgenic line is reported to exhibit reduced bodyweight and balance difficulties. For these reasons, heterozygous mice will be used wherever possible and rearing conditions may be altered to mitigate any adverse effects. In all cases, the intention is not to study these effects and monitoring of the animals would be tailored in a graded and targeted way, to be best placed to intervene before any undue suffering. Clear end points will be agreed in advance with the local Veterinary Services and/or Home Office Inspector. Xenopus laevis breeding will use a mild protocol in which the eggs are collected from a female

Xenopus laevis and mixed with a male's sperm in vitro. This maximises fertilisation success and, after the appropriate time, the female can be re-used once they have been assessed by a vet and confirmed to have a healthy appearance, normal feeding behaviour and a successful breeding history. Although uncommon, an expected adverse effect is that the egg collection can make an animal more susceptible to infection. This is minimised by the adoption of aseptic methods, by minimising stress associated with handling and by close monitoring of the animal's behaviour and skin. Our main experimental approach is to manipulate synapses in the brain by altering synaptic activity through changes to the animal's environment or by using targeted molecular-genetic modifications. Altering the animal's environment will not normally lead to expected adverse effects, although in some instances could lead to anxiety. For this reason, the least disruptive alterations to the environment will be used and monitoring of the animals will be tailored to be best placed to intervene before any undue suffering. The molecular-genetic manipulations of synapses will involve performing surgery on a subset of the animals under general anaesthesia. For example, a subset of the mice, rats and Xenopus laevis tadpole embryos will receive an injection of substances (e.g. to label cells or activate genes) whilst under appropriate anaesthesia, analgesia and aseptic surgical techniques. To study aspects of early brain development, one technique will involve injecting substances into the brains of developing mouse embryos, which requires surgery to be performed on pregnant female mice. Certain adverse effects are expected with these surgical procedures, including post-operative pain and the risk of infection/inflammation. These adverse effects will be mitigated through appropriate surgical technique, the use of pain-relieving drugs and careful post-operative monitoring. Various measures will be used to detect signs of adverse effects before they emerge fully, so as to reduce their frequency and severity. In a small number of cases, and only where it is experimentally essential, brain activity will be monitored in the non-anaesthetised state. In mice, this will involve mounting a lightweight device on the head, which could be associated with some initial discomfort. This will be mitigated by close monitoring for signs of altered behaviour, the use of analgesics and by providing opportunities for habituation to the apparatus. At the end of all of the experiments, animals will be killed using a Schedule 1 procedure or terminal anaesthesia and used for either in vitro electrophysiology or histological analysis. Outcome measures will include measurements of synaptic function in in vitro models, plus the assessment of molecular and cellular markers in postmortem brain tissue.

Application of the 3Rs

Replacement

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

Replacement

Neurons are not present outside the animal kingdom, so an animal is required. There is no alternative that would entirely replace the use of a living animal that would allow all of the research objectives to be met. Although computer models continue to provide great insights into neuronal network behaviour, the models currently available in the field are not yet sufficient to address our key objectives here as many of the basic variables are still unknown to us (e.g. synaptic connectivity between neurons, activity patterns in certain brain regions). Cell culture systems, which provide large benefits to other areas of science, do not recapitulate the full gamut of neuronal activity and often form aberrant synaptic connections. As such, the proposed project necessarily involves experiments on animals.

Reduction

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

Reduction

The number of animals used will be kept to a minimum at every stage of the project and by several means. Firstly, by careful planning of breeding and experiments (e.g. power analysis) in close contact with staff and animal technicians we will minimize animal numbers. Secondly, the designs of our experiments are such that we maximize the usage of tissue and minimize the number of animals. For example, a single animal can be used to generate 12 in vitro brain slices and each of these can be used to record 4 neurons allowing the recording of up to 48 neurons from a single animal. Any surplus slices can furthermore be used for immunocytochemical studies. Thirdly, we will use animals from all genders and both homozygotes and heterozygotes wherever possible. Lastly, we will techniques to genetically alter single animals, which will alleviate the need to generate new genetically, modified mouse lines, which typically require the breeding of many generations 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

The choice of mice and rats for the project is based on the fact that these are very well established species for understanding molecular and cellular processes in the mammalian brain. Nerve cells in mice and rats have similar morphologies and use the same chemical transmitter systems as higher mammals, including humans. The mouse and rat are two of the least severe species that will produce satisfactory results because (i) as systems to understand mammalian development they have a

relatively low neurophysiological status, (ii) they are well established as organisms for understanding brain development, and (iii) the mouse has become a species of choice due to the use of techniques for genetic modification in which specific proteins can be manipulated in specific groups of neurons. The Xenopus laevis tadpole is chosen because it makes it possible to study aspects of embryonic development in a vertebrate system, which would be much more difficult in mammals. The Xenopus laevis tadpole is largely transparent, which means that imaging of the nervous system is feasible and can be directly correlated with other cellular measurements. The proposed techniques are the most appropriate to address the scientific questions and all are at the forefront of research. The methods have been developed over many years in close collaborations between scientists, animal technicians and vets. A series of general measures are designed to minimise welfare costs. These include the use of aseptic surgical methods under appropriate anaesthetic and analgesic regimes, the use of the least invasive methods for delivering genes to small numbers of neurons and the use of the most temporally and spatially refined measurements/manipulations of neural activity.

PROJECT 166

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.

Word limit; 1000 words

Project Title	Molecular mechanisms that regulate vertebrate development and cancer cell behaviour
Key Words	Vertebrate development, In vitro cancer cell behaviour, Cell-cell interactions
Expected duration of the project	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):

The long-term goal of this project is to elucidate the molecular mechanisms of cell-to-cell interactions during vertebrate development. We will also examine cell-cell interactions during cancer development in vitro. Cell-to-cell interactions play crucial roles in development, and identification of cell-to-cell signalling molecules and their functions is one of the major goals of developmental biology. However, the molecules that are involved in those processes and their functions have not been fully identified.

In this project, we will focus on specific molecules that have been shown to play important roles in vertebrate development and regulation of cancer cell behaviour in our previous studies. We will continue our investigation on the function of those molecules in the present project.

Our approach to study the function of those molecules in development involves two steps. First, the expression pattern of a particular molecule(s) will be altered, by using targeted gene disruption in mice or by introducing ectopic genes into chicken embryos. Second, the effects of alteration of expression patterns will be examined, by histological and molecular biological techniques.

We expect that results from the proposed studies will provide novel insights to the molecular mechanisms that establish the complex structure of the vertebrate body. In addition, since many congenital disorders affect neural development and organogenesis, they will also provide essential information on the pathogenesis of such 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)?

Our body has a complex three-dimensional structure, and precise development of the structure is essential for proper functioning of our body. In development of our body, different cells (a functional unit of life) communicate with each other by using a variety of molecules, and such cell-to-cell interactions play important roles in establishment of our body structure. Cell-to-cell interactions also play important roles in cancer development and understanding these interactions is necessary to develop new targets for cancer therapy. Our projects aim to elucidate the functions of specific cell-cell interaction molecules in establishment of correct structure of our body and in regulation of cancer cell behaviour. The projects are likely to have clinical implications. Since functional defects of those molecules have been shown to cause abnormal development in animal models and have been implicated in regulation of human cancer development. Therefore that results from our studies will provide important insights into the mechanisms of human congenital disorders and cancer development. In addition, we expect that our results will also lead to development of novel therapeutic approaches for human cancer.

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

Mouse: 1,500 Rat: 50 Chick egg: 500 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?

The expected level of severity is mild or non-recovery. The mice (normal and genetically altered) used in our studies behave normally. Young mice may be injected with a tracer substance to show the position of developing cells and nerve fibres. In ovo electroporation and injection into chick embryos could cause a very low level of abnormal embryonic development and premature embryonic death. At the end of the studies the animals will be humanely killed and tissues analysed.

Application of the 3Rs

Replacement

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

Replacement

The vertebrate body has a complex three-dimensional structure, and currently no alternative systems are available that faithfully reproduce the in vivo structure and are suitable for our studies. Therefore, the use of animal models is essential for our research project.

We will use rodents and chickens as model systems, since basic information (anatomy, physiology, development etc.) on the nervous system and other organs is available. In addition to the animal work, we will also perform non-animal studies, including tissue culture experiments, to study biological functions. We expect that about 70% of work will involve animal usage.

Approximately 50% of our experiments will be carried out using early stages of chick embryos (before E10). Much of our work does not involve animals covered by the Animals (Scientific Procedures) Act, and all of our work at this current point in time is complete before chicks hatch.

Reduction

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

Reduction

Based on our previous experience in similar studies, the number of animals to be used in our studies will be maintained at the minimal numbers required.

We will also use appropriate statistical tests to ensure that the animal numbers to be used will be minimal and scientifically reasonable. In most of our studies, we only use ex vivo tissues or fixed tissues. When living animals are used, the 3Rs will be applied.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 our project, we will use mice, rats, and chick embryos. Those animals have been well-studied as model organisms, and extensive basic information (e.g. development, anatomy, physiology) has been described, which is necessary for our studies. In most of our studies, we will use embryos and very young animals.

The expected level of severity is mild or non-recovery.

The experimental procedures (e.g. mouse colony management, injections, electroporations) will be carried out by very experienced researchers/staff.

We have carefully considered the planne use of anaesthesia, analgesia and other pain relieving methods.

PROJECT 167

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.

Word limit; 1000 words

Project Title	Autophagy and other modulators of proteinopathies
Key Words	Neurodegeneration, Proteinopathies, Autophagy, Proteostasis, Physiology
Expected duration of the project	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):

Proteinopathies are diseases where proteins within cells do not form and function correctly. This includes many of the major neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease. Although these diseases are caused by different types of mutations, they show a common feature in neurons and their support cells: proteins clump into large "aggregated" structures which are toxic and result in neuron death.

There is an urgent need for new drugs and therapies to halt, delay and prevent proteinopathy diseases. This project focuses on two areas that regulate proteins in cells. The first is the cellular process called "autophagy", which is a way that cells can break down protein aggregates and recycle them. The second is chaperone biology. Chaperones are a group of proteins that correctly fold other proteins so that they do not clump into aggregates.

Several studies have showed that modulating autophagy or chaperones can be beneficial to treat neurodegenerative diseases and help neurons to cope with protein aggregates.

Moreover, it has also been suggested by several research groups that, when autophagy or chaperons function less efficiently in a cell, the nervous system is more prone to develop neurodegenerative diseases.

Therefore our project has three aims:

1. To further develop the potential of increasing autophagy in cells, in order to remove disease causing proteins and aggregates, as a therapeutic strategy for the treatment of proteinopathies.

2. To investigate the physiological (normal) function of autophagy and how changes in it may lead to disease progression. This expands our understanding of how this process works and will provide new avenues to manipulate autophagy for therapeutic purposes in the future.
3. To further our understanding of chaperone biology with particular reference to how this might be perturbed in disease and how modulating the function of these proteins may be beneficial in neurodegenerative diseases.

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

At the first level, we are testing approaches and compounds for their therapeutic benefit directly in models of neurodegenerative disease. In the long term we hope that these studies will be used as the basis for further translational therapeutic trials in human patients. At the second level, the main benefit from work carried out under this licence will be scientific advancement, particularly in the areas of autophagy and chaperone biology. This will be published and presented to the global scientific community in particular, providing pioneering information for discussion and review. Overall we hope to identify new genes and compounds that allow us to better understand and manipulate these processes. This will benefit and guide future therapeutic studies.

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

We plan to study at least three mouse models for neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington disease. Moreover we will study several transgenic mice to understand the function of autophagy and chaperons in mammals. Given the wide approach of our research, we plan to use up to 20 000 mice in five years. They will be disease models (transgenic) and wild type.

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

Over the period of the license, we may generate new mouse lines using standard protocols for superovulation and embryo implantation into pseudo-pregnant females. We will also carry on breeding of genetically altered animals that will allow us to study the role of autophagy and chaperone biology in neurodegeneration. For this purpose, mice are likely to undergo mild to moderate procedures. In parallel to our in vivo studies, we will also generate primary cells, including neurons, glial cells and fibroblasts. For this reason, we will use mouse embryos or pups (P1-P3) that will be killed by Schedule 1 method and will not exceed subthreshold severity. To investigate the role of new autophagy and chaperone modulating pathways in neurodegeneration, we will use mouse models for Huntington's disease, Alzheimer's disease/tauopathy and Parkinson's disease. All these models over-express mutant proteins found in patients with familiar types of the respective disease and show age-dependent disease relevant moderate phenotypes. We will administer compounds

that modulate autophagy or chaperone related pathways through the least invasive method (intraperitoneal/ subcutaneous/ intravenous injection, food/drinking water, oral gavage, osmotic mini-pumps) and perform studies to measure relevant disease phenotypes. Our approach will include measurement of the levels of the respective mutant protein, histopathological examination, behavioural analysis and ageing studies. Successful compounds will be further tested for their suitability for translation into patients. This will require studies to validate drug effects at concentrations relevant to those that can be achieved in humans. For those studies, the mice are likely to undergo subthreshold to moderate procedures. Our previous work has identified novel pathways that regulate autophagy and chaperone biology. To further understand their role in autophagy and chaperone modulation in vivo, we will characterise genetically altered mice for the relevant genes. These mice will be tested by behavioural, biochemical and histopathological studies. We will also mate these mice with our mouse models for neurodegeneration or with autophagy reporter mice. For those studies, the mice are likely to undergo subthreshold to moderate procedures. 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

We replace all possible elements of our mouse research with cell culture work and a variety of *in vitro* approaches.

However, there is no single alternative to assessing disease models in the context of a mammalian brain, especially if one wants to have a proof-of-principle model for testing potential therapeutics. As such, our cell culture and alternative model approaches guide all elements of our work that cannot be replaced. This includes both aspects of our aims for scientific advancement and therapeutic approaches.

Reduction

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

Reduction

We use the minimum number of animals through careful planning of our experimental design and breeding the minimum number of animals to meet this target. We have a dedicated member of staff to identify the genetic background of every mouse produced to ensure we can correctly define and use each mouse experimentally. We also maximise our use of animals beyond their lifecycle by

routinely collecting and storing tissues for later re-use in other experiments as part of histological and biochemical 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

In addition to our mouse models, we also work with and are continuing to generate zebrafish models to replace our work with mice where suitable. However, mice are a powerful animal model as they are amenable to genetic and compound modulation. Our animal models of neurodegeneration develop either the clinical signs seen in human disease – such as our Huntington's model, or human pathology characteristics as in all our disease models. They show clear behavioural, biochemical and histopathology changes that we can measure and aim to modify. In the case of our Huntington's disease mouse model, we have carefully established processes with monitoring to ensure that we minimise their suffering as a result of the disease.

Our emphasis is on understanding cellular processes in mice as a step towards modulating these processes for treating human disease. Our experimental approaches are guided by cell and zebrafish model work such that we carefully build on a solid scientific foundation when aiming to generate and modify new mouse models for understanding and modulating proteostasis.

PROJECT 168

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.

Word limit; 1000 words

Project Title	Research on products used in prevention and therapy of tuberculosis and malaria
Key Words	BCG, tuberculosis, malaria, safety, potency
Expected duration of the project	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;
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.

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 broad objective is to provide quality assurance that vaccine and therapeutic products used in medicine for tuberculosis and in some cases, malaria, are safe and likely to be effective. This also includes studies of how these medicines work in a living body.

What 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 is to provide assurance that vaccine and therapeutic products used in medicine for tuberculosis and in some cases, malaria, are safe and likely to be effective. A high level of assurance is particularly important for vaccines given to healthy people. If public confidence in vaccine safety/ effectiveness falls, vaccine uptake and coverage may be reduced and diseases that were previously well controlled by vaccine can re-emerge. Other potential benefit includes improvement of understanding whether a new vaccine or therapeutic product is likely to be safe, how it works and how its quality to be regulated. The knowledge gained will be used to inform regulatory process in the UK and worldwide.

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

The project involves use of small animals only (mice, guinea pigs and rabbits). For the duration of the project (5 years) it is expected that up to 5460 animals will be used (maximum of 5000 mice, 460 guinea 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?

The project including 8 protocols consist of procedures of moderate severity level. The animals may receive substances such as vaccines by standard routes (for example subcutaneous and oral). Animals may be challenged with malaria or TB including malaria by mosquito bites which is carried out under anaesthesia. Blood samples may be taken. Non-invasive imaging may be carried out under short duration anaesthesia. The majority of animals are not expected to experience more than mild to moderate side effects (for example skin irritation or swelling at injection sites). In rare occasions, uncontrolled infection would result in gradual loss of body weight and reduced physical activity. Frequent monitoring and supportive husbandry measures are used to reduce the impact of these side effects. At the end of all tests, animals are humanely terminated. The impact of these side effects is reduced as far as possible by frequent observations of the animals by experienced staff and the application of recognised humane end points where necessary.

Application of the 3Rs

Replacement

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

Replacement

Some protocols used in this project are performed according to methods described in regulatory guidance. In some cases, no suitable non-animal alternatives are currently available. In particular, protection and safety of a biological medicine cannot be fully examined without the use of animal procedures, as multiple factors contribute to protective response and the immune system itself is too complex to be modelled in cell culture. Efforts are continuing to develop suitable alternatives to some of the methods that are retained in this project licence.

The detection of biomarkers of protection could in the long-term result in progression towards suitable *in vitro* detection systems to replace some of the *in vivo* work.

Reduction

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

Reduction

For some protocols used in this project, the number of animal groups and size of each group are based on validated methods that are described in regulatory guidance. The number of groups and/ or group size of the other protocols are based on previous experience on small pilot studies, or on information from published literature or collaborators gained with the similar procedure and the advice of experienced biostatisticians. Many tests require the use of one or more control groups and where possible, many test samples will be included together in a single

experiment to maximise the use of these control groups and therefore minimise the total number of animals used during the project. New whole body imaging method is also introduced for monitoring distribution of biological medicine within a sedated live animal in order to reduce number of animals sacrificed at various 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

The protocols involving infection with tuberculosis and malaria are designed to reduce signs of disease and severity. Humane end points are determined such that animals can be euthanized at the earliest stage of unexpected adverse events to minimize the impact of infection on animals. Frequent monitoring by experienced staff, including out-of-hours checks for some procedures help to ensure that animal welfare is maintained wherever possible. As part of routine and ongoing animal welfare measures, animals are housed in caging suitable for the species used, with a range of varied and appropriate environmental enrichment to allow natural behaviours.

PROJECT 169

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.

Word limit; 1000 words

Project Title	Regulation of embryonic lineages and stem cells
Key Words	Embryo, Pluripotency, Stem cells, Gastrulation
Expected duration of the project	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):

The overall aim of this project is to understand how cells in the mammalian embryo are instructed and controlled during development, and how these cells can be harnessed and used to model development in tissue culture. This is important because our ability to generate specific tissues in culture would hold great promise for replacement of damaged tissues, but we need to be sure that such tissues will function normally. Understanding the process of mammalian development is important for the design of strategies to generate specific tissues in culture and is also of wider scientific interest because the decision-making processes involved in forming an embryo are also commonly adopted for other biological 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)?

There are several reasons why we want to understand how mammalian embryos develop. Firstly, although in vitro fertilisation (IVF) programmes have helped many patients to conceive, disappointments still occur, usually during the early stages of pregnancy. Our research using preimplantation mouse embryos will enhance understanding of how the essential tissues are generated and regulated for normal development and implantation. This knowledge will also benefit production of embryos from livestock and as non-human mammalian models for research. There is already widespread use of stem cell lines and specific tissues produced from them in biomedical research. However, the full repertoire of cells in the body is not yet available in a dish. To generate the best culture models for disease modelling or drug screening we need to understand how they are formed in the developing embryo. Our research is directed at understanding the important early stages of the process. Finally, if tissues generated in culture are to be used to replace damaged

tissue in patients, it is essential that the donor cells behave as intended and do not malfunction after transplantation, or turn into cancers.

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

We expect to use a total of 13,500 mice during the 5 years of this licence. Of these, the majority (up to 11,500) may be genetically modified.

In the context of what you propose to do to 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 without any abnormalities for the production of early embryos to be used in short term analysis. Of the ~2,000 expected to be used in surgical procedures, up to 250 may receive transplants of cells or tissues that may form tumours. None of these tumours, should they form, 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 only 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 replace the use of animals with cultured cell lines whenever possible. However, we sometimes need to breed mice carrying particular genetic modifications to provide early embryos for short-term experiments or to generate new stem cell lines. Unfortunately, there is currently no culture alternative that exactly mimics normal development. For this reason we sometimes transfer embryos that we have manipulated in culture into foster mothers for further development. We also occasionally need to determine the full repertoire of tissues that a cell line can produce in the context of a living body, in which case we may transplant the cells into an adult mouse.

Reduction

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

Reduction

We minimise the numbers of mice we need to use primarily by making sure our breeding programmes are the most efficient for providing the genetically modified

embryos we require. Wherever possible, and particularly for the purposes of training new staff and students, we use spare mice generated from our breeding programmes to mate for provision of early embryos to reduce the need to bring in extra mice. Our extensive experience in managing breeding programmes for the generation of embryos for research contributes considerably to the efficiency with which we can reduce the numbers of mice we maintain.

The methodologies for generating genetic modifications have now evolved sufficiently to enable us to delete specific genes in an inducible manner. This feature is particularly relevant for most of our work involving transgenic mice, since we are interested in the function of genes required to enable normal early mammalian development. Conventional deletion of the copies of such essential genes from both parents (known as 'homozygous null') in an embryo results in developmental failure. This means that homozygous null embryos can only be produced by mating male and female mice carrying one deleted copy of the gene each, and therefore, by Mendelian genetics, only 25% of embryos would be expected to be homozygous null. However, being able to breed mice carrying special modifications on both copies of the gene of interest that allow its deletion only when the embryo is exposed to a deleting agent means that all the derivative embryos from a mating have the potential to be made homozygous null. As a result, up to 75% fewer genetically modified mice need to be maintained to generate as many homozygous null embryos as would be required from animals carrying only a single deleted gene.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 animals for our research because they can provide all the genetic modifications we require. Furthermore, they are small and easy to breed, reaching sexual maturity within two months from birth and have the capacity to produce large numbers of embryos. Because of the biomedical relevance of our work, we need to use a mammalian system, and current scientific evidence indicates mice have a significantly lower potential for pain perception than other mammals. The majority of animals on this project are not expected to be subject to any pain, suffering, distress or lasting harm. A small proportion of our animals undergoing regulated procedures (<20%) will be subjected to surgery, which involves preparation for procedures required for making female mice receptive to transplantation of embryos, or for extending the period of development just before implantation (known as 'diapause'). All animals undergoing surgery will be provided with pain relief. Adult mice receiving transplanted cells to be tested for their potential to generate tissues will be carefully monitored daily for signs of ill health. If signs of ill health are

apparent, or developing tumours are causing anything more than minor discomfort, the animals will be humanely killed.

PROJECT 170

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.

Word limit; 1000 words

Project Title	Modulation of Wound Healing
Key Words	wounds, skin, trauma, chronic, therapies
Expected duration of the project	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:
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):

Tens of millions of people (worldwide) experience traumatic wounds every year. For many severe trauma patients the control of blood loss is essential for their survival, for others, it reduces the need for transfusion and extended hospitalisation. Scar tissue is the end result of the wound healing process – a tissue that is both functionally inferior to normal skin and a common cause of disfigurement.

The NHS spends approximately £5billion annually on the treatment of chronic non-healing wounds, such as leg ulcers, pressure ulcers and diabetic foot ulcers. These wounds are debilitating, painful and decrease patients' quality of life. They are slow to heal and usually afflict the elderly. With an aging population the burden on the NHS will increase in the years to come.

A significant complication of both traumatic and chronic wounds is that of infection. The increase in bacterial resistance to antibiotics has led to a dramatic rise in the number of both community and hospital-acquired infections - with limited means of treatment.

The control of blood loss and scarring after traumatic injury, and the numbers of chronic and infected wounds are all significant burdens - to patients, to those responsible for providing treatment, and to those responsible for paying for that treatment.

Current treatments for these conditions are largely ineffective – more effective therapies are required.

The aim of our work is to assist in the development of new therapies that minimise blood loss and reduce disfigurement after traumatic injury, promote the healing of chronic wounds (e.g. leg ulcers) and clear wound 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)?

It is anticipated that work undertaken on this project will help in the development of new, more effective, therapies able to - reduce blood loss and scarring after trauma, promote the healing of chronic wounds and control wound infection.

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

We expect to use up to 3600 mice, 1100 rats, 300 rabbits and 260 pigs over the 5 year period 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 expected adverse effects include discomfort due to wounding and treatment, unplanned infection, implanta-tion of materials and inoculation of wounds with bacteria. Procedures likely to cause pain or distress to animals will be performed under general anaesthesia, and all animals will be given post-surgical pain relief. All animals will be frequently monitored for the develop-ment of adverse effects and signs of ill health and any an-imal found to be displaying signs of distress or discomfort, that does not respond to remedial actions, will be hu-manely killed. Our experience has shown that the procedures to be fol-lowed in this project are well tolerated by the animals we use. We have not previously observed and do not expect any significant physical or behavioural deviations from normality. While the maximum likely severity for all procedures is moderate, it is expected that the majority of animals will experience mild and transient discomfort. All animals will be culled at the end of any given study.

Application of the 3Rs

Replacement

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

Replacement

The way in which the body heals wounds and deals with wound infection is very complex; involving cells and processes both at the wound site and in other parts of the body.

Currently, it is impossible to replicate all the events involved in wound healing *in-vitro*, therefore studies on live animals must be undertaken to evaluate new therapies prior to their use on patients.

Only agents considered suitable, according to existing data or after preliminary tissue culture studies, will be investigated in this work.

Reduction

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

Reduction

Our group sizes are the lowest possible to allow for the infrequent “in study” loss of animals (due to complications); whilst maintaining a high probability that a study will be sufficiently powered to detect a statistical difference. This reduces the likelihood that animals will undergo unnecessary procedures in statistically underpowered studies.

We also ensure that the minimum number of animals are used by:

Performing prior *in vitro* testing, to screen-out inappropriate investigational agents.

Running small scale pilot studies – to screen out less effective formulation variants.

Using control data from previous studies (wherever possible) – which reduces the need for control animals in each study.

Using multiple wounds per animal (where possible) – this reduces animal usage by permitting several investigational treatments and control treatment to be tested in the same animal.

Using single strains of animals matched in terms of age and gender - which reduces variation between animals - reduces the number of animals required to detect significant 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 undertake our work on mice rats and rabbits, because they have been widely used, and techniques well developed, to screen wound healing therapies prior to human use. Some studies will be carried out in pigs; as their skin is very similar to

that of man and wound healing studies in pigs are highly predictive of effects on humans.

The size of the wounds we create, the number of interventions undertaken, the volumes of test materials applied, and the number of repeat assessments over time - are kept to the minimum necessary to generate scientifically valid data. Wherever possible, environmental enrichment will be provided to animals (e.g. forage food, nesting materials and wooden chew-sticks for rodents; forage food, talk radio and balls for pigs).

PROJECT 171

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.

Word limit; 1000 words

Project Title	Hypoxia and Cancer: Molecular Mechanisms and Therapeutic Strategies
Key Words	Hypoxia, cancer, metastasis, immunotherapy
Expected duration of the project	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):

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.

PROJECT 172

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.

Word limit; 1000 words

Project Title	UK Reference Laboratory activities for rabies and other viruses
Key Words	Inocuity, Virus, Isolation, Disease, Diagnosis, Zoonotic
Expected duration of the project	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;
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) highe/r education or training for the acquisition, maintenance or improvement of vocational 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 aim of this project is to support the research and diagnostic activities for statutory functions on rabies, arthropod-borne and wildlife viruses of veterinary and zoonotic importance. It does this through the objectives detailed below.

What 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 outputs from the objectives defined in this license will have the following benefits: 1) Inocuity testing of inactivated biological products to enable removal from high containment will enable further studies with biosafe material derived from high consequence pathogens. 2) Isolation of virus of high scientific value in mice will enable characterisation of novel viruses which will help inform policy and vaccination requirements. 3) Production of positive control material for diagnostic assays will support diagnostic testing to UKAS accredited standards.

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

A maximum of 650 mice over the course of 5 years.

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

There are two protocols on this license; both have a maximum severity of moderate. Through frequent monitoring of animals and pre-emptive end points it is hoped to restrict actual severity to moderate although with the nature of the viruses being used this means clinical progression speed of onset and development of disease is variable. Where isolation of novel pathogens is involved, clinical score sheets with

humane endpoints will be followed. All animals will be humanely killed 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

For protocol 1, mouse models are only used in situations where in vitro methods of inocuity testing are not available.

For protocol 2, mouse models are only used where previously, the isolation of virus in vitro has failed and for the generation of authentic control material and in vivo work is required.

Reduction

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

Reduction

For some viruses, intracranial infection will provide the most reliable method to ensure sensitive and consistent infection. This will reduce the number of animals required to achieve the required outcome. Expert statistical advice will be sought for each study to ensure the minimal number of animals is used to get a meaningful result. All studies at the establishment meet with ISO9001 (research) and ISO17025 (diagnostics) quality standards and comply wit 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 appropriate model to use for work with viruses as they are easily handled within the laboratory setting.

For inocuity experiments, if one mouse develops clinical disease then we are likely to be able to terminate any remaining mice without allowing development of disease.

Analgesia prior to general anaesthesia will be utilised wherever intracerebral inoculation is required.

PROJECT 173

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.

Word limit; 1000 words

Project Title	The cell biology of zebrafish infection
Key Words	Shigella, infection, zebrafish
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) trans/lational 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):

Infection continues to be a major global health issue.

Whilst research in vitro using tissue culture has provided information on events at the cellular level, it cannot reflect the complex interactions in the entire organism. The results from this proposed study are expected to provide fundamental advances in our understanding of the biology of cellular immunity in the entire organism (i.e.zebrafish).

Therefore, we aim to discover new ways to control bacterial infection and antimicrobial 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)?

Bacterial infections continue to be responsible for immense human suffering and mortality throughout the world. We hope that this project will provide vital clues towards our understanding of the mechanisms of bacterial disease and for other human diseases arising from a dysfunctional host immune response, such as Crohn’s disease. There is also the exciting possibility that novel treatments against inflammatory diseases, infection and antimicrobial resistance may be discovered as a result of this project.

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

Approximately 6,500 zebrafish 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 are breeding adults to generate larvae to use in our research. Occasionally adults need to be humanely killed due to old age or illness. Where adults are used for infection studies, the effects are moderate and at the end of the study they will be humanely killed. Expected adverse effects include: abnormal swimming behaviour, separation from the shoal, abnormal scale position, abdominal swelling, abnormal skin outgrowth. Fish will be regularly monitored for such effects and killed using a humane method if such effects 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 host response to infectious disease is a complex trait, influenced by multiple factors, including

virulence of the pathogen and the host genetic make-up. Teasing out the multiple factors that

contribute to infection in humans is extremely complex as many of the variables remain unknown.

Using animal models to study infection, the researcher can focus on individual variables during the infection process in order to establish their relative importance.

We use zebrafish instead of mice because

•They develop fast

•They are vertebrates and have a fully developed immune system (larvae have innate immunity only, adults can be used to study role of adaptive immunity)

•The eggs / larvae are clear and easily observed and manipulated at the microscope

Reduction

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

Reduction

All experiments will be designed so that the minimum number of animals will be used. Breeding strategies are carried out by qualified personnel and aimed to avoid unnecessary animal generation (animal surplus).

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 will be used because they are easily genetically modified, fast breeders and the larvae are small and transparent which make it possible to follow individual cells and their behaviour using a microscope.

Animal suffering will be minimised by regular checking of the animals for relevant symptoms that constitute the end point of the experiment. All breeding and husbandry procedures will be performed by trained staff, and the fish monitored regularly.

PROJECT 174

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.

Word limit; 1000 words

Project Title	Organisation of neuronal dynamics in cortical microcircuits and related structures
Key Words	Rodent, cortex, neural dynamics
Expected duration of the project	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):

Neuroscientists seek to explain how brain activity gives rise to perceptions (e.g., recognising pictures, sounds and smells), movements (e.g., getting up from a chair), and memories (e.g., recalling your yesterday's whereabouts). These cognitive processes occur in a fraction of a second, so most neuroscience research is focused on neural signalling that happens on this and faster timescales. Some neural processes, however, happen on much slower timescales. Sleep is one such example: one cycle takes about 24 hours, a duration that is controlled by endogenous neuronal processes and persists even in complete darkness.

In fact, neural dynamics is not limited to one or just a few specific timescales but has prominent features on all timescales - seconds, minutes and hours included. Unlike fast timescale neural activity, the functions and mechanisms of the slower changes in neuronal activity received very little attention and are not well understood. The aim of the present project is to understand the mechanisms regulating the activity of individual cortical neurons on timescales of tens of seconds to several minutes, as well as the ways in which the activity of individual neurons is interrelated with the activity of large ensembles of cortical neurons. This will give us new insight into cognitive processes that happen on these timescales, such as changes in attention, motivation, introspection, vigilance, as well as how these slow processes modulate the fast cognitive processes.

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

This work will answer fundamental questions about nerve cell functions, however there is an additional important reason for needing to understand the slow changes

in activity of cortical neurons. This reason concerns 'functional magnetic resonance imaging' - the most advanced method for non-invasively ob-serving neural activity, which is widely used in both science and medicine. Functional magnetic resonance imaging (fMRI) measures neural activity indirectly, via its effect on the blood supply to the brain, and is consequently limited to revealing only the slowest components of neural activity. As a result of fMRI's widespread use, much is known about slow changes in activity of cortical areas, and this information is beginning to be used for diagnosis and treatment of neurological conditions. What is sorely lacking, however, is an understanding of how individual nerve cells give rise to the high level activity patterns. Our study will help filling this gap in our knowledge, and thus provide a better understanding of the origins of fMRI signals.

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

Mice: 100-200 / year Rats: 15-20 / 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 main experiments in this project involve monitor-ing or influencing their neuronal activity using elec-trophysiological or imaging techniques. To allow ac-cess to the brain, mice undergo one or two surgery procedures. During surgery they are fully anesthe-tised (and do not experience pain). The animals are also implanted with small bars that allow to keep their head in a fixed position for a duration of a few hours (mice can still adjust the position of their body with respect to their head). This is necessary because some of the equipment used to look at the neural ac-tivity (e.g. a microscope) weighs many kilograms and cannot be used otherwise. The experiments themselves are performed several days after the animals recover from the surgery and are not painful. After the experiments are complete, the animals will be humanely euthanized. We need to record in drug-free (awake or naturally sleeping) animals because anaesthetics significantly modify neuronal activity, therefore it is impossible to fully understand the mechanisms of brain activity in humans and other mammals if one only studies brain activity under anaesthesia. The severity of the above procedures is 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

Measurements of neural activity are currently only possible in live animals.

Non-invasive approaches, such as fMRI, cannot be used to address the questions we are studying. In fact one of the main aims of the project is to better understand the physiological mechanisms behind the fMRI signals.

Computer simulations of neural networks also cannot give us the information we seek, as the understanding of physiological mechanisms which have to be simulated is lacking. In fact furthering our understanding of such mechanisms is the very goal of our research.

Reduction

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

Reduction

We strive to use the latest technology for recording from large numbers of neurons simultaneously (tens to hundreds of neurons). This makes it possible to use much fewer animals than in previous 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

Among other reasons (such as genetically altered strains), rodents were chosen out of the attempt to use the least sentient animals in which the scientific questions can be studied. Furthermore, their needs can be more easily met in laboratory environment.

Some of the methods for recording the activity of large ensembles of neurons (a reduction approach mentioned above) are only available in genetically altered strains of mice.

The health and wellbeing of the animals will be closely monitored. Appropriate anaesthetic and analgesic procedures will be used. The animals will be provided with environmental enrichment and when possible they will be group housed.

PROJECT 175

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.

Word limit; 1000 words

Project Title	Dairy Precision Farming and Nutrition
Key Words	Precision farming, production diseases, metabolic disorders
Expected duration of the project	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:
Yes	(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;
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);
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 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 bec ause 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 teste d 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 us e known information from the traits of interest such as standard deviation for milk yiel d .

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.

A senior statistician from REDACTED will be consulted to ensure that all planned studies use a statistically supported 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 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 manageme nt to reduce number of sampling from the same animals.

PROJECT 176

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.

Word limit; 1000 words

Project Title	Tolerability and PK profiling of Compounds
Key Words	Pharmacokinetics, Tolerability, Infection, Antimicrobial
Expected duration of the project	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;
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):

This licence will enable us to provide support services to the Pharmaceutical and Biotechnology industries to assist in the development of novel antimicrobial compounds.

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

There is an acute shortage of compounds to treat microbial organisms that do not respond to available antimicrobials and newly-emerging diseases. The WHO recognises that antimicrobial resistance and infections are some of the greatest threats to humans. This licence will allow development of novel antimicrobials which will primarily benefit humans and animals.

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

The number of animals used will be dependent on the service requirements of clients and the number of drugs in the development pipeline but will be approximately 50,000 Mice, 25,000 Rats, 5,000 hamsters, 5,000 cotton rats, 500 Guinea pigs and 500 rabbits over the 5 year period of the licence.

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

A safe dose required for preclinical studies will be identified by an iterative process starting with a low dose, identified from in vitro studies, will be given to two mice and if it is tolerated the next two mice will be given a higher dose or lower dose if it was not tolerated. This will be repeated until a safe clinically relevant dose is found. In our

experience this requires 2-4 cycles. Following treatment the mice will be closely monitored for any ill effects such as prolonged change in breathing, hunched and ungroomed appearance or display fits. Any animal showing adverse effects will be humanely euthanized. All mice at the end of the study (typically 24h post-treatment), will be humanely euthanized and post mortem carried out to look for any damage/change to internal organs. Based on our experience over the past 5 years ~80% mice will be in the mild severity band and ~20% will be in the moderate severity band. Pharmacokinetic studies are pivotal to drug development as it is critical to have sufficient drug present at the target site to inhibit the microbe causing an infection. Pharmacokinetic studies will use treatment doses that are well tolerated and so no adverse effects of the treatment are expected. Blood and at times tissue samples, are collected post dose to allow measurement of drug. When only blood samples are required, whenever possible animals will have micro samples of blood taken at different times during the study. When larger samples or tissues are required terminal samples are taken at the time of euthanasia. At the time of euthanasia ~90% of animals in PK studies will be in the mild severity banding and ~10% will be in the moderate severity banding.

Application of the 3Rs

Replacement

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

Replacement

We run an extensive range of *in vitro* assays (termed ADME and DMPK) to identify the most suitable compounds to progress into animal studies. The sets of assays performed are designed to prevent compounds with unsuitable physical properties, pharmacokinetic properties or toxicity progressing into animal studies. We use large panels of assays that include:

- Drug uptake studies through human intestinal and lung cells
- Drug degradation studies following exposure to liver cell or cell metabolic enzymes
- 3D Hepatotoxicity assay to screen for liver toxicity
- 3D Combined hypertrophy and structural cardiotoxicity assay (uses beating cardiac 3D spheroids derived from human stem cells).

However, currently there are no *in vitro* models or mini-host systems that can fully replace animals that more closely mimic the clinical spectrum seen in humans. Comparative gene expression studies and immunological responses show substantial differences with vertebrates so can limit translation when using non-animal alternatives.
We offer clients *in vitro* biofilm and hollow fibre models for screening of compounds. In addition where possible antimicrobial of interest are screened in *Galleria*

mellonella (wax moth) larva infection models for efficacy confirmation before going into animal studies. Compounds that show no or limited efficacy are rejected at this stage. However, pathogenicity, response to therapy and importantly pharmacology differs greatly between *Galleria* compared to human and animals and therefore animal use is unavoidable.

Reduction

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

Reduction

The experimental design and analytical methods are fully supported by a statistician.

Prior to all experiments literature is reviewed to ensure best practice in terms of experimental design. We aim to publish all models both successful and failed ones which should help reduce animal use.

Where possible we collect multiple samples from a single animal to reduce the number of animals required. In addition where possible we combine multiple drugs into a cassette to reduce animal usage.

For all experiments we include:

- Statement of the objectives.
- Description of the experiment.
- Deliverable statement.
- No protocol will be executed until approved by senior staff

Refinement

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

Refinement

Whilst mice will be our first choice for development of new antimicrobials there are likely to be instances where this species is not relevant. Specific examples where mice might not be appropriate would include host specificity (where it is not possible to establish an infection in mice), or where blood sampling is required to assess biomarkers for assessment of disease progression. Where blood sampling is necessary the rat is a better species to use, both because serial sampling is possible and multiple samples of sufficient volume to analyse can be collected. Further, the metabolism of the target test drug may require the use of a species where it is more likely to reflect that in the human. In these cases the species that more closely mimics the human infection or can provide the volume of samples required for

bioanalysis will be used. For example, cotton rats are most suitable for chronic nasal colonisation with *Staphylococcus aureus* due to natural carriage and are one of the few non-human hosts of RSV.

We will use the following to minimise harm to animals:

- Ensure that where possible animals are kept in their social/cage mate groups.
- Only trained competent personnel carry out procedures.
- Ensure that administration and sampling limits are adhered to.
- Where pain is likely then prophylactic analgesic agreed with the named vet is used.
- Use rigorous monitoring of clinical conditions to ensure animals are euthanized within agreed severity bandings.
- Continually assess published literature to ensure latest refinements are used and avoid duplicating work.

PROJECT 177

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.

Word limit; 1000 words

Project Title	Genetic and epigenetic control of development
Key Words	Neurodevelopmental disorders, chromatin, signaling, cancer
Expected duration of the project	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):

The overall aim of this project is to understand how genetic and environmental factors control brain development, to understand how gene mutations and environmental changes can lead to abnormal brain development and function and give rise to conditions like autism and learning difficulties. The genes we study are also important in specific brain cancers that affect children, and we seek to understand their functions in brain cancer with the aim of identifying potential treatments. Where our studies identify the mechanisms whereby these factors cause disease, we will evaluate potential drugs for their ability to treat these conditions.

Our current objectives are to:

1. Identify and characterize the behavioural and disease phenotypes in mouse mutants
2. Determine mechanisms underlying specific defects identified in mouse mutants
3. Apply this knowledge to investigate further research hypotheses towards a more in-depth knowledge of mechanisms or to test specific treatments in pre-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)?

This project will identify the causes of specific developmental defects associated with human syndromes and neurodevelopmental disorders, which will lead to improved genetic counselling of families. An understanding of the mechanisms by which these gene mutations and environmental factors result in specific brain diseases will

represent a major advance in the field. These findings will improve diagnosis and may allow preventative treatments to be started early. In the longer-term, these findings are expected to eventually result in treatments for devastating conditions, which include autism, intellectual disability and cancer.

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 21,000 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?

The majority of animals (>75%) used in this project will be used in breeding protocols where no adverse or painful effects will be experienced. Adverse effects include developmental anomalies that occur as a result of the genetic mutation, including but not limited to phenotypes of mild severity such as incoordination, balance problems, loss of hearing, vision or smell and/or moderate severity such as brain tumours. Complications may rarely (<0.1%) arise as a result of surgery to introduce DNA or viruses into the brain, or administration of drugs. At the end of the procedures, animals will be provided to other project holders or recognised sources for brain imaging, or killed humanely and tissues collected for analysis, where appropriate.

Application of the 3Rs

Replacement

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

Replacement

Brain development is a complex process that involves many different cell types and often involves the migration of cells or extension of cell processes over long distances. These complex processes cannot be modelled fully by studying isolated cells in a dish. Furthermore, to understand how altered brain development results in specific psychiatric disorders such as autism or learning difficulties, we need to perform studies in animals. The experiments we are proposing relate to function in the conscious behaving animal for which there is no suitable alternative. It is not ethical to manipulate either the genetics or the environment, especially during early development, in humans. Therefore, there is no feasible alternative that would entirely replace animals.

Where possible, certain aspects of our work will be modelled in cells in a dish. For example, we have access to human cell lines that can recapitulate some aspects of brain cell development and human and mouse cancer cell lines that can be used to study certain aspects of tumour cell behaviours. We are also able to perform certain

short-term experiments in slice cultures where tissues are kept alive for a short time to replace the use of animals. Finally, we are exploring the use of human minibrains that can develop from human embryonic stem cells as alternative methods to study certain early stages of brain development. These alternative approaches will be employed where possible (e.g. where we are only interested in a short developmental time window) to replace animal work.

Reduction

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

Reduction

The majority of experiments will involve the breeding of genetically altered animals. Wherever possible, we produce mouse lines with specific genetic mutations that allows us to significantly reduce the number of mice needed. Where possible, experiments are designed such that multiple parameters are assessed per sample to reduce the number of mice and samples that are needed to achieve robust and clear data. After behavioural studies, we collect the brains from these animals for ex vivo MRI and histological analyses, thereby reducing the number of animals produced specifically for these studies.

Statistical analyses are done after each experiment to ensure that experiments are only repeated until statistically significant data are obtained. Unnecessary production or import of genetically altered mouse lines will be avoided by searching specific databases. We make use of published findings and our own expertise gained over many years of research to use the optimal number of animals to yield statistically significant 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 mouse is by far the best experimental model system for modelling genetic disease and development of the mammalian brain. Optimised methods are available for all the analyses we intend to perform during the course of our studies. Most adult animals used in this project will be subject to minimal stress, as many of the experiments will involve only breeding and maintenance of mice, followed by killing to obtain tissues. In some cases it is necessary to administer agents to the animal (or to the pregnant dam) in order to label or identifty cells and tissues, to alter the expression of genes, or to test possible drugs for treatment - this will mostly involve no more than injection or feeding of animals, often on one occasion only. Similarly,

alterations in the environment to be used in this project have been tested and shown to cause minimal stress to animals with no significant impact on animal health and well-being.

In some cases, we may kill animals by decapitation of pups, up to the age of 10 days, or we may kill by perfusion and/or fixation under terminal anaesthesia, in order to obtain the best possible quality brain tissue for laboratory analysis. In some cases, we may harvest blood or tissues under terminal anaesthesia. Depth of anaesthesia will be monitored at all times.

The most invasive techniques proposed in this project involves administration of substances straight into the brain. These studies constitute a very small part of the work undertaken, very few animals (<100 each per year) will be used in each of these protocols and animals are expected to recover easily from the surgical procedures required for these experiments.

PROJECT 178

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.

Word limit; 1000 words

Project Title	Breeding and therapy of the delta E50-MD dog model of DMD
Key Words	Duchenne Muscular Dystrophy, Therapy, Muscle, Brain
Expected duration of the project	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;
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.

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):

This project aims to maintain a colony of small dogs with naturally occurring muscular dystrophy that will be used to test promising therapies prior to clinical trial in patients with Duchenne muscular dystrophy (DMD). DMD is a disease of young boys caused by mutations in an X-linked gene that is critical for muscle function but also causes behavioural and cognitive problems. Sufferers are confined to a wheelchair by the age of 12 and all die in their late 20s or early 30s due to progressive wasting of all muscles including the heart. Optimal medical management has improved quality of life and increased lifespan from an original age of death at 16, but can do little to prevent the relentless muscle wasting. Pet dogs also are affected with this condition, so our work will also benefit pet dogs and their owners in the future.

The development of treatments for this condition mostly relies on cells in culture and the *mdx* mouse model of the disease. The *mdx* mouse is a good biochemical model but does not show the clinical signs typical of the disease in boys. Consequently, there is doubt about the ability to directly translate results in the mouse into a human clinical trial. In contrast, dystrophic dogs show similar clinical and pathological progression to humans and so can serve as a final test to enable rationale decisions about which treatments are most likely to be successful 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)?

Although most treatments are initially developed and evaluated in cells in culture, it is not possible with such experiments, fully to replicate the complex environment of the diseased muscle and its interactions with other parts of the body such as the

immune system. Thus, mice are used to test which experimental treatment is effective in the whole animal. The dystrophic dogs will only be used to test the most successful treatments arising from the mouse studies, in order to confirm that they have real potential for effective treatment in humans. Treatment will be given via the local (intramuscular) or systemic routes (either by intravenous injection or by oral administration) and by direct injection into the nervous system (intrathecal) as these are the routes that may be used in treatment of human patients. Dogs will be provided with a similar or better level of medical care that human patients and pet dogs with this problem receive with the difference that for these dogs there are defined humane end points at which they will be euthanised. The effects of administration of experimental therapies will use similar assays as used in man with the possible exception of a few more muscle biopsies compared to children with DMD. These will all be performed under general anaesthesia with post-operative pain relief. Although DMD is a genetic disease that can be inherited, the gene responsible is very large and has a high new mutation rate that means genetic counselling cannot be used to prevent future cases of DMD. In addition, the frequency of this neuromuscular disorder does not vary significantly worldwide, meaning that there will always be patients needing effective therapies. As the disease can be detected by screening of newborn infants, a successful treatment could effectively “cure” this disease thus massively improving both quality of life for patients and carers as well as a very substantially increased lifespan for DMD patients

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

We will use dogs with a naturally-occurring form of Duchenne Muscular Dystrophy. We will only breed animals that are required for maintaining the colony, studying the disease and and for the therapeutic trials. The colony will be maintained for the duration of the programme and we anticipate that over the duration we will use up to 120 dogs in trials. Additional animals that are bred that are not required for the trials will be rehomed to loving owners whenever possible.

In the context of what you propose to do to 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 any particular adverse effects of the therapies or research that we are conducting. All therapies will have first been tested in cell culture and in rodent models. Of the procedures that we are performing, muscle biopsy is associated with mild discomfort in people that can readily be controlled with pain killers. We will routinely use pain killers in our dogs whenever needed or other medication as recommended by vets. Other procedures are not expected to be associated with any discomfort. Some procedures (for example MRI imaging) are conducted under general anaesthesia. Muscular Dystrophy in humans is not associated with pain and we have not detected pain from the naturally occurring disease in our dogs. As in

humans, they become weaker as the disease progresses and sometimes have problems with swallowing, so we carefully monitor these aspects in particular and have defined humane endpoints so that these problems do not compromise the welfare of the animal. At the end of the study all affected dogs are euthanased. Normal animals that are not required for studies are rehomed wherever possible either at weaning or when they have completed the study protocols.

Application of the 3Rs

Replacement

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

Replacement

All therapies will have first been tested in cell culture before animal studies. Because mice do not display clinical signs of Duchenne Muscular Dystrophy, we cannot assess response to treatment in mice, and instead need to study a model that displays the weakness and muscle problems that are seen in humans.

Reduction

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

Reduction

For each study we will use the minimum number of animals that are required to prove or disprove the efficacy of a possible treatment. We use the results of previous work, and where possible, stored tissues to further 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

Whilst other dog models of Duchenne Muscular Dystrophy are used in research, the model we use (unlike others) has a naturally occurring mutation that is in the region of the dystrophin gene that is most commonly mutated in humans. This means that this model is more applicable to several of the most promising treatments currently being evaluated. In addition, the dogs we use weigh less than other dog models, and as a result, they are less affected by the muscle weakness that develops as they get older.

We take welfare aspects of this project especially seriously. All dogs are socialised and have access to outside runs and paddocks to play with other animals and humans and they are taken for short walks on a daily basis. We monitor the dogs very closely for signs of progression of their disease and make decisions to end the studies before the dogs reach the end stages of disease that occur in humans with this disorder.

PROJECT 179

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.

Word limit; 1000 words

Project Title	Pesticide residues in animal tissues and other matrices
Key Words	Pesticide, Residue, Feeding, Metabolism
Expected duration of the project	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.

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 overall aim of this work is to generate data to be included in the dossiers submitted for the registration of plant protection products. Livestock feeding and metabolism studies are required under EC Council Directive 91/414/EEC when residues of a pesticide are expected to be present in livestock feed and might cause detectable residues in livestock. The conduct of the studies, including minimum numbers of animals, dose levels and duration, is specified in guidance documents.

The use of live animals is needed in order to determine transfer of pesticide and/or their metabolites into meat, milk and eggs. Farm animals (cows, hens, etc) are required by the regulator as the objective is to measure potential dietary exposure to pesticides in their edible tissues. The minimum number of animals required is specified in the relevant guidelines and the minimum number will be used wherever possible. In some cases, the use of one particular type of animal removes the need to generate data for other animals. For example, in most cases the results of a cattle feeding study can be used to establish tolerances in goats, pigs, horses and sheep. Similarly, data on chickens is normally accepted in lieu of data on other livestock poultry, e.g. turkeys, geese and ducks. This licence allows the use of farm animal species (cattle, goats and poultry) to achieve the regulatory requirements.

All planned studies are assessed by an ethical review committee to determine if the study can proceed and all studies will be subject to ongoing review. Studies will be undertaken under commercial husbandry conditions and according to the relevant Defra Codes of Recommendation for the Welfare of Livestock.

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

The primary purpose of studies conducted under this licence is to provide accurate data which is acceptable to regulatory authorities. The data is likely to be included in dossiers submitted to the regulatory authorities when registering plant protection products. This data will enable accurate decision making with regard to labelling of the chemicals, for example.

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 200 lactating dairy cows, 800 laying hens and 30 lactating goats over the five year timescale 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?

Adverse effects they are expected to be mild in nature and the incidence is expected to be low. Blood sampling of laying hens during metabolism studies will lead to haematoma in approximately 30% of cases. However, our experience is that these heal rapidly with no observable consequences to the birds. All other adverse effects are expected to be infrequent i.e. an incidence of less than 1%. All animals will be euthanased by a humane 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

Livestock feeding and metabolism studies are not required for all crop protection chemicals and clients usually make strong representations to the regulator to avoid the need to conduct them. However, once the regulator decides that animal feeding or metabolism studies are necessary they usually specify that they need to be carried out to OECD Guidelines for the Testing of Chemicals, Test No. 505: Residues in Livestock or Test No. 503: Metabolism in Livestock respectively. The use of animals is specified in these guidelines. For each study conducted under this licence we will ensure that, as part of the Ethical Review Process, the client has made representations to the regulator questioning the need to conduct an animal feeding study.

Reduction

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

Reduction

The minimum number of animals as recommended in the guidelines will be used unless there is a strong case to use more. If more than the minimum recommended number is requested, this will need to be justified by the sponsor and/or regulator and statistical advice will also be taken. In all cases, any increase over the minimum recommended numbers will need to be approved through the Ethical Review Process.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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, model and method is rigidly laid out in the OECD guidelines, OECD 503 for metabolism studies and OECD 505 for feeding studies:

For metabolism studies, OECD 503 indicates that separate metabolism studies should be conducted in ruminants and poultry. The species of choice are lactating dairy goats and egg-laying hens.

For feeding studies, OECD 505 indicates that separate feeding studies should be conducted for ruminants and poultry whenever residues are likely to occur in the feeds of these classes of livestock. The species of choice are lactating dairy cows and egg-laying hens. Data on residues in milk from dairy cows will usually equally apply to dairy goats. In most cases the results of a cattle feeding study will be used to establish animal commodity maximum residue limits for goats, pigs, sheep and horses. Within the poultry group, data on chickens will usually be accepted in lieu of data on other livestock poultry e.g. turkeys, geese and ducks.

It is unlikely that any toxicological effects will result from administration of pesticides at the levels to be used in such studies. In all studies conducted under the previous project licences no adverse effects from the pesticides were observed. It is likely that the sponsor will already have conducted some livestock studies, results from these will inform the REDACTED AWERB on the likelihood of adverse effects being observed in the residue study. However, twice daily observations of the animals for adverse effects which may be associated with the pesticide will be conducted.

Animals will usually be acclimatised in their individual pens for a minimum of one week, usually two, before dosing / feeding of the pesticide commences. Individually penned animals will be located within sight of each other to minimise any distress caused by individual penning. For metabolism studies involving one goat, a companion animal will be located within sight of the study goat.

For feeding studies administration of the pesticide on top of the daily compound feed allowance for dairy cows has been successfully implemented on most occasions. This refinement means that cows do not have to be restrained for administration of a bolus twice a day for 28 days. This refinement was trialled for laying hens but failed due to palatability problems. This refinement is not possible for metabolism studies as the method of administration is prescribed in OECD 503.

PROJECT 180

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.

Word limit; 1000 words

Project Title	Myocardial infarction and heart failure
Key Words	heart, signalling, infarction, remodelling
Expected duration of the project	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:
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):

The purpose of this project is to ultimately benefit patients by reducing myocardial infarction and its main consequence, heart failure.

Although there have been major advances in the prevention and treatment of myocardial infarction (MI) it still remains the most common cause of death in developed countries and is becoming more common in developing countries. Myocardial infarction is caused by a sudden blockage of the blood vessels supplying the heart muscle also known as the myocardium. Patients can die within minutes to hours of the blockage due to direct injury to heart muscle. However many patients survive these first few hours only to die days or even years later due to heart failure. The processes that cause initial heart muscle death and then late failure are not completely understood but they involve changes occurring in the various cell types that make up the heart itself as well as in the hormones and nerve impulses that influence the heart but arise from outside it. These nerve impulses and hormones arise from organs such as the brain, kidney and gut but also from glands such as the adrenals, near the kidneys. The complexity of the cell types within the heart in combination with the effects of nerves and hormones means that simpler models in cultured cells or in isolated hearts cannot completely replace experiments in intact animals.

The majority of experiments will be done on isolated cells or hearts to model conditions during MI. These experiments involve the administration of a high dose of anaesthetic to achieve a very deep level of anaesthesia. Once this is achieved the heart is removed.

For the reasons described above we cannot avoid experiments in living whole animals to look at the late changes occurring in the heart that cause it to progress towards heart failure. Studies we will carry out in animals include coronary artery obstruction induced by tying a ligature around a coronary artery under general anaesthesia. Animals will then be allowed to wake up and changes in the heart monitored for up to 6 months through the intact chest using echocardiography (sound waves), magnetic resonance imaging, X-ray computed tomography and other “non-invasive” imaging techniques that are also used in patients to monitor heart failure after MI and allow multiple measurement to be taken from a single animal. These investigations will be done under anaesthesia as described in our protocols and may also involve the administration of agents to improve image quality (so called contrast agents and tracers).

At the end of the period of observation, which maybe up to 6 months, animals will be subject to a final deep anaesthetic for further measurements before the heart is removed and analysed. Based on previous work we will need up to 200 mice and 50 rats per year for experiments of this type. In this way we hope to identify factors that reduce MI 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)?

Signalling proteins, or Kinases, are known to be crucially important to heart function during and after myocardial infarction (MI) and during the swelling of the heart that leads to heart failure. REDACTED The proposed benefit of this project of work is to identify and manipulate these kinases in order to change the way the heart responds to stress. If this were possible it would add to the therapies currently available to reduce MI, post-MI remodelling and their associated mortality.

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

We expect to work mostly with mice, using a small number of rats to verify key findings or to use techniques that do not give clear results in the mouse because of their small size. In total, we expect to use approximately 12,000 mice, and 700 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?

We have explicitly designed all experiments and protocols to allow us to answer scientific questions with the minimal discomfort to experimental animals, as described below. The most common intervention will be manipulation of isolated hearts obtained from animals that have been humanely killed. Myocardial infarction (MI) and the cardiac remodelling which can ensue in humans is a severe disease, and is one of the leading cause of adult mortality and morbidity in the UK adult population. As such, modelling MI and cardiac remodelling is a severe procedure,

and may cause weight-loss and moderate distress in animals for several days following surgery. These effects will be minimised by close monitoring and administration of pain killers. However, our lab has methods to assess the development of injury during the surgical procedure, and without recovering from general anaesthetic, allowing us to answer several scientific questions without the animal experiencing post-MI distress. Wherever possible, we will perform this non-recovery surgery, thus subjecting animals to mild, rather than severe procedures. 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

We have performed, and continue to perform, many experiments using cells derived from patients and animals, rather than animals themselves.

However, the complex interplay between different organs seen in the disorders we are investigating means that the processes cannot, therefore, be completely modelled in cell culture or in the isolated heart. Both cell culture models and the isolated heart are however used extensively to screen for relevant signals.

Within our research we use a variety of these models in which MI is simulated using chemicals and oxygen starvation.

Reduction

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

Reduction

We have performed statistical tests based on previous work performed in our and other laboratories to model the minimum number of animals and experiments necessary to demonstrate benefit, if it can be seen.

We will also make maximal use of tissue from individual animals following experiments, so that wherever possible, multiple questions can be answered 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 and rats are the least sentient animals we can use to model the clinical conditions we are investigating, while still remaining relevant to humans.

Our cardiac remodelling work has been refined by modifying the surgery in accordance with the latest literature to reduce death and distress following recovery, and, wherever possible, to answer experimental questions under general anaesthesia without recovery, to avoid the stresses associated with post-MI morbidity and minimise any associated suffering.

PROJECT 181

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.

Word limit; 1000 words

Project Title	Characterization of Novel Antimicrobial Agents
Key Words	Infection, antimicrobial, antibacterial, antifungal, antiviral
Expected duration of the project	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;
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):

This project licence will enable us to provide support services to the Pharmaceutical and Biotechnology industries to assist in the development of antimicrobial 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)?

There is an acute shortage of agents to treat microbial organisms that do not respond to available antimicrobials and newly-emerging diseases. This licence will allow development of novel antimicrobials, disease modifying therapeutics and vaccines, which will primarily benefit humans and animals.

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

The number of animals used will be dependent on the service requirements of clients and the number of drugs in the development pipeline but will be approximately 100,000 Mice, 75,000 Rats, 25,000 hamsters, 25,000 cotton rats, 5,000 Guinea pigs and 5,000 rabbits over the 5 year period of the licence.

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

Animals will be infected with suitable microbial organisms (bacteria, fungal, viral and parasites) to mirror human infections. Typical symptoms observed in these studies will be loss of appetite, lethargy, change in body temperature, pain or respiratory distress. The aim for most models is to replicate the clinical disease observed in humans so disease specific symptoms are likely to be present e.g. diarrhoea following Salmonella infection, acute airway disease following RSV infection and

blindness following ocular infection with toxoplasma. In many cases if the infection was left unchecked the animals would eventually succumb to infection. To ensure that this does not happen, a range of assessments and monitoring strategies developed over 25 years are implemented, including use of analgesia, regular clinical assessments, body weight change, change in body temperature and early termination as soon as clinically valid results are obtained. These measures enable us to euthanize animals before they show significant signs of the infection. Based on experience over the last 5 years, at the time of euthanasia ~40% of animals will be in the mild severity banding, ~50% within the lower limits of the moderate severity banding and ~10% will be near the upper limit of the moderate severity banding. Monitoring and assessment strategies are continually reviewed and revised to ensure they are fit-for-purpose, i.e. the frequency of observation and clinical observations are amended as required. In all cases we humanely euthanize animals before they reach severe endpoints. At the end of all studies animals will be humanely euthanized and infected site/tissues removed for culture to quantify the infection burden.

Application of the 3Rs

Replacement

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

Replacement

Currently there are no in vitro models or mini-host systems that can fully replace animal infection models that more closely mimic the clinical spectrum of the infection seen in humans, sepsis for example. Comparative gene expression studies and immunological responses show substantial differences with vertebrates so can limit translation when using non-animal alternatives.

We offer clients biofilm and hallow fibre models for screening of compounds with compounds of interest screened in our *Galleria mellonella* (wax moth) larva infection model for efficacy confirmation before going into animal studies. Compounds that show no or limited efficacy are rejected at this stage. However, pathogenicity, response to therapy and importantly pharmacology differs greatly between *Galleria* compared to human and animals and therefore animal use is unavoidable.

Whilst there are no suitable alternatives at this time, REDACTED staff regularly attend scientific meetings and constantly review the published literature for other potential non-animal models.

Reduction

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

Reduction

The experimental design and analytical methods are fully supported by an appropriately trained statistician.

Prior to all experiments literature is reviewed to ensure best practice in terms of experimental design and analytical methods. A database is used that captures outcomes of all of the models and microorganisms to avoid unnecessary repetition

For all experiments we include:

- Statement of the objectives (both primary and secondary).
- Description of the experiment, including background, design, endpoints, treatment, surgery, dosing procedure, harvest of samples, list of standard operating procedures and reference used etc.
- Deliverable statement including how data will be presented and statistical analysis.
- No protocol will be executed until approved by senior staff.

A report, detailing the outcome, is written and circulated to senior staff. We aim to publish all models both successful and failed ones which should help reduce animal use.
Wherever possible we publish in journals which support the ARRIVE guidelines which in turn underpin good experimental design and all the benefits that brings to the reduction in the use 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

Whilst mice will be our first choice for development of new infection models there are likely to be instances where this species is not relevant. Specific examples where mice might not be appropriate would include host specificity (where it is not possible to establish an infection in mice), or where blood sampling is required to assess biomarkers for assessment of disease progression. Where blood sampling is necessary the rat is a better species to use, both because serial sampling is possible and multiple samples of sufficient volume to analyse can be collected. Further, the metabolism of the target test drug may require the use of a species where it is more likely to reflect that in the human (see example c below).

a) Cotton rats are most suitable for chronic nasal colonisation with *Staphylococcus aureus* due to natural carriage (the only other natural hosts are humans and pig).

b) The Golden Syrian hamster *Clostridium difficile* model is regarded as the most relevant model for screening of efficacy of antimicrobials.

c) Guinea pigs have significant advantages at times due to differential metabolism of drugs (e.g. voriconazole rapidly hydrolysed in mice and rats whilst stable in guinea pigs and humans) or susceptibility to the pathogen (e.g. fungal infections of skin).

d) Rabbits are an excellent model for many human fungal infections and for some bacterial and fungal species have similar levels of susceptibility to infections as humans (for instance *S. aureus* and *Candida* meningoencephalitis).

We will use the following to minimise harm to animals:

- Ensure that where possible animals are kept in their social/cage mate groups.
- Only trained competent personnel carry out procedures.
- Ensure that administration and sampling limits are adhered to.
- Where pain is likely following infection or procedure that prophylactic analgesic agreed with the named vet is used.
- Use rigorous monitoring of clinical conditions to ensure animals are euthanized within agreed severity bandings.
- Continually assess methods in the public domain to ensure we use the latest refinements as well as ensure that we are not duplicating model development work.
- Selection of microbial strain/inoculum that causes the least pain, suffering, distress and lasting harm but provides meaningful data.

After every experiment we will critically appraise what we do to seek out any ways to improve our models to reduce harm to animals.

PROJECT 182

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.

Word limit; 1000 words

Project Title	Neurobiological basis of neuropsychiatric disorders
Key Words	Neuropsychiatric disorders, serotonin, acetylcholine, brain function, sleep
Expected duration of the project	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;
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):

Neuropsychiatric disorders and symptoms including depression, anxiety, psychosis, drug addiction, eating and sleep disorders, and learning and memory difficulties are common, long-lasting problems which have a big impact on the quality of life of those who experience them. The treatments currently available for these disorders and symptoms don't work very well. Medicines and talking therapies take a long time to have any effect and in many cases they don't help very much at all. In addition, medicines can have nasty side-effects.

Designing new therapies relies on scientists having a better understanding of how the brain works normally, how and why it can go wrong, and how treatments can make the brain work normally again. The purpose of this project is to provide information about the brain systems and regions involved in neuropsychiatric disorders and how they work normally in the healthy brain

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

The potential benefits of the project are that we will get a better understanding of how the different parts and different chemicals in the brain work to control our mood and the behaviours. In the end we hope that through our work we will be able to identify targets for new medicines and other treatments for neuropsychiatric disorders.

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

We will use rats and mice in our project. Over the 5 years of the project we will use up to 350 mice and 450 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?

We are interested in understanding normal brain structure and function. In particular the normal physiology and pharmacology of specific brain systems (neurones, transmitters, nuclei, neuronal circuits and pathways) which may not work properly in neuropsychiatric disorders and/or may be the targets of treatments for these disorders. In order to investigate these questions • we will breed some genetically altered mice. The mice appear normal and do not suffer any adverse effects from their genetic changes. We will need to genotype the mice which involves taking a small blood sample from the tail or tissue from the ear. This might cause brief pain. Overall this procedure is mild. • We will inject some mice and rats with special viruses to introduce genetic material into cells. The animals will be anaesthetised while the injections are made into specific parts of the brain. They will also be given analgesics to prevent them feeling pain from the surgery wounds afterwards and will be allowed to recover for several weeks before we do our experiments. This procedure is moderate • Some animals will have their behaviour measured in their own cage or in a special arena or maze. During the testing, they may have drugs injected so that we can evoke a particular behaviour, or test the sensitivity or function of a brain system. These procedures are usually very mild but some of the drugs we use may cause animals to feel more anxious or otherwise alter their normal behaviour. This procedure is rated as moderate. • will undergo procedures under general anaesthetic to measure brain activity or chemicals. The animals will not be allowed to recover from the anaesthesia. Adverse effects are not likely. At the end of each experiment, animals will be humanely killed. The procedure is rated as non-recovery. Other animals will be killed by methods which allow us to preserve the brain tissue for experiments. We will kill some animals by anaesthetising them very deeply and then replacing their blood with a salt solution. Other animals will be killed by rapid decapitation without an anaesthetic. This allows us to examine the brain tissue which hasn't been exposed to anaesthetics which can affect the brain in many ways.

Application of the 3Rs

Replacement

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

Replacement

Our research concerns complex behaviours and the impact of the internal and external environmental on brain function. Whilst research in humans is possible, there are many ethical issues. It also impossible for us to examine their brain function in detail while they are alive.

To find out about brain function we must use experimental animals.

Reduction

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

Reduction

It is important to use large enough numbers of animals so that if there is an important biological effect, we will be able to detect it. Using knowledge about how variable our measures are will calculate the numbers of animals needed to see important effects. We can reduce variability and so reduce numbers of animals needed by doing our experiments very carefully, using animals of the same gender and age, and carefully controlling the housing conditions of the animals. Often we can also make more than one measure in each animal which reduces the overall 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

For our studies we will use rats and mice. We already know a lot about their brains and behaviour and know that in many ways they are very similar to man.

In all experiments we will ensure that animals are handled by experienced and skilled persons and that trainees are closely supervised while acquiring the necessary skills. We will use appropriate anaesthetics and analgesics for surgical procedures. All animals undergoing treatments will be monitored to ensure they are well and are not experiencing adverse effects. We will ensure that we choose doses of drugs and give them to the animals in such a way to minimise the stress to the animal. Where animals have surgical procedures from which they will recover, surgery will be done under sterile conditions and the animals will be carefully looked after and given pain relief as necessary as they recover.

PROJECT 183

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.

Word limit; 1000 words

Project Title	Pharmacodynamics of Antibacterial and Antifungal Agents
Key Words	antimicrobial, antimicrobial resistance, antifungal, drug development, antibacterial
Expected duration of the project	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:
Yes	(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.

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):

This project provides knowledge about the use of new antibacterial and antifungal agents. We are especially interested in new compounds that are effective for antibiotic and antifungal resistant infections. The overall aim is to provide the experimental evidence for antimicrobial activity that enables new molecules to be tested in humans. The project also provides the justification for dosages that will be used in the very first patients enrolled in clinical trials.

A deep understanding of the relationship between the dose of a drug, the concentrations that are achieved in the body and the ultimate effect in terms of killing fungi and bacteria in the body is a mandatory requirement for the development of new drugs. Regulatory authorities such as the MHRA (UK), EMA (EU) and FDA (US) require such information.

What 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 to provide patients with life-threatening infections new therapeutic options. Currently, these patients have few if any options and die as a result. This project will allow academic groups, small-medium enterprises (SMEs), biotechnology companies and larger pharmaceutical companies to develop promising new compounds in an accelerated manner. The project will also prevent compounds with limited or suboptimal efficacy being tested in humans. The project will involve examining the efficacy of approximately 20 new antibiotics and antifungal agents with ultimate aim of identifying safe and effective dosages for humans.

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

Mice and rabbits are the only species being used. We plan to use 18,000 mice and1150 rabbits 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 animals in this project have treatment that damage their immune systems prior to being infected with a bacterium or fungus. These models closely mimic human infection of skin and soft tissue infection (e.g. surgical site infection), pneumonia (bacterial and fungal), and meningitis (bacterial, fungal and tuberculous infection). The laboratory animal models are mimics of neonatal and adult infection. Animals then receive new antibiotics and antifungal agents that are being developed for humans. Most of the animals used in this project have mild to moderate symptoms. Nevertheless, most protocols in this project are graded as severe reflecting the fact that we expect to see (on occasions) animals that are significantly unwell. The grading reflects the fact that we are modelling severe human infections where the mortality is generally 50% and on occasions higher. Most the adverse effects are related to the infection rather than drug related toxicity, which is screened out in early testing.

Application of the 3Rs

Replacement

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

Replacement

We use a range of non-animal models (i.e. in vitro) of infection to help understand how antibiotics work. A wide range of experimental models that include both laboratory animals and in vitro models of infection are required to obtain a complete understanding of the potential benefits of new antibiotics. In vitro models are especially useful for understanding how resistance to antibiotics develops. However, in vitro models cannot completely replace laboratory animal models for several reasons that include: (1) the inability to understand how antibiotics work in complex infections within tissues such as the lung and brain. The pattern of infection in those body sites may have an important bearing on the activity of an antibiotic in humans (e.g. sometimes antibiotics just will not work in the lung despite working elsewhere in the body); (2) the inability to assess the additional benefit of the immune system over and above the effect of the antibiotic; (3) the inability to understand how an antibiotic penetrates into tissues (e.g. parts of the brain) where some infections may reside; and (4) some infectious processes simply cannot be modelled using an in vitro system. For these reasons, a wide range of model systems are required to fully assess the potential benefit of a new antibiotic.

Reduction

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

Reduction

We will minimise the numbers of animals in several ways. We design experiments collectively so they yield the maximum amount of information with the fewest animals. We plan experiments precisely so that we minimise technical error and therefore minimise the number of experiments that need to be repeated. We seek statistical advice and input when necessary to ensure experiments are adequately powered to address the scientific question that is being posed. We progressively learn from experiment to experiment so that we maximise the use of previously obtained knowledge. We use all the data that is obtained. This means that all animals used in the experiments contribute information and allow the primary scientific question to be addressed in an efficient manner.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 most refined models possible that enable us to obtain a realistic estimate of drug activity and an insight into the dosage that is likely to be effective for humans. We use analgesia routinely. We perform invasive procedures under general anaesthesia. We use catheters if appropriate to minimise the number of needlesticks that are required to administer drugs or obtain blood samples. We inspect and care for animals very carefully and are available around the clock. We use the shortest possible models to obtain the necessary information. We minimise the number of invasive procedures through careful experimental design.

PROJECT 184

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.

Word limit; 1000 words

Project Title	Analysis of VHL and HIF function in the zebrafish
Key Words	
Expected duration of the project	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):

Cells have what is known as signalling pathways, i.e. communication lines that allow the cell to talk to other cells as well as internal communciation. This project aims to understand one of these pathways known as the “Von Hippel Lindau/Hypoxia Inducible Factor” pathway during the development of an embryo to adult stages. In addition, many signalling pathways can mis-communicate during the development of cancer and therefore we will also study this pathway to understand what goes wrong in cancer with respect this communication pathway. Oxygen is essential for the survival of most animals, and the aforementioned pathway is an “alarm signal” that detects if oxygen levels are too low. We want to understand how this signal is working in a normal animal, and how it communicates with other signals in our body. It is important, because inappropriate activation of the signal may cause -for instance- kidney cancers, and may help other cancers to thrive. Our work may help to clarify why inappropriate function of this pathway can cause 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 work will result in a detailed understanding of the role of the VHL gene and “low-oxygen” signalling during organ development and tumour formation. It may suggest ways in which we can alter their role and this could benefit Von Hippel Lindau disease patients. For instance, we have discovered a role for VHL in DNA repair, this might lead to cancer in patients but might also lead to vulnerability of tumour cells to particular chemicals. Such chemicals can be indentified using zebrafish VHL mutant embryos. This could on the longer term produce clinical leads, which may be developed for use in patients. VHL and “low-oxygen” signalling plays a role in may other diseases where oxygen supply is disrupted (stroke, heart attacks, other

cancers), a precise understanding of this pathway will increase our understanding of those diseases as well.

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

The great majority of fish (>90%) will simply be used a breeding stock to produce, embryos of the correct genotype, which we will need for our experiments (max 17000 fish over 5 years). Occasionally a biopsy of their fin may be taken to determine their genetic composition. Some fish may be used to determine the effect of loss of function of a gene in a small part of their body (max 1450) or may be treated to induce tumour formation (max 800). In all these cases, any fish that show significant impairment of their quality of life will be euthanized.

In the context of what you propose to do to 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 all our fish will have mild adverse effects at most. For instance, we may observe tumour formation in some fish where we try to test the effect of our candidate genes on this process. We generally do not expect significant adverse effects from other procedures, like tail fin biopsies, transient anaesthesia, or behavioural observations. All animals will be euthanized after the experiments are completed.

Application of the 3Rs

Replacement

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

Replacement

We are working with human cell culture, and are also collaborating with people who do, to help inform our experiments in the zebrafish. This helped us for instance to suggest a mechanism of how glucocorticoids interact with our “low-oxygen” signal. However, in several cases it is difficult or impossible to recreate the correct conditions in culture. Often multiple cell types need to interact, or cultured cells loose characteristics that are essential to understand how low-oxygen signalling works. For instance, liver cells are known to lose their unique “liver” identity with respect to glucose usage and storage. Therefore, it remains important to study our signal in whole animals

Concerning the latter, in the majority of cases we will use embryos or young larvae for our experiments, which have a very low awareness and do not fall under the UK legislation that regulates animal usage in science.

Reduction

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

Reduction

In the great majority of cases we will work with the eggs that the fish produce. Animals are mainly used to produce these embryos and we are not experimenting on these. The number of animals we use is therefore mainly determined by safe stock keeping considerations, and our need to reliably get sufficient numbers of embryos.

In cases where adults are needed, we have consulted with statisticians at REDACTED to determine the minimal number of fish needed, and we will do this again if we intend to change our experimental set-up.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 a vertebrate model organism with the lowest level of awareness. In addition we will mainly use immature forms of these animals i.e. embryos where the brain is less developed.

All our fish are regularly monitored for health, and we increase monitoring if experiments are done where health may be affected. We screen larvae that have been manipulated for defects, before allowing them to develop past the point of protection. Any fish that shows significant effects on welfare will be euthanized.

For both embryos and adults, whenever we need to do observations, or manipulations like recovery of gametes animals will be appropriately anaesthetised and monitored until they are completely recovered.

If adults need to be taken out of the water for observation they will be placed in wet sponges to minimize damage to the protective slime layer on their skin.

PROJECT 185

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.

Word limit; 1000 words

Project Title	The neurobiological mechanisms of pain
Key Words	pain, neurobiology, inflammation, arthritis, postnatal development
Expected duration of the project	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;
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):

Everyone experiences pain following an injury, however this is normally short lasting and protects you from further damage while the tissue repairs. There is evidence that in some diseases the pain people experience is not protective and is part of the disease. This pain doesn't serve a protective purpose and stops peoples' normal everyday life. We know that the ways in which pain is generated and detected and processed are different in different types of disease and that the treatment of these types of pain requires different drugs to the ones we take everyday for a minor acute pain. In some diseases such as arthritis people start with acute short lived, intermittent pain form the joint and overtime this becomes more regular, more intense and more debilitating pain. This change is not just because the disease in the joint is worsening, we need to understand why this happens so that new treatments can be developed. We aim to identify the mechanisms occurring at a sub-cellular, cellular and tissue level that lead to chronic pain. We will achieve this by studying models of major clinical chronic pain problems in society, these are inflammatory pain, neuropathic pain and musculoskeletal pain.

There is increasing evidence that people experience of pain is shaped by both events that take place in early life and interactions with anxiety. For example, newly-born term and pre-term babies are exposed to a number of tests which will activate the pain pathways, it is now clear that these events may influence the experience of pain in adulthood. We will investigate how pain in early life effects the way the nerves, spinal cord and brain responses to pain in adulthood. It is clear from clinical data that mental health, in particular anxiety, modulates responses to acute and chronic pain. Why people with higher anxiety experience greater chronic pain is an important question which can be studied using animal models. At the same time our

work under this authority will be complemented by studies in clinical samples and populations as well as cell based approaches to the study of pain which we and others 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)?

Chronic pain is a major worldwide clinical problem that impacts hundreds of millions of people every year. Pain arises following trauma and disease or as a consequence of medical interventions like surgery or as a side effect of drug treatments. Acute (short lived) pain is a necessary survival mechanism alerting us to tissue damage, however chronic pain outlasts any tissue damage and has no beneficial purpose. Pain can negatively impact the lives of anyone regardless of their age or sex. Chronic pain states are more commonly seen in older people (in diseases such as osteoarthritis) however it is also seen in the youngest children born prematurely. This research will advance understanding of how early life pain changes the way the central nervous system matures, and why this alters pain responses for the rest of an individual's life. This new information will allow us to identify new ways to prevent these changes and hopefully prevent altered pain responses in adulthood. In later life, pain can arise following inflammation, injury to sensory nerves (neuropathic pain) or from diseases of later life such as osteoarthritis (OA). In terms of the mechanisms leading o these conditions, understanding what is similar and what is different between these different types of chronic pain will allow us to identify new ways to treat these chronic pain states. People in chronic pain are more likely to suffer from anxiety and other mental health problems such as depression. The mechanisms by which pre-existing anxiety can exacerbate chronic pain are poorly understood, understanding how these interactions occur will enable the future development of new treatments. The research in this application will shed new light on these questions and directly influence clinical research.

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

We will use rats (Sprague-Dawley, Wistar and Wistar-Kyoto strains) and some genetically modified and wild-type mice. This will be over the entire course of the licence period. We have calculated a MAXIMUM of 9200 rats and 2500 mice will be used.

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

We will use models of inflammatory, arthritis and neuropathic pain. Adverse effects of these models vary; the models of inflammation cause some tissue swelling at the site of injection and this doesn’t spread to another site. The inflammation lasts for a few days and cause some changes in movement, but this won't alter the animals’ ability to access water or food. The animals have small but biologically important

changes in their pain responses to a fixed stimulus applied to the site of inflammation. The models of arthritis and neuropathic pain involve either a short surgical procedure under anaesthesia or an injection of a substance that causes damage to cells that lead to the injury of the joint or the sensory nerves. The effects of these models last longer (weeks to months) than the models of inflammation, they also lead to changes in pain responses to fixed stimulus applied to the site of injury. These models may cause some short-lived reductions in weight gain and some reduction in mobility following the model induction. Between a third, to one half, of the animals used will be the controls for the surgery or treatment to induce the model, and so the adverse effects will be less in these animals. Some of the animals receive drug treatments aimed to reduce the pain, which will also reduce the burden of severity. The maximum severity of our work will not exceed moderate. At the end of all studies 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

Pain arises due to complex interactions between cells in many different parts of the body including the peripheral and central nervous systems, the immune system, circulation and endocrine systems. These cannot be replicated in vitro. The central nervous system in particular is exceptionally complex and something that cannot be replicated in vitro, similarly in silico approaches rely on obtaining large datasets from in vivo studies before they are useful. Laboratory rodents are least sentient species in which these studies are able to be performed. They are vertebrates, like humans, and share the basic anatomical and physiological responses to pain that are seen clinically in man. Pain relies on the integration of nocuous information into complex spinal and brain systems which are not present in invertebrates.

Reduction

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

Reduction

Our previous experience of performing these types of studies in the field of pain research have provided us with valuable information which can be used to perform calculations during the design of research studies so as to ensure the minimum number of animals are used in a study. All studies have to be designed and planned well in advance by each experimenter and a written plan with appropriate power calculations presented for inspection by the licence holder and/or deputy. We are

therefore confident that with this step included in our procedure we can minimise 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
To ensure clinical relevance of our studies it is important that these experiments are performed on mammalian species. Rodents have become the ideal choice for pre-clinical research as they have proven to be reliable models of humans in many aspects. As rats and mice have been so often used their anatomy, physiology, and welfare are extremely well understood. This facilitates and expedites research that can be done into the understanding of pain and pharmacology, all whilst being able to ensure that animal welfare is conserved. Our research aims to build upon pre-clinical work performed over the last century and so the majority of models and tests we plan to use have been extensively developed, refined, and validated. We aim to use a range of models that reveal unique insights into neurological mechanisms whilst maintaining animal welfare as much as possible. The durations of the pain models vary, in part due to the duration over which individual mechanisms act, and in part due to the nature of the injury. The shortest models are the inflammatory models which usually resolve within a week, whilst the longest are the arthritic models which we have previously studied for durations approaching 30 weeks. In all cases the length of the study will be determined based on what the particular aim of the study is; however, some models will be limited in duration to ensure animal welfare is upheld.

We will minimise unwanted pain responses due to potential irritation following repeated dosing of drugs, or post-operative pain due to a surgical procedure. This is achieved through the limited use of repeated injections into the same site and the use of local anaesthetic cream (EMLA) at the sites of an incision for example for the generation of models of OA or neuropathic pain. In cases where a recovery surgery is performed prior to the induction of a pain model, systemic analgesics will be given to minimise any pain. In all cases we will attempt to minimise suffering by ensuring that upon completion of surgery animals are placed in a recovery cage or their home cage and will be provided with bedding, warmth, and mash to ensure that they are comfortable and that their environment is enriched. Experimenters are closely observing the animals to ensure they are recovering appropriately.

The models of pain used are associated with changes in thresholds to painful stimuli which are measurable when stimuli are applied to freely-behaving animals. These are the same tests as used clinically in people with chronic pain. They provide use with very useful data whilst causing minimal distress. If performed at a high frequency, however, the tests may contribute substantially to the cumulative distress of the animal. To minimise this risk frequency limitations have been laid out for each test.

The other behavioural tests are also well refined and used in many labs, these tests measure activity or weight distribution on the limbs, they are not expected to cause any harm or stress even when repeated often no limitations are necessary for the frequency of testing. With measures of anxiety we have selected tests that do not induce anxiety themselves and thus ensure welfare is maintained. Again, limitations to frequency will be adhered to in order to ensure animal welfare is not compromised.

We have incorporate best practices for ensuring the welfare of neonatal animals is maintained for a broad range of pain models. In each of the pain models the refinements that have been made for the adults will be incorporated alongside appropriate adjustments for volumes administered. Furthermore, when appropriate we aim to make specific changes that may benefit the younger animals, for example inflammatory substances will be administered to the dorsum of the hindpaw to minimise the impact that the pain model has on the pups ability to develop motor skills and compete for food.

PROJECT 186

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.

Word limit; 1000 words

Project Title	Hypoxia and its role in the transcriptional modulation of physiological response
Key Words	Oxygen, hypoxia, heart attack, stroke
Expected duration of the project	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):

The aims are to better understand how low oxygen levels in tissues can impact how those tissues survive and function. We aim to understand how the interruption of blood flow, which occurs in both heart attack and stroke, can lead to oxygen deprivation, and how that in turn can cause changes to tissues; those changes can allow adaptation to reduced blood flow and oxygen supply, but sometimes lead to tissue damage, something we would like to understand.

What 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 significant killer of people in the UK is heart disease and stroke. Both of these occur because blood flow is interrupted to the heart and brain, respectively. When blood flow is halted, oxygen does not get to these tissues, and as a result tissue damage and even death occur. A better understanding of how tissues respond to loss of oxygen will help treat these diseases more effectively, and help us better prevent the damage that loss of oxygen gives rise to.

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

We use mice, and anticipate using 20,000 over the five year course of these experiments. Our experiments involve very complex genetic modeling, with many genes altered simultaneously in the same animal; all of the genes we work on are involved in the complex responses of tissues to oxygen deprivation. To obtain these extremely complex mice, equally complex breeding schemes are necessary that give rise to significant numbers of mice. We use state of the art genetic monitoring to

minimize the numbers created and used, however, and keep them to the absolute smallest number required to gain scientifically reliable results.

In the context of what you propose to do to 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 many of these experiments, the adverse effects are mild, in that animals do not suffer undue stress by being subjected to the levels of oxygen employed by us experimentally. They will experience some increase in breathing rates and slightly lower levels of activity, much as is experienced by mountain climbers and other people at high altitudes. Some animals will have surgically implanted units used to monitor parameters such as blood pressure and heart rate. When this is done, all procedures will be carried out with aseptic surgical techniques and appropriate levels of anesthesia, and pain relieving drugs will be administered during recovery from surgery. On occasion, humane techniques will be used to obtain blood and urine samples, and drugs will be administered in some cases as well. Animals will be killed humanely 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 response to low oxygen is very complex, and to better understand how it impacts tissues during cardiovascular and other diseases, it needs to be studied in whole animals rather than in tissue culture/cell systems alone. . This is particularly true when one wishes to understand, as we do, how the interaction of multiple tissues at once gives rise to coordinated responses by the body to low oxygenlevels]. Additionally, we use cell co-culture systems wherever possible to model simple cell-cell interactions. Such co-culture systems are an important mechanism for replacement of living animals. We will continue to explore such systems for co-culture of multiple cell types to allow us to replace use of animal models wherever possible.

Reduction

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

Reduction

We always use the minimal number of animals necessary based on statistical calculations and then using only those animals needed to obtain a reliable result. We do pilot experiments with small numbers to gain an initial understanding of the

degree of change our experiments cause in the response to low oxygen, and only after understanding that do we carry out a larger experiment designed to use the smallest number of animals necessary to get a scientifically and statistically reliable understanding.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 pain relievers and other agents to minimize effects of procedures on animals, whilst in pursuit of our experimental objectives. We use mice, as they are a species that has high relevance to humans in regards to physiological response to low oxygen, and their genes can be readily modified to enable us to investigate specific pathways/genes of interest in a way that cannot, for ethical reasons, be completed in humans. Our animal models are state of the art and engineered to directly address pressing questions related to cardiovascular health.

PROJECT 187

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.

Word limit; 1000 words

Project Title	Analysis of genetic eye disease and new therapies
Key Words	genetic, eye, disease, development
Expected duration of the project	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):

The overall aim of this project is to characterise novel genes involved in genetic eye disorders and understand their role in eye development, normal function and disease in order to develop potential therapies to treat patients suffering from visual impairment and blindness. Worldwide 285 million people are visually impaired, of this 39 million are completely blind. The majority of conditions causing blindness have a strong genetic component; 60% of blindness in infants is caused by genetic eye disease, and inherited retinal diseases account for the most common cause of certifiable blindness amongst working age adults in the UK. Only less than 25% of patients with genetic eye disease receive a molecular diagnosis. There is no effective treatment available to the majority of patients.

With more advanced technology, genes are being detected but little is known about their normal function in the eye. Initially, genetic analysis using whole genome sequencing will be performed on affected patients, if a novel disease-causing gene is identified, and there is a lack of knowledge on its role in human disease, we will use the zebrafish model to study gene function in the eye and any related sites of expression. Zebrafish share 70% of their genes with humans, and their eyes are closely related in cellular structure and colour vision, therefore by employing experimental techniques to manipulate genetic function, we can gain a greater understanding of the disease processes to better characterise patients. Through this research, we can identify targets for potential therapies against eye disease. *In vitro* human cell cultures will be used initially to assess treatment efficacy, and if successful, it will be translated to zebrafish disease models to examine the outcome *in vivo,* before future clinical application.

What 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: 1. Improved genetic diagnosis for patients and subsequent genetic counselling and family planning. 2. Improved knowledge of molecular mechanisms of disease leading to identification of therapeutic targets 3. Development of potential therapies for genetic eye disease 4. and the Sight Loss and Vision Priority Setting Partnership list ascertaining the causes and prevention of MAC within the top 10 priorities for research into childhood onset eye disorders (www.sightlosspsp.org.uk). The majority of MAC patients with an unremarkable prenatal history lack a genetic diagnosis, and are thus managed without insights into the molecular cause. Consequently, families fail to receive accurate genetic counselling. Early diagnosis can reduce morbidity and maximize function through supportive treatment, providing significant health economic benefits. The main clinical benefit of this study will be enhanced diagnosis and improved genetic counselling for patients and their families. The major application will be transferring knowledge to understand normal eye development, related birth eye defects, adult genetic eye disorders and how the same gene affects other organs in the body. Once we understand the genetic mechanisms of abnormal development, a real focus on preventative treatments can be developed in the future. Beneficiaries will be patients and families with genetic eye disease, and in particular those with ocular maldevelopment, clinicians providing their care, researchers in the fields of Ophthalmology, Genomic Medicine and Developmental Biology as a whole. Plus, public sector charities supporting childhood, eye and rare diseases, and policy makers, including those in the UK Government and the European Union. The global burden of sight loss extends to 285 million people worldwide. Nearly 20% of the 25,000 children registered blind or visually impaired in the UK suffer from ocular maldevelopment, making it the most common cause of visual disability. Approximately 50% of these children have additional disabilities with special educational needs. From the Sight Loss UK 2013 RNIB report, the total direct NHS expenditure on eye health was £2.64 billion. The direct cost of care for children with ocular maldevelopment is unquantified and lifelong, including frequent NHS outpatient appointments, surgery, prosthetics, prescriptions for eye drops, multidisciplinary team input and social care for the whole of their lives. Indirect costs, which include total cost of unpaid care and reduced employment, particularly relevant for those caring for children with sight loss, costs the UK economy £5.3 billion. In order to reduce these costs, fundamental research into the aetiology of eye disease and development of subsequent therapies are required. Potential treatment targets will be licensed and commercialised in partnership with industry. In this way the research will also contribute to the nation's wealth by fostering scientific and economic competitiveness in the United Kingdom. Policy-makers setting the research agenda will benefit as this research is directly relevant to patients, and demonstrates how investment in genetics and translational stem cell research can

potentially change lives. It also guides allocation of NHS resources through specialised commissioning and establishing formal European Reference Networks.

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

We will be using zebrafish, approximately 10,000 embryos and 10,000 adults over the 5 year period, together with human cell models for our experiments. The statistical advisory service will support us with the minimal number of zebrafish required for each part of this study (the sample size required to determine a meaningful and significant result). From previous experiments characterising the disease features and treatment investigations ~30-50 zebrafish per experiment will be required. It is best practice to ensure the total cohort of fish are derived from 3 separate breeding crosses. To look at all the genes involved in a normal developmental pathway or disease process using a new technology called RNA-Sequencing, we use equations to determine the number of fish required based on natural genetic variation and the size of the effect to be measured (e.g. prevention of retinal degeneration or not), and this suggests we need approximately 25 zebrafish per group. We plan to use the minimal amount of fish necessary over the next 5 years, refining techniques, and replacing their use with human models where appropriate. We have already removed several protocols that were present in our last project license as they were too harmful or have been superceded with more milder and effective 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 overall workflow in this project involves breeding genetically altered fish, which are not expected to develop a harmful phenotype. Drugs, administered in concentrations not expected to cause adverse effects, will be administered to the water (for embryos) or directly into the eye under anaesthesia (for adults). Toxicity will be assessed by studying behaviour, including swimming, balance and feeding. Non-invasive behavioural studies and imaging under anaesthesia will be performed to assess for response to treatment, and then fish will be humanely killed. If a harmful phenotype is seen, the animal will be humanely killed. Anaesthetics and/or analgesics will be used to minimise suffering. Animals will be humanely killed if they show adverse effects that are more than transient. The majority of work will be performed on embryos less than 5 days post fertilisation (dpf), when they are incapable of independent feeding, and if any signs of distress are observed, they will be humanely killed. This protocol has been introduced as we are now seeing disease phenotypes manifesting in adult zebrafish and so therapeutics can be delivered locally to the eye to observe for treatment effect, rather than as an embryo when this is not realistic or wholly applicable to adult-onset disease. New technologies also permit less invasive analysis of disease signs e.g. using genes to encode harmless fluorescent proteins to visualize developing blood vessels and nerve cells. Patients

and cells in a dish will be used to study clinical features and treatment response, respectively. Limited information can be gained about the molecular basis of genetic eye disease from patients, mainly due to inaccessibility of tissue, hence the zebrafish will be required to supplement the work as it cannot be modelled in any other way. This research project will add to our growing knowledge and understanding of genetic eye disorders and normal ocular function with a direct aim of developing treatments to prevent blindness in patients. All fish 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

Birth eye defects develop within the first few weeks of pregnancy, and degeneration of the retina can occur from infancy through to late adulthood. We cannot assess the molecular basis of genetic eye disease as access to human tissue, particularly the retina, is not possible. Most genetic studies require analysis of the whole eye to determine function and rescue through treatment and these cannot be *modelled in a dish as* yet. The genetic code, related databases looking at genes and protein, and the zebrafish community (such as ZFIN) provide information and other resources for all genes in the fish. This information helps us to select between various disease-causing genes for functional analysis, rather than having to perform preliminary analysis on all candidates. All potential therapeutic strategies will be tried on cell models first to determine safety and toxicity, if a benefit is seen, then the treatment will be applied to animals. The retina is a complicated cellular model with several layers, hence, although cell cultures allow us to study one particular cell type, we need to evaluate entire retinal structure and function. At the moment we still do need to use fish in our research, we regularly review the scientific literature to ensure that we can respond to new developments in model design particularly where newly emerging in vitro techniques could replace animal use. However, at present the use of animals is essential.

Reduction

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

Reductio

The number of zebrafish used in each experiment will be kept to the minimum required to achieve statistical significance, and formal statistical advice from the Statistical Advisory Service will be sort to conduct appropriate power calculations. From previous advice between 30-50 zebrafish embryos per experimental condition

will be required to determine a difference between, for example a treatment response such as increase in survival or prevention of retinal degeneration. The zebrafish will also be maintained at the minimum number required to generate sufficient animals of the appropriate genotype. When a line is not temporarily required, sperm will be cryopreserved until needed. This will eliminate the need for continuous breeding to maintain lines and importing fish from other PPLs. To minimise the number of fish we need to breed, females, from which eggs have been previously harvested, are re-used for in-vitro fertilisation. Multiple tissues and cell types will be used from all zebrafish that have been killed, to reduce the number of fish required. To further reduce the amount of animal work, in-vitro models will be used.

The majority of experiments will be performed on embryos less than 5 days post-fertilisation, when they are incapable of independent feeding. Where possible, we will further reduce the amount of *zebrafish used by adopting* the use of explanted tissues and generating induced pluripotent stem cell lines from patients. The number of animals used will therefore be kept to the minimum required to achieve the goals of this project.

Over the past 2 years we have also adopted the use of non-invasive optical coherence tomography imaging of the zebrafish retina using safe infra-red light (the same as used to image a patient's retina in clinic) to allow for long-term monitoring of the eye, directly reducing the number of fish sacrificed for experiments. We published this protocol in the peer-reviewed journal *Zebrafish* to enable the scientific community to follow suit.

Our experimental design strategy is also 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 (Animal Research: Reporting of In Vivo Experiments) guidelines, to ensure the minimal numbers of animals are utilised in order to gain valid experimental outputs. We also publish in journals that support the use of 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

We have chosen the zebrafish model to aid our studies into the genetic basis of eye disease, due to a very similar structural eye appearance and sharing of approximately 70% of genes with humans. The zebrafish retina is composed of the same light-sensing cells, rods and cones, as humans and this puts it at an

advantage compared to the mouse model (predominantly rods). We study birth eye defects and as the zebrafish lays its eggs outside of the body it allows us to directly visualize the developing eye non-invasively, unlike with other animals. The zebrafish eye reaches an adult form by 72 hours of age, therefore most experiments can be performed before the model is capable of independent feeding, therefore minimizing animal suffering. Also, any zebrafish displaying signs of distress will be humanely killed. Our ability to refine the experiments we perform is progressing due to the development of new technologies that allow less invasive analysis of phenotypes. For instance, traditional labelling of the nervous system to visualise living neurons or axons rely on injection of dyes or other substances to specific regions of the CNS potentially causing minor CNS trauma (albeit at early developmental stages). Instead, genes encoding fluorescent proteins to non-invasively routinely monitor neurons and blood vessels in living fish can be employed. These genes/proteins are entirely harmless to the fish and allow us to visualise many developmental processes without any harm to the animal.

In order to identify genes involved in early eye development, one approach is to mutate genes randomly throughout the genome using a harmful chemical called ethyl nitroso-urea (ENU), and then analyse fish carrying these mutations for defects. We have replaced this method, which used to cause substantial harm, with a gene editing method with less harm (mild procedure). These procedures are widely followed by the zebrafish community and communication in forums, such as ZFIN, over previous years has helped to refine the protocols, reduce the harmful effects on the fish and improve efficiency of the genetically altered fish.

We undertook a study where we compared the retinal structure of histological sections of the zebrafish with the images obtained through optical coherence tomography, the results were equivalent. This is a refinement in technique as it is non-invasive, rapid and also reduces the use of zebrafish.

We are constantly striving to improve and refine the use of zebrafish by regularly reviewing the techniques and outcomes of our research, another example is the addition of analgesia to the tank water following fin clipping for genotyping and after drug administration into the eyes of adult fish. This was a refinement published in the scientific literature that helps to reduce any pain in the zebrafish following the procedure with improved zebrafish recovery. We will also endeavour to use skin swabs instead of taking a small piece of fin for genetic identification as this is a mild and non-invasive procedure.

PROJECT 188

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.

Word limit; 1000 words

Project Title	Regulation of neuronal and neuroendocrine function across the life course
Key Words	Epigenetics, Transcription, Neurotransmitter, Neuroendocrine
Expected duration of the project	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;
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):

This project seeks to increase scientific understanding of how nerve cell signals and hormones influence the development and functioning of the brain through identifying the changes to gene activity which they produce and learning more about the biological and behavioural consequences of these changes. In addition, we wish to identify novel small molecule drugs that may alter the functions of the proteins encoded by some of the genes affected by these nerve signals and hormones, and which may be of value in treating nervous system 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 work will lead to increased understanding of how the vertebrate nervous system grows and develops. Our proposed studies will also shed light on the functions of genes implicated in human nervous system disorders. The proposed small molecule screens may provide novel drug leads that could be of value in developing much-needed new therapies for neurological disorders.

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

Animal usage will be kept to a minimum. Most of the adult fish required for this project will be used for breeding purposes to produce genetically altered embryos and larvae that will then be used in experiments, at developmental stages prior to the onset of independent feeding. For each gene of interest, a stock of ~120 adult fish are needed to provide a reliable supply of embryos and larvae. We estimate that ~4000 adult fish will need to be bred per annum. Adult fish are killed humanely at the

end of their breeding life (~2 years of age). Overall, we expect to to use 25,500 fish over the period of this project 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?

Breeding stocks of genetically altered fish are not expected to exhibit major physical or behavioural abnormalities. Fish recover well from the biopsy procedures used to obtain DNA samples from fish. Fish exhibiting any unexpected adverse behavioural responses to compound treatments will be humanely killed. Any fish exhibiting signs of abnormal balance, physical posture, a failure to feed or to breed, and other physical manifestations of ill-health such as the presence of tumours, will be humanely killed. All animals will be humanely killed at the end of each experimental procedure.

Application of the 3Rs

Replacement

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

Replacement

Crucial hallmarks of the developing vertebrate nervous system are the orderly assembly and integrated functioning of nerve cells as components of neural circuits in the whole animal. This process is not fully understood, and it cannot currently be modelled with any accuracy in vitro using cultured neurones or in silico models. Understanding of how neural circuits are built and how they function together absolutely requires in vivo animal studies. Most experiments will be performed on embryos and larvae under the age of protection. A few experiments will use juvenile or adult fish. Alternative model vertebrates, such as the chick and mouse are of higher neurophysiological sensitivity than zebrafish, and they do not have the combination of genetic and pharmacological tractability, as well as the optical clarity for live and fixed tissue imaging, that the zebrafish possesses. For these reasons, the zebrafish an excellent replacement for the mammalian models that have traditionally been used for understanding the development and function of the vertebrate nervous system.

Reduction

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

Reduction

We will maintain sufficient numbers of adult zebrafish to ensure that a reliable supply of embryos and larvae are available for the proposed experiments. For chemical

treatment and behavioural studies, we will perform small scale pilot studies to inform the power calculations that will be needed to enable the definitive experimental studies to reach statistical significance. Where appropriate, molecular data obtained from in vivo studies will be confirmed in cultured mammalian cells.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 provides great analytical power for understanding how the vertebrate brain is built and how it works. The human and zebrafish genomes are highly similar, so insights learned from zebrafish genetic studies are likely to be relevant to understanding human brain development and function. Many sophisticated genetic tools are available to manipulate gene function in the whole animal, and the optical transparency of externally developing zebrafish embryos and larvae allows individual cells and their behaviours to be observed in the intact animal in real-time, with great precision and resolution. The chemical permeability of zebrafish embryos also allows in vivo drug discovery research using compounds that are diluted directly into the water. Zebrafish aquaria and husbandry techniques are developed and implemented with fish health and welfare foremost in mind. Aquarium and veterinary staff involved with the project are involved with relevant aspects of research aimed at improving welfare and ensuring standards of best practice are improved. All new experiments on protected animals are assessed internally by Individual Study Plans, and pilot studies are initially undertaken to assess feasibility and inform larger scale experimental design, ensuring that fish usage is kept to a minimum.

PROJECT 189

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.

Word limit; 1000 words

Project Title	Novel vaccine development for lyssaviruses and flaviviruses
Key Words	Vaccines, rabies, lyssaviruses, flaviviruses, challenge
Expected duration of the project	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;
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):

1. The development of new vaccines that are able to protect against multiple viruses (lyssaviruses, flaviviruses) following vaccination.
2. Assessment of the ability of existing rabies vaccines to protect against divergent lyssaviruses and recombinant viruses expressing different lyssavirus glycoproteins

What 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) Potential identification of novel human vaccines that will go on to further pre-registration studies. If successful these vaccines could revolutionise human and animal vaccination strategies the lyssaviruses and flaviviruses. 2) Increase knowledge base on the protection afforded by rabies vaccines, and inform policy on the applicability of different vaccines that target lyssaviruses and flaviviruses.

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

Potentially up to 800 mice on each of the 3 protocols 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?

Protocol 1- Assessment of the pathogenicity of lyssaviruses and relevant flaviviruses in different strains of mice- involves pathogenesis assessments for viruses to use as challenge viruses post vaccination and to simply establish pathogenesis of isolates. Severity is expected to be mild or moderate. Should the outcome be deemed to have

been exceeded, for example where an unexpected accelerated disease progression has caused death during the overnight period, the severity limit of the licence will be re-evaluated. Similarly for flavivirus infection, clinical outcomes are used to define humane end points. Protocol 2- Serological assessment of vaccination without challenge- involves assessment of vaccine doses in mice to determine antigenicity and optimal vaccination strategies. This only includes vaccination and serological assessment of animals and as such is rated as mild. No adverse effects of vaccination are predicted. Protocol 3- Vaccination challenge experimentation with lyssaviruses and flaviviruses- mirrors protocol 1 in that it involves virus challenge with different viruses and although it is expected that all animals will be maximally be moderate. Should this limit be exceeded then the severity rating may be reviewed appropriately. Animals from all 3 protocols are euthanased by a schedule 1 method 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

Initial assessment of vaccine candidates will be done using serological neutralisation of virus in vitro. However, to truly define the antibody response to vaccination and pathogenic virus infection, animals are required. Further, to assess any protection afforded by vaccination, the infection of vaccinated animals is required.

Reduction

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

Reduction

Local ethical review, including by a biostatistician, of proposed in vivo studies will ensure that the minimal number of animals is used. All studies performed for research activities are carried out to ISO9001 quality standards. Our establishment is committed to complying with ARRIVE guidelines. Futher, all studies are scrutinised by the AWERB onsite at our establishment.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 hamsters represent a good choice for these studies as there are established models of lyssavirus vaccination and pathogenesis which results can be validated against. We have a state of the art research facility within which to conduct animal studies to the highest level. Welfare is key to all of our studies and refinements have been made across different experimental platforms to minimise harms including refinement of techniques, improvement in enrichment and environmental elements and increased post inoculation observations and human endpoints.

PROJECT 190

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.

Word limit; 1000 words

Project Title	Resuscitation and management after traumatic injury
Key Words	Trauma, Blood-loss, Resuscitation
Expected duration of the project	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;
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 overall aim of the research is to reduce the number of people that die as a result of severe injury resulting from trauma. This will be achieved through the development of new treatments. Treatments need to be effective for people who are injured in remote and/or poorly resourced environments to reduce secondary injury and inflammation arising from the body's own response to trauma.

Objectives:

1. Evaluate impact on survival, blood clotting, whole body physiology and inflammation of different therapies such as fluids (used for resuscitation), drugs, resuscitation strategies and devices (emergency equipment) given or used *after* traumatic injury has occurred;
2. The same evaluation as objective 1, but this time for treatments *given before traumatic injury* (pre-treatment);
3. Provision of blood and/or blood products for resuscitation.

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

Results will provide the evidence base for new early treatments of trauma to be used by Defence Medical Services (DMS) and potentially the civilian sector. The benefits to the patient will include: • Improved survival. • An improvement in physiological, coagulation and inflammatory state during the early in-hospital phase that will allow greater flexibility in choice of surgical procedure. • Reduction in post-operative complications that result in patients needing longer periods of intensive care. • Reduced morbidity (degree of 'sickness') and enhanced recovery (improved quality

of survival). The benefit to health systems will be: • A reduction in hospital stay required by casualties and therefore a reduction in the burden of trauma to health systems. • Guidance for DMS regarding the clinical resources needed to manage casualties. • Guidance to the civilian sector when planning for medical treatment of victims of terrorist incidents. Work conducted under previous licences has supported clinical changes in military and civilian resuscitation practices. These changes have been credited with saving lives in Afghanistan, and are currently being translated into civilian practice in the UK.

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

Approximately 400 pigs, 50 rabbits and 400 rats over the 5 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?

Animals that have a blood sample taken before the injury phase may have some bruising at the sampling site. Animals that receive drugs before the injury phase may experience mild side-effects. Animals that receive an anaesthetic to allow . blood sampling before the injury phase for example, may experience a very short period of dizziness or disorientation. All other procedures will be performed under anaesthesia, and the animals will be killed humanely at the end by overdose of anaesthetic. None of the animals will therefore be aware of any major adverse effect. During the injury phase there will be tissue damage and initiation of widespread inflammation in most of the animals, and there may be early death, as these are features of the response to trauma in people. The purpose of the study is to find treatments that reduce the adverse effects of trauma. However, is must be stressed that the animals will be unaware of any adverse effect during the injury phase as they will be anaesthetised throughout.

Application of the 3Rs

Replacement

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

Replacement

These studies examine the complex interaction between a range of body systems e.g. cardiovascular, respiratory, inflammatory and clotting. It would be impossible to assess these integrated responses without using living animals.

It would be possible to conduct some anatomical studies using cadavers (including human cadavers), but adverse responses relating to tissue/organ function could not be investigated without a living system.

Techniques using living organs or isolated cells can be used to screen drug action and for some studies of local mechanisms. However, when the research question requires an interaction between several body systems, and the whole body alterations inherent in trauma states, then the only model that can be used are whole living systems.

Wherever possible we have been developing models in human volunteers. These are being used to study the impact of mild levels of injury and assess drug action. However, it would be unethical to model severe injury in human volunteers, and the new treatments that are the subject of this licence application need to be evaluated in anaesthetised animal models before a clinical trial on human patients.

Reduction

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

Reduction

If several treatments are being assessed that require the same “comparator” group (a good example of a “comparator” group would be the current standard of care to see if a new treatment is better or worse), experiments will be designed wherever possible to allow the sharing of “comparator” groups between studies, and hence reducing the overall number of animals required.

We will use a stringent statistical approach to ensure a clear answer with the least number of animals.

Data from our studies will be provided to other researchers to avoid the need for animals to be used in their 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 least sentient species consistent with the aims of the study will always be chosen, principally dictated by the degree of monitoring and granularity of sampling needed as well as fidelity of the model to the human condition being investigated. Suffering that would be associated with the injury is eliminated by the use of terminal anaesthesia.

When social animals such as pigs are used we will endeavour to house these animals in groups of 2 or more prior to commencement of the experiment. If this is not possible we will ensure that single housing is for as short as time as possible and

where practical single housed animals will have visual contact with others of the same species.

Tissue collected from our studies may be used by collaborators to increase the information derived from our studies.

PROJECT 191

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.

Word limit; 1000 words

Project Title	Bacterial infections in farmed animals
Key Words	Bacteria, Pathogen, Disease, Zoonoses, Safety
Expected duration of the project	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):

The primary objective of work under this licence is to reduce the burden of intestinal disease in farmed animals and humans. The bacteria *Campylobacter*, *Salmonella* and *E. coli* cause around half a million cases of gastroenteritis in humans each year in the United Kingdom at a recurring annual cost of >£1bn. Farmed animals are key reservoirs of these organisms and human infections often arise from consumption or handling of contaminated animal products. Reducing the prevalence of such bacteria in farmed animals will improve food safety and lower the incidence of human infection. However, effective vaccines for these agents are lacking and antibiotic use is restricted to limit the evolution of resistance and entry of drug residues into the food chain. Some types of *Campylobacter*, *Salmonella* and *E. coli* can also cause disease in animals and impair productvitiy and welfare. In addition to these pathogens, we also propose to study *Clostridium perfringens*, which causes necrotic enteritis in poultry. Our main aims are to:

1. Identify bacterial factors required for colonisation of farmed animals and disease. This will involve analysis of mutated bacterial strains in animals compared to wild strains &/or mutated strains that have been repaired.

2. Identify the responses of animals that are associated with clearance of natural infections and vaccine-mediated protection. This will involve measuring immune responses of farmed animals to infection or vaccination, as well as work to modify the immune system to understand which responses are needed for protection.

3. Identify genetic variation among farmed animals that is associated with resistance to colonisation by bacteria or resilience to disease. Farmed animals often exhibit

heritable differences in their response to pathogens and we will associate variation in their genetic make-up with their resistance to colonisation or disease.

4. Define the role of microbial communities naturally found in farmed animals in resistance to disease and as a reservoir of antimicrobial resistance genes during infection and drug 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)?

Identification of bacterial factors required to colonise their animal hosts and produce disease can be used to design drugs that inhibit their function. Moreover, such factors can be purified and used as ‘subunit’ vaccines that raise immune responses in animals that will neutralise the function of that factor if infection occurs. Factors required for virulence can also be removed from harmful strains to produce vaccine strains that are alive but weakened. Understanding the immune responses of animals that are required for natural or vaccine-mediated immunity will help us to devise better vaccines. Once we know the key constituents of pathogens that induce immunity, and which types of immune response are vital for clearance, we can devise ways of boosting those responses and making them long-lasting and effective against circulating forms of the microbes. Knowledge of the animal genes associated with resistance or resilience will allow breeders to select for animals with improved responses to the pathogens under study. In the long-term, identification of the precise genetic variation that confers resistance may allow the production of engineered animals with beneficial changes to their genome. Understanding the role of gut microbial communities in resistance may identify beneficial microbes that can be used as probiotics in animals or humans, or whose growth could be encouraged with dietary additives. Most microbes cannot be cultured in the laboratory and the role of this ‘dark matter of microbiology’ in harbouring drug resistance genes requires study if we are to reduce the spread of drug resistance in animals and people.

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

Over five years we anticipate that, at most, we would use 5000 chickens, 100 pigs, 100 cattle and 500 mice, mostly in procedures of mild or moderate severity. This use should be balanced against the vast scale of global animal production, the extent of animal suffering due to bacterial diseases, and the potential to improve food safety. The potential for our research to enhance societal and economic prosperity is evident from the ongoing investment of funding agencies and industry.

In the context of what you propose to do to 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 under this licence cannot enter the food chain or be released to the environment as they will typically have been inoculated with bacteria that are harmful

to humans and which may have been genetically-modified. All experiments must therefore be conducted in biosecure accommodation and at the end of licensed procedures animals will be humanely killed. For context, it is estimated that 60 billion chickens are killed worldwide each year for meat. Many of the infections studied produce little or no clinical signs (e.g. Campylobacter or non-typhoidal Salmonella in poultry; E. coli O157 in cattle). Some types of these organisms can cause diarrhoea in farm animals and spread from the gut to cause systemic illness. We expect work involving these types to be rare and to be able to meet objectives before the onset of clinical signs of moderate or substantial severity.

Application of the 3Rs

Replacement

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

Replacement

Wherever feasible, animal use will be avoided through the use of immortalised cell cultures and a *Galleria mellonella* (wax moth) model for analysing bacterial virulence. As disease originates from farmed animals, we believe it is necessary to study the activities of pathogens, the nature and consequences of immune responses, and the role of genetic variation and natural microbial communities, in the relevant animal.

Reduction

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

Reductio

We will reduce animal use by interacting pathogens with tissues or cells from farm animals that are cultured in the laboratory. Often such cells can be obtained from animals undergoing other procedures or from abattoirs. We will continue to make extensive use of approaches to reduce animals use, including methods to screen the activities of large numbers of bacterial mutants in pools, co-infection studies to define how mutant and parent strains compete, and surgical approaches that permit the testing of multiple strains or treatments in 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

We strive to reduce the impact of licensed procedures on animals used in our research and have refined monitoring and clinical signs used to evaluate severity and define human end-points. Moreover, with each experiment estimates of variance in the measurements taken are used to calculate the minimum number of animals of the target species that are required to achieve statistical significance and give confidence to users of the data.

PROJECT 192

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.

Word limit; 1000 words

Project Title	Biochemistry, genetics, virulence and drug action against trypanosomes and Leishmania
Key Words	parasite, infection, treatment, diagnosis,, host-pathogen interaction
Expected duration of the project	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.

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):

This project aims to develop new ways of combatting human and animal diseases caused by specific parasites. The parasites, called trypanosomes and Leishmania, are single celled organisms that live in our blood and some other organs. Current drugs are of limited use against the diseases caused by these parasites, which afflict millions of people and also millions of animals in parts of the world where food is in short supply. Trypanosomes are transmitted by biting flies, particularly tsetse flies which feed on the blood of humans and other vertebrates. Different species of trypanosome cause disease in humans and domestic animals in Africa. Leishmania parasites are transmitted by tiny sandflies. Different leishmanial species cause a range of diseases in the tropics and sub-tropics. Depending on the species humans and various animal, incuding dogs, can be affected. The trypanosomiases and leishmaniases have been classified as neglected tropical diseases, given the lack of incentive for pharmaceutical companies to develop drugs for use amongst the world's poorest people. It therefore falls primarily to academics, funded through government research agencies, charities and non-governmental organisations to work towards new treatments for these diseases, although a few pharmaceutical companies do retain an interest. We aim to learn more about the biology of the parasites in order to develop new drugs and to test new chemicals for their ability to control infections, as a starting point to take those new molecules forward to cure the diseases. We also hope to find new ways of diagnosing the presence of specific parasites and understanding the processes by which they cause disease, offering other ways of controlling virulence.

What 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 that during this project we will make progress in the development of new drugs to treat the African trypanosomiases (human and animal derivatives) and also the Leishmaniases. New effective medicines are desperately needed for the millions of people afflicted with these diseases and also to preserve the livestock afflicted to help maintain food supplies in afflicted regions.

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

We will primarily use rodents: Mouse – 4600 over 5 years Rat – 100 over 5 years Hamster – 100 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 anticipate minimal adverse effects beyond those associated with predictable courses of infection and the level of severity for all procedures is mild or moderate. All animals will be euthanased humanely after experiments.

Application of the 3Rs

Replacement

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

Replacement

Although trypanosomes and Leishmania parasites can be grown in in vitro systems, when it comes to assessing the ability of drugs to kill them it is necessary, ultimately, to determine whether activity found in vitro is retained in vivo where the complexity of mammalian biology impacts upon chemicals in ways that can inactivate them. Parasites can be difficult to reach in mammals (for example African trypanosomes can invade the central nervous system which is protected by the blood brain barrier and Leishmania parasites can reside within difficult to reach granulomas that cannot be reproduced *in vitro*). We also propose explicitly to understand the impact of parasites on mammalian physiology and to date no substitute for the whole organism has been created that enables assessment of parasites on mammalian physiology.

Reduction

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

Reduction

We will only test compounds against parasites where activity is already proven using in vivo systems (other than where there is explicit evidence that the in vivo system

may activate a compound into a parasticidal derivative). Hence all compounds for testing will first be screened in vitro and the viability of parasites modified in predicted virulence factors screened in vitro before using animals. Using an in vivo imaging system allows the same animal to be followed over time rather than having to euthanase animals at different time points which reduces numbers needed in drug testing experiments. Statistical analysis will be performed for any experiment requiring multiple animals to determine the correct number to offer sufficient statistical power to make meaningful conclusions from experiments and the minimum number of animals always used. We will always use in vitro cultivation of parasites where adequate numbers can be attained for specific 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 offer well understood models of both trypanosome and Leishmania infections hence we will confine most work to this species to offer predictable infections and the most readily system to enable comparison to existing literature in this area. Occasionally rats will be used where larger parasite numbers are required for analysis. For *Leishmania donovani* and *infantum*, suitable mouse models do not always exist and hamsters are used. We will use well established hamster models to ensure our results are readily comparable to existing literature.

PROJECT 193

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.

Word limit; 1000 words

Project Title	Defining haemoplasma host immunity and establishing improved haemoplasma detection methods
Key Words	rodent, immunity, bacteria, detection
Expected duration of the project	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;
No	(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):

This project aims to characterise the immune-response to blood-borne microorganisms that cause disease in animals, including humans. This information will help develop rapid diagnostic tests for use in clinical scenarios. Potential vaccines to protect against naturally occurring disease will also be developed. As these organisms cannot be grown in culture in the laboratory, we want to explore novel cultivation methods to reduce the need for experimental 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)?

Animals will benefit from rapid diagnostic tests that will reduce suffering (as treatment can be started earlier in infected animals) and reduce unnecessary antimicrobial use in non-infected animals. As these blood-borne pathogens cannot be grown in culture, we have to grow them in animals. However, by using a novel rodent model, we hope to be able to reduce the number of other mammals used for similar studies. Ultimately, our understanding of bacterial pathogenesis will be broadened in a previously poorly explored group of pathogens, and their host’s immune response to infection will be determined. This interaction cannot be studied without the use of animals.

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

We expect to use up to 600 mice, 60 gerbils and 60 rats over a period of 5 years. The numbers will be minimised by careful experimental design and appropriate statistics.

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

Following habituation to handling, laboratory rodents will be inoculated with small volumes of blood containing bacterial pathogens and/or novel vaccine candidates. They will then have multiple small volume blood samples taken, which will be positively reinforced by food treats. We will carefully monitor for excessive blood loss and signs of clinical disease. Most mice show no discernible signs in response to infection and only experience mild transitory pain during blood sampling. However, sudden death without any preceding clinical signs may occur in around 1% of animals. When collection of maximum blood volumes is needed at the end of the experiment, animals will ultimately be killed. Some uninfected rodents may be rehomed as pets. When collection of maximum blood volumes is needed at the end of the experiment, animals will ultimately be killed. Some uninfected rodents may be rehomed as pets.

Application of the 3Rs

Replacement

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

Replacement

The pathogens of interest cause variable degrees of disease in their animal hosts. They cannot be grown in culture, so animals must be used to grow them Non-protected animals are not hosts for these pathogens and so cannot be used in these studies. One of the objectives of our study is the characterisation of the host immune response to infection, which can only be performed using an animal model.

We have recently been able to establish a rodent model of infection, which we now want to use for development of novel diagnostic and prevention methods, successfully replacing the use of other research mammals in the early phases of research.

Another objective of our study is to replace the need for live animals to grow these bacteria, by exploring novel methods of *in vitro* cultivation for use in the laboratory. Initially, these studies do require live animals to grow the organism.

Reduction

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

Reduction

The minimum number of experimental animals will be used in each study. For each experiment, the minimum number of animals required to confirm or refute statistically significant differences on the basis on previous experience will be determined (our findings, or from the literature). Where previous experience is lacking, pilot experiments will be set up and numbers increased to the minimum required for significant results, once pilot data have been obtained.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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, such as rats and mice, are the species selected for these studies since we have very recently established a reliable experimental model of infection in these species. This experimental model serves to replace the use of other ‘higher’ mammals in the early phases of research developing diagnostic assays and vaccines. Using laboratory rodents offers the advantage of reducing animal numbers by using already developed and validated analytical assays (e.g. to assess the immune response) and by being able to control for host variability by using inbred strains of rodents.

For each experiment, adverse effects and humane end-points have been defined. Animals will be monitored for adverse effects following each experimental step and at least daily for ill health throughout the experiment. We have not observed any adverse effects related to infection or experimental protocols during establishment of the rodent model and we also have taken care to optimise experimental procedures such as blood draws by positively reinforcing the experimental steps and allowing time for habituation to frequent handling. Naturally, we will abide by these principles and seek to further improve them in the future. Veterinary care will be sought if any adverse effects or signs of ill health are observed.

PROJECT 194

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	The role of human and mouse tissue stem cells in development and cancer
Key Words	Development, Cancer, Stem cells, Breast, Metastases
Expected duration of the project	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;
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 overall purpose is to understand human tissue and tumour development, the cellular hierarchy of stem, progenitor and differentiated cells and their interactions with the tissue microenvironment. These goals are relevant to malignant progression in cancer and metastasis (cancer spread) where an increased understanding will lead to new modalities of treatment which would potentially impact clinically.

What 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 UK, for example in breast cancer, there are more than 50,000 new cases diagnosed each year and there are greater than 12,000 deaths from the disease. In other major tumour types, the mortality is similar, if not worse. Identification of the regulators of tissue and tumour cell stemness versus differentiation is important for elucidation of cellular signalling pathways that could be targeted to regulate tumour development, progression and metastatic spread. We believe the proposed pre-clinical experiments based on understanding mammary development and in vitro CSC colony formation and co-culture studies will lead to advances in our understanding of how to tackle cancer progression and its metastatic lethality in patients.

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

We will use up to 1325 mice over the 5 year period 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?

Procedures will involve the transplantation of human cells into immune-deficient hosts, monitoring their growth and testing therapies aimed at interfering in stem cell regulation, progenitor proliferation and the cellular differentiation programme. These therapies may target the tissue or tumour itself or the supporting cells around the tumour implant with the aim of reducing tumour growth or cancer stem cell activity that drives tumour initiation, recurrence and spread to other tissues. Adverse effects are mostly mild or moderate and mice will be killed humanely if limits are exceeded or at the termination 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

Transplantation of human stem cells into a mouse model is currently the gold standard for testing them based on twenty years of experience in the human haematopoietic and other tissue stem cell fields. There has been recent development of in vitro stem cell models such as sphere colony assays but these have several short-comings primarily that the tissue stem cell environment is not fully modelled. It is clear that tissue support cells have important interactions with the stem cells and the developing tissue, the complexity of which is not modelled outside of an animal. Thus, there is a need for demonstrating stem cell capacity in mice, in order to test the efficacy of factors for stem cell-specific effects in the local environment.

Reduction

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

Reduction

By the use of stem cell colony and 3D organoid culture studies using normal and cancer cell lines and primary cells, the number of mice needed has been reduced over the course of the previous licence. All of our stem cell studies are started without using mice by using sphere colony assays, 3D organoids and FACS analysis to test effects of regulators or inhibitors on stem cell activity. We will continue this process in this proposed project licence by experiments to mimic tissue environments outside of animals but using the tissue of origin, eg. lung, liver, bone, brain, etc. where normal tissue and tumour cells grow in the body.

For determining the effects of the environment, firstly, small scale pilot experiments will be performed to assess the size of the effect of a particular factor. Number of animals to be used will be determined by the type of experiment and this will depend upon the observations of experiments performed in the lead up to 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

Mice are the appropriate species for transplantation of human normal and malignant cells since immune-deficient mice are able to host the tissues in a mammalian environment that models the human microenvironment in multiple ways.

We will continue to refine experimental protocols wherever possible, to minimize both the number of mice used and their suffering. We will at all times monitor tissue and tumour growth and the health status of transplanted mice. Any mice that have health related issues related to surgery, transplantation or experimental therapy will be given immediate attention with a view to alleviating symptoms or discomfort and will be killed humanely if adverse effects are considered too severe to be treated.

PROJECT 195

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.

Word limit; 1000 words

Project Title	Cellular and molecular mechanisms underlying the pathogenesis of arthritis
Key Words	Arthritis, Obesity, Fibrosis
Expected duration of the project	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;
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):

Rheumatoid arthritis (RA) is a progressive and disabling chronic disease with unknown cause that affects over 400,000 adults in the UK. Many patients with arthritis do not respond well to current treatment, representing an unmet clinical need. Arthritis is more severe in patients who have other associated diseases like obesity and cardiovascular diseases, and inter-relationship between the mechanisms of these diseases is unknown. This requires better understanding that will lead to new options for therapy for RA as well as for the other associated conditions. In our work on RA patients' cells we identified several pathways that are aberrant compared to healthy individuals. We generated *in vitro data* using patients' cells that suggest that these aberrations may be involved in driving arthritis.

Thus, **aim** of our research is to test whether these cellular and molecular mechanisms cause or propagate arthritis and associated conditions.

Objective

1. To model individual parts of inflammatory pathways contributing to arthritis and RA associated diseases such as obesity, cardiovascular diseases and tissue fibrosis using genetically modified mice, *e.g.* mice that do not have a component of specific pathway.
2. To test the potential adverse effect of interfering with pathways that drive arthritis by looking at remodelling and allergy models.

These animal experiments will help us better understand mechanisms of RA and identify new 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)?

Short-term benefits: This work will advance basic scientific knowledge on important cells regulating our immunity. This information will be valuable for scientists. Middle term benefits: Our work will help to reveal what drives arthritis and associated conditions. This information will be valuable for scientists working on understanding mechanisms of diseases. Long-term benefits: Our research can lead to the identification of potential novel therapeutic targets for the treatment. This information will be valuable for pharmaceutical companies.

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

Over the period of 5 years we are going to use 10,000 animals. We will use genetically modified animals in the molecules that are potentially involved in arthritis based on our preliminary work on patients' cells.

In the context of what you propose to do to 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 standard and any interventions will be risk-assessed for expected adverse reactions. There are robust measures to monitor adverse reactions. Briefly, I will use specific substances that I will inject into mice via different routes to induce arthritis (skin, joint and paw injections) or asthma and lung fibrosis (lung injection) or skin fibrosis (skin injections). I will also put mice on high fat diet to induce cardiovascular disease and obesity. Most of these procedures last from a several days to 12 weeks and will make animals uncomfortable but they will not cause suffering. The exception is arthritis, which can spread to majority of joints causing pain and preventing animal movement. In our hands, this can happen in 1 of 10 animals. If this occurs we will humanely kill affected animals. Other impacts include weigh loss. If this reaches 20% of the initial body weight, the animal is terminated. In the animal models, we will also use multiple interventions such as injection, repeated blood sampling and anaesthesia. Most of the injections can cause swelling or irritation. This response is transient and should only last for 5-10 minutes and then settle down. Mice injected intranasal route (lung) may rarely experience increased respiratory rate. Those will be killed if not normalised. Repeated blood sampling of animals has the potential to cause anaemia and pain and distress to some animals. Therefore, blood sampling will not exceed 15% total blood volume in any month. 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

The mouse data complements the work on patients derived cells. We first test our hypothesis *in vitro* and only after obtaining positive results we move into *in vivo* models.

In detail our strategy is

1. identification of the aberrant genes in patients,
2. testing their role in the cell behaviour using cells from healthy donors and patients,
3. investigating the therapeutic potential of targeting an identified gene in cells derived from patents and
4. testing the role and therapeutic potential of an identified gene in animal models.

Thus, we use animal models to generate a final proof of concept.

Reduction

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

Reduction

Minimal numbers of mice to show statistically valid data will be based on power analysis and our experience gained by performing all the models on previous licence (60-4430). This would be typically determined for 20% change in measurements, assumed 80% power and 5% significance level.

As part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material; and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and the treatment differences that are to be estimated).

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 don’t normally develop rheumatoid arthritis, cardiovascular disease, obesity or skin fibrosis. These models therefore require immune-priming or stimulation or

disruption of cells or molecules involved in inflammation. We used the models that are widely acceptable by scientific community, with the most up-to-date refined protocols, to represent human disease. This is necessary because the clinical complexity of human disease cannot be adequately reproduced using *in vitro* culture systems. We have several measures in place in order to reduce animal suffering, such as daily monitoring, extra bedding, and a welfare scoring system.

PROJECT 196

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.

Word limit; 1000 words

Project Title	Impact of chronic intermittent hypoxia on atrial electrical stability
Key Words	Hypoxia, Atrial fibrillation, Reactive Oxygen Species, Mitochondria, Ion Channels
Expected duration of the project	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):

This research will characterise a new mechanism showing how repetitive exposure of the heart to low oxygen causes atrial fibrillation. We will also evaluate whether the detrimental electrical changes can be protected against by specific anti-oxidant drug therapies or genetic manipulation.

What 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 be in a better position to understand the cause of atrial fibrillation in patients with sleep disordered breathing or coronary artery disease. We will know if the electrical changes can be prevented by drug therapy, potentially leading to an early stage clinical trial.

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

500 mice 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 repetitive short periods of low oxygen (chronic intermittent hypoxia) induces long-term changes to the cardiovascular system, however, the animals exhibit normal behaviour and do not show signs of adverse effects. There may be a small amount of weight loss (less than 10%) as a consequence of the CIH. Cardiovascular measurements made on awake animals are short-lasting and generally non-invasive (e.g. nothing greater than mild restraint for 15 minutes), causing very mild and short lasting stress, with no lasting harm. Respiratory

measurements made on awake animals will last for a maximum of 3 hours and the animal may experience mild stress due to constraint within the recording chamber. Animals will be able to acclimatise to the chamber before any measurements and bedding will be provided from the animal's home cage to reduce stress. Animals may experience some transient mild discomfort due to daily I.P. injections, but these are expected to cause no lasting harm. Some animals will undergo surgery for implantation of a slow release device for antioxidant drug delivery. Surgery will be performed under anaesthesia, using aseptic conditions and pre, peri and post-operative analgesia will be provided to minimise any pain. Tissue collection for in vitro experiments are performed on deeply terminally anaesthetised animals and so the animal will not suffer any pain or distress. Animals will be terminated by an authorised and humane 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

Loss of electrical integrity in the heart is often caused by a combination of multiple factors. The loss of atrial electrical function caused by CIH is likely to be a consequence of multiple stressors, including the local effect of on CIH on the atria as well as high blood pressure, changes in nerve activity delivered to the heart and enlargement of the ventricles. This experimental programme is therefore centred on integrative physiological responses and requires *in vivo* experimentation.

We will undertake regular reviews of the literature in this area of work so that we will be aware of any developments in this area of research where *in vivo* techniques could replace animal use

Reduction

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

Reduction

Our proposed plan of experiments have been developed in line with ARRIVE guidelines. Appropriate animal numbers (based on power calculations) and our plan of experiments have been peer reviewed and we have received funding from the BHF to perform these experiments within the proposed duration of the project license. A variety of approaches will be used to reduce mouse numbers:

- Experiments will be performed in a way that to reduces variability in genetics, age, gender and behavioural background. Thus data collated is more meaningful.
- Experiments and data analysis will be performed blinded to therapeutic interventions and animal genotype to minimise investigator bias.
- We will use well-established and the most refined experimental methods to reduce variability.
- Several procedures will be performed using a single animal E.g. measurement of blood pressure and ECG to reduce the number of animals to be used.

We have access to independent statistical support, available at University of Birmingham to ensure that we 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

The animals used in this licence are all rodents (mice). These are the lowest order of animals that show the complexity of integrative physiology to allow modelling of human physiology relevant to this licence. Mice give greater access to genetically altered animals to help investigate proposed mechanisms in greater detail.

The integrative nature of these experiments requires animals of sufficient neurophysiological complexity to ensure results are meaningful and are able to be interpreted in the context of humans. We intend to investigate certain hypotheses in GA animals and these currently tend to be available in mice only. Thus, all animal experiments will be performed in mice.

The measurements made on conscious animals are all short-lasting and induce minimal stress to the mouse. We also use a number of non-invasive techniques in preference to surgical approaches. For example breathing measurements will be performed on freely moving, conscious mice using state of the art small animal plethysmography. Furthermore, non-invasive blood pressure and ECG measurements will be made with dedicated small animal platforms that do not require the need for anaesthesia and measurements only take 5-10 minutes. Heart isolation will be rapidly performed (maximum 5 minutes) under deep general anaesthesia without recovery thereby removing any suffering to the animal in the surgical phase of the experiments.

For our *in vitro* experimentation, we use innovative technologies to refine our experimental techniques including optical mapping and RNA sequencing as well as

current gold standard techniques for measuring atrial electrical function including microelectrode, Langendorff apparatus and patch clamping. These techniques are all well established in the current laboratory, are highly reproducible and have been validated by extensive peer-review. The high level of accuracy achieved with these techniques as well as the strong reproducibility will reduce the number of mice needed for experimentation.

At the end of each experiment, we will undertake a critical review to see how it can be refined going forward.

PROJECT 197

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.

Word limit; 1000 words

Project Title	Fish telemetry investigations to inform effective management actions
Key Words	Fish, telemetry, migration, movement, behaviour
Expected duration of the project	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.

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):

Fish populations in the UK, including salmonids, coarse fish and conservation species (e.g. lamprey, eel and shad), face many pressures in the freshwater environment, including exploitation, fish stocking, pollution, abstraction, navigation, power generation, flow regulation and habitat modification. Understanding these pressure, which will be locally specific, is crucial to the implementation of effective management actions and targeted rehabilitation projects driven by legislation. Fish will be implanted with marks and tags, using well established methods and techniques, to monitor their movements, behaviour, and habitat use in order to target and assess improvements to the aquatic environment aimed at enhancing fish, eel and lamprey populations in the UK.

What 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 beneficiaries of knowledge arising from this research is anticipated to be governing bodies (e.g. the EA in England and their equivalents across Europe), who will be able to use outputs to inform and revise policy, regulation or operational best practice. Implementation of policy, regulatory or operational advancements will ensure freshwater environments worldwide are sustainably managed within legislative frameworks to deliver positive environmental outcomes.

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

Freshwater fish, migratory salmonids, shad, eels and lampreys. <10,000

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?

The procedures in this protocol will either be very brief (subcutaneous and fin marking and tagging) or carried out under general anaesthesia and fish will therefore be subjected to no more than mild and transient stress arising from capture and handling and may experience mild post-operative discomfort.

Application of the 3Rs

Replacement

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

Replacement

This study is focused on wild fish in a locally specific situation; therefore a non-animal alternative approach cannot be used and all non-tagging alternatives are technically inadequate.

Reduction

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

Reduction

Non-animal techniques will be used were appropriate to minimise the numbers of animals, including using modern techniques like underwater video monitoring and hydro-acoustics.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 tagging protocol will be carried out under anaesthetic to ensure no pain is experienced, except during minor procedures when holding fish during and after anaesthesia and/or clearing the anaesthetic after sedation are considered more stressful to the fish. In some instances the procedure will involve a small incision on the belly of the fish, an inert and sterile transmitter inserted and the incision closed with a suture to ensure full recovery and minimal suffering.

PROJECT 198

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.

Word limit; 1000 words

Project Title	Develop and treat ALS/MND using rodent models
Key Words	MND, ALS, Neurodegeneration
Expected duration of the project	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):

The main objective of the project is develop rodent models of human diseases such as Motor neurone disease (MND) and Amyotrphic lateral sclerosis (ALS) such that we can better understand how these diseases occur and also to test potential treatments to ensure that they are safe and provide benefit before taking them forward to human patients.

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

The project will allow us to better understand how specific genetic mutations lead to ALS/MND and on that understanding we can develop new treatments that can be tested in the animal models. Safety and efficacy can be tested before they are tested in human patients.

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

We plan to use mouse in these studies. We plan to use a maximum of 1200 mice during 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 animal model developed are to mimic the human ALS/MND. Hence, the animals show symptoms of paralysis when the disease is fully developed. However, the majority of the work will be done during early disease stage when the symptoms are mild. These include changes in walking, ability to balance, wheel running activity, muscle strength. Some animals will be treated like a human with therapies (gene or

drugs) to determine if they can alter disease course for the better. Some animals will be utilized to measure the amount of the therapeutic in the CNS to measure their levels where it is supposed to work.

Application of the 3Rs

Replacement

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

Replacement

We use in vitro and in vivo methods to model motoneurone disease and to understand the genes and pathways involved in this disease. We use zebrafish in my lab to identify novel drugs that stimulate or interact in ALS/MND pathways. However, to validate these novel treatments we need to use a model that more closely models the human anatomy and physiology, therefore testing our novel treatments in rodents is required to understand the complex interactions of the whole biological system.

Reduction

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

Reduction

We will use validated statistical tools to identify the minimum number of animals used. This will reduce the number of animals used both for breeding to generate the experimental models, but also each experiment will be designed to ensure that the correct number of animals are used to reach meaningful and significant conclusion that ultimately can be translated to the clinic for further validation. Our drug screening programme utilises zebrafish as they lend themselves to high throughput analysis, therefore only drugs and treatments that have already shown promise in the fish model will be tested in rodents.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 in collaboration with others robust parameters to evaluate the animal's disease progression, this detailed scoring system allows us to monitor disease progression closely and where possible use early time-points to end the experiment and reduce distress. In addition to this scoring system animals are

provided with a modified home cage environment that can reduce the distress suffered, where animals have access to food and water in an easy to reach and digest format. We will continue to refine our scoring system to monitor end stages and to develop equivalent parameters that we can detect earlier with the ultimate aim of ending experiments at earlier timepoints to alleviate distress and improve the wellbeing of the animal.

PROJECT 199

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.

Word limit; 1000 words

Project Title	Immunity to parasitic infections.
Key Words	Immunology, Parasites, Vaccines
Expected duration of the project	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):

Parasitic worm infections are some of the biggest health problems in humans and animals worldwide. The World Health Organization estimates that over 25% of the world's population are infected with parasitic worms, with the majority in low and middle-income countries, and this burden of disease has remained almost unchanged in the last fifty years. Worm infections are also a significant problem in domestic animals, causing substantial losses in live stock productivity due to poor animal growth and health, both in the UK and worldwide. In addition there are also associated food safety risks caused by several types of animal worms that can spread from animals to humans through consumption of infected meat.

The current approach to control both human and animal infections is almost exclusive focused on drugs aimed at killing the parasites, however, increasing resistance to the currently available drugs are of major concern. No effective vaccines are available that can protect humans or animals from these infections, and efforts need to be greatly intensified in order to generate new treatments and vaccines to combat these debilitating 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 primary purpose of this project is to gain new knowledge regarding how parasitic worms are able to infect and survive inside their host for such long periods of time. The worms are very long-lived with each worm surviving for several years inside the host and we know that the worms produce a number of immunosuppressive molecules, which prevent the immune system from attacking them. The current project is aimed at characterising in detail the interaction between worm and host

and finding out exactly which immune mechanisms that needs to be stimulated in order for the immune system to be able to kill the parasite. Based on this information we can then design and develop new vaccines to prevent infections in both humans and animals. In parallel we are also testing new types of drugs to combat the emerging resistance to the existing drugs. Furthermore, the results of our studies will provide new knowledge regarding the immune system in general as well as for other specific conditions such as allergies and asthma.

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

Only mice will be used and we expect to use 2450 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 order to replicate the chronic infections seen in infected humans, mice will be infected with parasites at a level that generally gives few symptoms. The parasites used are natural mouse parasites which resemble the worms found in humans, have similar life cycles and similar symptoms. In some cases an inflammatory response may lead to moderate symptoms, such as light diarrhoea or minor weight loss. The numbers of animals will always be chosen to be the minimum compatible with sufficient statistical power to generate scientifically sound results. At the end of procedures mice will be culled 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

Due to the complex nature of the immune system in mammals it is impossible to achieve the features of an immune response outside of a complete animal model. Furthermore, parasitic worms can not survive outside a living mammalian host so it is not possible to grow them in the laboratory without using animals. For drug development work however, we can obtain large numbers of worms from one animal meaning that we can screen a large number of compounds using minimal numbers of mice.

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 scientifically meaningful results. We minimise group sizes by reducing sources of variability (by using inbred strains of mice, age matched and housed in individually ventilated cages). Pilot studies define the minimum number of mice per group required to give robust, repeatable and statistically significant 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 are natural hosts for all of the parasite species used in this project and develop chronic low-level morbidity disease similar to that seen in humans and larger animals. In many cases the infection is almost symptomless and mice may harbour asymptomatic infections for many months, even for life, similar to that seen in human infection. Our work is aimed at replicating the low level chronic infections seen in nature in a laboratory setting thus allowing us to systematically address the functions of individual components of the immune response using cutting edge technology, including the use of conditional knockouts and other transgenic technology. As such we are confident that these mouse models are excellent representatives of the chronic low-level disease evident in nature. Most of 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 are housed together in groups in cages with environmental enrichment that allow them to burrow and build nests and they have wood chewing sticks and tunnels to explore and hide in, stimulating natural behaviour and maximising well being. All staff undertake regular refresher training in animal handling and experimental techniques, and skills sharing between researchers is encouraged.

PROJECT 200

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.

Word limit; 1000 words

Project Title	Treating Kidney and Eye disease using immune based therapies.
Key Words	Complement, Kidney, Eye,
Expected duration of the project	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):

Drug interventions are being developed that could slow or reverse progression of both kidney and eye disease, or protect kidney (or other organs) before transplantation but these require significant testing before they can be given to man. In the program of work

- We will test many of these drugs with a particular focus on kidney disease but may test efficacy of some in the eye;
- We will further establish the role of dysregulate immune response on the mechanisms of injury in kidney and eye disease;
- We hope to identify potential drug targets and develop new biomarkers of these diseases;
- We will establish whether suspected triggers of a rare kidney disease are a likely cause or do contribute to disease onset.
- We will also evaluate the importance of disease modifiers in the course of disease to reveal additional drug targets or evidence of mechanisms.

What 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. This programme of work will focus on testing of cutting edge drugs already developed and therefore, generate data to determine whether these drugs progress into clinical testing, conceivably leading to marked improvements in patient care in the next 5-10 years 2. Use of and development of models with the closest resemblance to human disease establishes powerful tools to analyse drugs that could replace the only existing immunotherapy for rare kidney diseases with a drug with a much greater safety profile. It should translate to improved patient quality of life within the next decade. 3. The work will increase our understanding of the

mechanisms that underlie immune system mediated damage to the kidney and/or eye, work will be published and presented to patient groups as it arises. 4. The work will identify new targets for drug development that will allow alteration of the course (and prognosis of progressive) renal and eye disease, again published as it arises. 5. This research, beyond helping to treat kidney disease, will contribute to treatments for diseases such as the eye disease age-related macular degeneration (a leading cause of blindness in the western world) and patients undergoing organ transplant; both of these could significantly improve patient care, saving the NHS many millions of pounds within 10-15 years. 6. This programme of work will advance our understanding of immune system function and the targeting of drugs, development of biomarkers which will likely benefit treatment of many indications i.e. Arthritis or Lupus and not just the organ specific diseases investigated herein.

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

Mice will be used to maintain the breeding colonies and to provide mice for the experiments planned (800/year). We expect to use an average of 500-600 mice per year over the course of this 5-year project for direct experimental work, with the majority of the remaining 200 – 300 ‘non-experimental’ animals per year used to provide tissues or samples for ‘indirect’ laboratory based experiments as well as some mice being used for breeding stock only. Mice will be a combination of typical ‘wild type’ mice, as well as genetically altered mice. They will be used specifically to study certain aspects of the immune and fibrosis systems as they impact primarily on kidney and eye functions. Statistical estimates have been used to ensure we use only the minimum number of mice for each stage of the 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?

The large majority of mice will be group housed and will be provided with cage enrichments (shelters and nesting material). Many of the mice will experience no ill effects. These mice will be humanely killed and tissues (serum, kidney and eye, lymphocytes, etc.) taken from the majority of the animals for study. These will allow the generation of additional reagents and allow for key experiments in the lab, which will allow strategic decisions on use of animals for experiments going forward. Some mice may be housed singly in special cages to collect urine to track disease for no more than a day and no more than 6 times overall, this may cause mild distress which may exacerbate disease. Animals will be closely monitored by non-invasive tests, i.e. weighting and urine dipstick tests. Much of the research planned within this study relies on mice developing kidney disease, either spontaneously or after induction. The level of kidney disease varies from mild (not obvious from animal behaviour but possible evidence on biochemical analysis of blood/urine) to that causing animals distress (and easily detected by urine dipstick test). In some cases, disease will develop spontaneously and rapidly (mimicking human disease) giving a

short window to evaluate effectiveness of the treatments or humanely kill animals. In one particular spontaneous disease model, there remains a risk of animal death before we can intervene during these studies, this will be carefully managed and only used were no other option will provide the data. Treatment with an immunotherapy used in man is successful in this model and gives us certainty we can protect animals from disease using the correct drugs after animals are identified as being at-risk. Notably, a degree of risk is reintroduced as we test drugs with unknown efficacy in this model. However, this a vital process to find better drugs to tackle these diseases. Non-invasive real time imaging or measurements of these animals will provide another mechanism to control for unwanted animal distress and so, we will develop new agents (using simple vaccination style techniques) and refine existing approaches in the programme of work. Both wild type and genetically altered animals will be used in these studies to increase success. Drugs would be introduced to animals via a number of routes, depending on the agent, with injection into the blood or tissue either before, during or after induction of kidney disease. This injection, in its self, will not cause more than transient discomfort. Induced kidney disease will be in the form of mild infection (symptoms analogous to the common cold) or agents that mimic mild infection or insufficiency of immune proteins or through chemicals designed to make subtle changes to kidney function or that mimic the conditions associated with organ transplant. Animals will only experience minor effects of disease before intervention is applied. Animals will be carefully monitored throughout. A small number of mice may experience complications after surgery and/or use of drug delivery devices which may induce distress. These can be managed effectively through use of pain relief and antibiotics. However, if infections or complications persist animals will be humanely killed. Modification of procedures will be carried out should these events arise. Non-invasive imaging of mice provides an ideal model to test the effectiveness of such technologies for following disease progression in man but these may contribute to adverse effects in certain models. Clear evidence of distress could arise and in these cases any imaging would be stopped, failure to improve would lead to animals being humanely killed and a review of imaging practises. The large majority of the animals at the end of experiments will have their blood withdrawn under deep anaesthesia followed by humane killing (without recovery from the deep anaesthesia). Animals suffering acute kidney failure (urine dipstick measure) or showing recognisable and significant pain/distress 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

Human tissue or cells can be used to answer simple questions/ outcomes but vaccination/immune responses are complex, involving multiple cell types and pathways; therefore, it cannot be modelled in the laboratory and animal studies are necessary to fully dissect mechanisms. GM mice allow us to find out if changes in normal protein expression in the body will have a major effect on kidney function or disease progression. We have included the option to generate unique monoclonal antibodies (through immunisation and subsequent laboratory work). These may be replaced by new laboratory technologies (phage display) and where at all practical we will use such technologies. We will always check commercial sources and thoroughly investigate the scientific literature for available antibodies to fulfil our plans before using mice for monoclonal or polyclonal antibody production.

Reduction

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

Reduction

Statistical techniques have been used to ensure we use only the minimum number of mice for each stage of the experimental procedure. i.e. to reduce animal use we will use power analysis or search the current literature to determine the minimum number of experimental animals. Where ever possible human tissue or cells will be used to minimise animal use (but as noted above this will be limited to simple questions).

Where possible tissues collected from previous experiments in mice (serum and kidney's etc.) will be used before new animals are used 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

The mouse is a well-established model for experimental kidney disease, and there is a large body of published data in this species. In respect to the immune system, it is well characterized in the mouse and is highly similar to humans. Although there are differences between human and rodent immune responses, these have only minor effects when studying kidney disease models and rodent models provide important insights into the causes of these diseases. Mice are the species with the lowest capacity to experience pain or distress that are likely to produce satisfactory results.

Genetically altered mice for which a specific gene is either deleted (knockouts) or reintroduced (transgenic) are very useful tools for the study of gene function. Use of

genetically altered mice with specific genetic alterations believed to affect disease development will allow us to identify and determine the influences of specific genes (and by consequence specific molecules) in kidney disease. Indeed, use of viral delivery or inducible gene conversions may allow us to move from models with uncertain outcomes to kidney disease models that are less likely to generate significant harm yet still provide models to test drugs destined to treat man.

Minimum severity: Protocols to be carried out adhere to “best practice” and current guidelines to cause minimum distress to the animals used. All protocols allow for appropriate supportive care in consultation with experienced technical and veterinary staff. Mice will be group housed and will be provided with cage enrichments (shelters and nesting material). Induction of disease will be carefully planned/staged to ensure the lowest level of useful effect of infectious or chemical agents is applied. Development of ‘trigger’ specific models will allow us to progress from using models with unpredictable outcomes and increase the usefulness of the studies as a whole

PROJECT 201

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.

Word limit; 1000 words

Project Title	Microvascular and Cardiovascular Biology in Cancer
Key Words	cancer, therapy, blood vessels
Expected duration of the project	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;
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):

We aim to study the differences between blood vessels in tumours and normal tissues, so that tumour blood vessels can be specifically attacked for cancer therapy, resulting in cutting off the blood supply to tumour cells and effectively starving them to death. For drugs already approved for this type of therapy, we need methods for identifying which patients would benefit from them most. We also need methods for protecting patients from unwanted toxic effects of 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)?

Our research will contribute to a scientific understanding of how tumour blood vessels develop and are controlled. Results will be of value to clinical scientists planning clinical trials and, if new molecular targets are identified, this would benefit development of new cancer medicines.

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

In order to know how new treatments and their combination with conventional drugs and radiotherapy affect solid tumours, we need to use animal systems, where there is a fully functioning system of blood vessels. All the animals used in this licence will be rodents: mice and rats because of the availability of a variety of tumour cell types that can be successfully used in them. We estimate that we will need approximately 2,100 mice/rats over 5 years for this research.

In the context of what you propose to do to 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 our experiments require implantation of tumour cells into mice/rats to model the human situation. This is expected to cause only minor discomfort. The trial treatments that we administer may cause moderately severe side effects, as many current cancer treatments do in humans. In addition, we sometimes need to carry out surgery under general anaesthesia, which will cause pain on recovery from anaesthesia, if untreated. At the end of an experiment, 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

We need to use animals in our research because there are no alternatives available that allow us to study the inter-relationships between tumour cells and the blood vessels that supply them with oxygen and nutrients.

Reduction

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

Reduction

We will minimise the number of animals that we use in two main ways. Firstly, wherever possible we will use techniques such as external imaging of animals to enable repeated analysis of the same animal over time. This circumvents the need for separate groups of animals to be analysed at different times after treatment, where a timecourse of treatment effects is needed. Secondly, we will use appropriate experimental design and analysis to ensure that optimum numbers of animals are used to provide definitive answers to specific scientific questions. For complex experiments, we will seek advice from specialist statisticians to make sure we get this right.

Refinement

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

Refinement

A mammalian species is needed to model the blood vessel development that occurs in human tumours. The mouse is a suitable species for much of this work because most previous work has been done in this species and so there is extensive

background information available and ready availability of suitable tumour cells and reagents for mouse research. Rats are also suitable for some surgical procedures because of their larger size. We do not allow implanted tumours to grow or spread to the point where they cause serious harm to the animals. Rather, experiments are planned so that the required measurements are made before the tumours reach this stage, at which point the animals are humanely killed. Drug doses are chosen from small pilot experiments such that side effects are tolerated without severe effects. Rapid recovery from surgery is aided by keeping animals warm and hydrated and providing pain-killing drugs.

PROJECT 202

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.

Word limit; 1000 words

Project Title	Cell therapy for diseases of striated muscle
Key Words	Muscular dystrophy, Fibrosis, Stem cells, Cell lineage, Tissue engineering.
Expected duration of the project	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):

We aim at developing a new therapy for muscular dystrophy, based upon stem cell transplantation. Muscular dystrophy affects patients' muscles and often also the heart; in its most severe forms it reduces quality and life expectancy. We conducted experiments in animal models that led to a first clinical trial, based upon transplantation of donor stem cells from a brother. The trial proved safe but only modestly efficacious. In order to reach clinical efficacy, we now plan to develop novel protocols to isolate the stem cells of the patient, genetically correct them and then re-introduce them in the same patient, after having optimized all the step of the transplantation. We will test this strategy first in cells in culture and then in dystrophic animals. The same experiments may help to develop therapies for other diseases of the muscle and to alleviate damage to the heart in muscular dystrophy.

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

Muscular dystrophies compromise quality and length of patient life, lack any efficacious therapy and carry a severe burden for the patients' families and for the NHS that needs to provide palliative and supportive care, often for many years. Even a partially efficacious therapy that would arrest or delay the progress of the disease would have an immense medical and socio-economical impact.

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

Mice and rats will be used. We will use the minimal number animals to reach statistical significance, use any possible alternative method, ranging from well-established cell cultures to ad hoc designed organ cultures. However, by taking all

the projects to be carried out be carried out, approximately 5,300 mice and 1,800 rats will be needed over a 5 years 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 procedures envisaged are injections, minor surgery, catheterization and therefore the level of severity will range from moderate to mild. The animals will be monitored for beneficial effect, in the absence of which, or in the presence of unforeseen complications, the animals will be humanely culled. In case of successful therapeutic outcome the animals will be monitored until one year of age, after which time they will also 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 first part of the experiments will be conducted on cells in vitro, thus significantly replacing part of the work that would otherwise be conducted on animals. However, there is no actual surrogate method that allows measuring the outcome of cell transplantation on the animal motility.

Reduction

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

Reduction

The use of cell cultures will greatly reduce the number of animals employed, since many answers will be obtained in vitro. Expert advise has been obtained to reduce the number of animals to the minimum sufficient to produce statistically significant 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 are the main species that model all the genetic diseases we intend to treat. Newly created dystrophic rats will also be used. We will use power calculation to use the minimal number of animals require to reach statistical significance of results.

Anaesthetic and analgesic treatments will be administered to reduce or eliminate stress and pain associated with the surgery the animals will undergo

PROJECT 203

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.

Word limit; 1000 words

Project Title	Study of the mammalian hypothalamic pituitary gonadal axis.
Key Words	Neurons, Fertility, Testes, Hormones, Ovary, Hypothalamus
Expected duration of the project	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):

This project is aimed at increasing our knowledge of the mammalian reproductive axis. Reproduction is of central importance in many aspects of human health and disease. Impaired fertility is an increasing problem in western society affecting around 10% of all couples. In addition, around 10-20% of pregnancies are lost as a miscarriage during the first 12 weeks of pregnancy. Thus, an understanding of normal fertility and pregnancy is essential to develop potential clinical treatments for these problems.

Mice will be used to investigate the way in which fertility is controlled and identify genes that regulate the reproductive axis. To do this, we will generate mice that carry defined mutations and analyse the effect of these mutations on their fertility. We will also look at the cells in the brain that initiate puberty and how substances such as hormones regulate these and how they communicate with other cells 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)?

This project is expected to provide novel information on the molecular processes that control mammalian fertility. It will advance our knowledge about the molecular pathways that control puberty and the production of eggs and sperm. This may allow the development of novel compounds that regulate the reproductive axis, which can be used as contraceptives or for the treatment of reproductive problems such as infertility or miscarriage.

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

Over the course of this licence, we will use a total of around 4,200 mice (approximately 860 mice each year). Of these, the majority (around 2,800) are used simply for breeding and these will have no detrimental effects caused by the genetic alteration. A further 400 mice will be used each year for the generation of the genetically modified mice and these will not have any surgical procedures performed on them. Surgical procedures (mainly removal of testes or ovaries) will be performed on around 650 mice with viral delivery into the brain be performed on around 350 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?

In general, mutations that affect reproduction produce non-painful effects specifically on fertility and the mice are healthy and uncompromised in other functions. The majority of mice will be used for collecting tissue samples for further analysis. These tissues will be taken from mice that have been humanely killed. A small number of mice will have surgical operations under general anaesthetic to remove their ovaries or testes to ensure low levels of hormones from which we can evaluate any responses to treatments. We will also deliver substances directly into the brain via a small hole in the skull and allow the mice to recover afterwards. These studies will allow us to study the effects on fertility of substances normally found in the brain but which cannot easily get into the brain from the bloodstream. Animals that have had surgery will be provided with appropriate pain relief and will be monitored regularly to check on their health. Any animal that loses too much weight or whose appearance indicates that their health is being compromised will be humanely killed. Adverse reactions to surgery will be minimized by using appropriate sterile techniques and are expected to occur less than 1% of the time. No surgical procedures will exceed loss of weight greater than 15%. All animals 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

Mammalian fertility is regulated by complex hormonal signalling that allows communication between several different body parts (eg brain, pituitary gland and the testes and ovaries . It is not possible to copy [this communication system using tissues growing in a culture dish necessitating the use of live animals to study this system. In addition, sperm and eggs will only form properly in a whole animal. Moreover, the processes within the brain that regulate reproduction can only be studied in live animals.

Reduction

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

Reduction

We apply statistical methods to minimise the possibility (to less than 5%) of making either a false positive or a false negative conclusion, and as part of these calculations we can therefore ensure that we do not use more than the necessary number of animals to achieve statistical power and significance at the 95% level. For some studies, it is possible to monitor responses in the same mice over a period of time so that paired tests can be used which typically require fewer animals. Prior to undertaking a new study, we will perform Power Calculations to assess how many mice might be required generally using a significance level of 5%, a power of 80% and a least practicable difference between groups of 25% and if this indicates that the number is very large we will try to modify the procedure.

We will also keep the number of breeding pairs to a minimum (usually only 2) when mice are being maintained. We will only increase the number of breeding pairs to generate cohorts of mice for a specific study and reduce the breeding numbers as soon as possible after this point.

Before generating genetically modified mice, we will search public databases and publication records to ensure that they do not already exist elsewhere from where they can be obtained. Breeding will be optimized to produce only the type of mice that we need for experiments.

We will use sterile male mice for some of our work and we will purchased these from a commercial source and house them in an animal facility where they can be shared with other scientists to eliminate the need to duplicate having these mice in multiple facilities.

We maintain our genetically modified mouse lines as inbred stocks to minimize genetic differences that could contribute to variation in the parameters being measured. We also try to ensure that cohorts of mice are age and sex matched to reduce variability. Some mice are gonadectomised to remove variability caused by different levels of sex steroid hormones.

When GM mice are not required for future experiments, we will freeze eggs and sperm cand embryos from the specific mouse line so that we do not need to keep the mice breeding 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

Mice are an excellent model to study mammalian reproduction. The main physiological processes are conserved between mice and humans and the hormonal regulation of fertility in particular is almost identical between these species. Thus, knowledge gained from studies in mice can be directly applied to understanding human fertility. In addition, the ability to generate transgenic mice, in which a single gene has been altered or removed, provides a very powerful tool for studying the role of a single gene in reproduction. This technology does not exist for any other mammalian species. In addition, the short gestation period of the mouse allows us to study aspects of reproductive function relatively quickly.

We may also test whether administration of sex steroids on the skin can be used rather than by injecting under the skin as a less painful delivery route. We have also developed an ultra-sensitive method for measuring a hormone in the blood, which works with very small volumes of blood [5 ul) so that we can reduce the amount of blood taken from the tail vein.

We will retain existing ear clip tissue taken for identification purposes and use this for identifying the genetic make up of the mice [and thus eliminate the need to re-earclip for genotyping alone.

Transfer of embryos into recipient female mice to generate genetically modified mice will normally be performed using an NSET (non-surgical embryo transfer) device, which is a less invasive method than esurgical transfer of embryos.

Where possible, we will use genetically sterile mutant male mice (eg *Hiat1* mutant mice) instead of vasectomised males.

PROJECT 204

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.

Word limit; 1000 words

Project Title	Induction, assessment and prevention of adhesions
Key Words	Adhesions, Post-surgical
Expected duration of the project	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:
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):

Post-surgical adhesions (PSAs) consist of fibrous tissue which sometimes grows excessively and can lead to constriction of the bowel and other internal structures, cause significant pain and even result in female sterility.

We will investigate the ability of new procedures, materials and/or devices to affect the formation of PSAs by applying them to pre-clinical models we have used and developed in house.

In a systematic review of 87 studies including 110 076 patients the incidence of small-bowel obstruction due to postsurgical adhesions was 9% which is equal to 9906 patients over a period of five years. If these figures are extrapolated to include adhesions at other sites (which have not yet been exposed to systematic review) it is likely that an excess of 10,000 patients per year could benefit from an effective postsurgical adhesion prevention strategy.

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

Reduction or prevention of post surgical adhesions in both humans and animals will reduce post-operative complications, enable efficient recovery to normal movement, reduce or remove the need to carry out subsequent surgery to remove adhesions and thus improve patient welfare, reduce hospital in-patient time and reduce the financial implications.

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

Initial studies will, where possible, be carried out in mice or rats and only if they show potential will it progress to rabbits, sheep or pigs. Over the 5 years of this licence we would aim to use approximately 400 mice, 400 rats, 300 rabbits, 100 sheep and 200 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?

The models we use create adhesions but we do not let these adhesions become painful to the animals as we treat just after creation to assess reduction or prevention and we know from our culture studies that the treatments we use have good potential to reduce or prevent PSAs so we would regard this licence as only moderate in its severity. Some animals will be recovered from surgery and will be monitored for up to 12 months after the initial surgery. This may include repeated anaesthetics for the purposes of biopsy and/or non-invasive imaging. Any animals who show excessive signs of distress will be put down and examined in an attempt to determine the cause and also to assess the affect of the treatment applied to them. At the end of each study the animals will be put down and the tissue taken and examined to assess the efficacy of the treatment, also, where possible, tissue will be taken for other studies and/or educational purposes in an effort to maximise the usage and reduce overall number of animals used.

Application of the 3Rs

Replacement

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

Replacement

The formation of adhesions is a complex process involving many different components within the body (blood, lymph, enzymes, etc) all interacting and as such a complete live animal is needed to form adehsions for evaluation and subsequent treatment. Prior to live animal studies, procedures, materials or devices to be assessed will, where possible, be tested on cells or tissues in order to keep animal use to a minimum.

Reduction

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

Reduction

The ability to remove organs and tissues under terminal anaesthesia from animals in one study to be used for in vitro or ex vivo studies or transplantation/implantation or

to be used for training reduces the need to retrieve these organs or tissues from dedicated donors thus reducing the number of animals required overall.

All potential treatments, procedures or devices transitioning from the laboratory into live animal testing will go via pilot studies involving small numbers (typically 3) of animals - this is to be sure that the laboratory prediction is borne out in live tissues.

For many of the studies carried out under these protocols, several sites of injury can be induced in the same animal which allows us to reduce the number of animals required to produce scientifically relevant data. Also, the ability to use adjacent or remote tissues from the same animal as internal or autologous controls again allows a reduction in the number of animals required overall.

For those studies carried out under Good Laboratory Practice (GLP) compliance, a regulatory process required by the MHRA and the FDA for all pre-clinical studies leading to requests for use in man, statistically robust appropriate information must be derived and this typically requires between 6 and 10 animals per experimental group to satisfy these parameters.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 we use have been evolved over the last 20 years and are as refined as we can currently achieve. We use the smaller species (mice and rats) for intial studies to confirm that the laboratory prediction is borne out in live tissues but often need to use more appropriately sized animals (i.e. a similar size to humans) for many studies. Using a range of assessments including non-invasive imaging (e.g. X-ray or Ultrasound) has further refined our techniques allowing us to obtain more information whilst minimising the impact on the animals' welfare.

For some direct application treatments the rabbit can be used to assess efficacy however, to establish representative sized defects and relevant treatment doses, large animals are required. Also, for the new procedures, instrumentation is designed for humans and a representatively sized animal will therefore have to be used. There are some areas of anatomy which are specifically recognised within different species as best models – e.g. for meniscal cartilage the sheep is deemed more anatomically similar to humans than is the pig, while for bowel and vasculature the pig is deemed more representative of the human than the sheep. Choices of species will be dependent on the anatomic site under investigation.

Appropriate monitoring of animals post-surgery and intervention if necessary with pain relief medication will ensure animal comfort. Our experience is that the animals are not in any pain during these studies probably because most are treated and those that are not are not allowed to progress to the level of adhesion formation where humans would present with symptoms.

PROJECT 205

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.

Word limit; 1000 words

Project Title	Reconditioning of marginal donor hearts for transplantation with machine perfusion
Key Words	transplantation, machine-perfusion, marginal-donors
Expected duration of the project	2 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;
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):

Although it has almost been 50 years since the first successful human heart transplant, it remains the gold standard treatment for end-stage heart failure. Unfortunately, the ever growing demand for heart transplantation vastly out-strips the limited number of available donor hearts. Consequently, more than half of the patients accepted onto waiting lists will never receive a heart transplant. Currently, of the donor hearts made available from "*donation after brain death*" (DBD), less than 30% are eventually retrieved and transplanted. This alarmingly low rate of donor heart utilization is due largely to the harmful effect of brain death on donor heart function, rendering many organs too damaged to be transplanted.

Our intention is to minimize and ameliorate these injuries on donor hearts thereby increasing the proportion of donated hearts that could safely be transplanted. In doing so, it would allow the wishes of more donor and donor families to be fulfilled. At the same time, it would afford more patients on the heart transplant waiting list the chance to undergo this life saving operation.

The process of brain death in the donor has many deleterious effects on the heart. In order to maintain an adequate blood flow to all the vital organs in the donor, intensive care units often have to use powerful drugs to drive these injured hearts to work harder, thereby compounding the injury. Once a donor heart has been surgically removed from the donor, it is placed in a "picnic" box packed with ice and transported to the recipient hospital to be transplanted. The heart receives no oxygen during this time and deteriorates further inside the cold box. This makes it hard for the transplanting surgeon to be confident that the donor heart will have sufficient power to keep the recipient alive once transplanted. In fact, as many as 1/3

of the carefully selected donor heart that are currently transplanted go on to develop so-called “primary graft failure” and put the life of the recipient at risk.

After years of systematic research REDACTED

We shall validate this approach in a large animal model, similar to the model that we have successfully developed for our DCD heart transplant programme. We have found this large animal model to be an invaluable platform in the process of translating our research hypothesis from bench to the bedside with an immediate positive clinical impact. Furthermore, with our experience of this experimental model and our expertise in the field of heart transplantation, we are very well placed to conduct this research.

We hope to move swiftly to deploy this innovative use of technology into a clinical programme. This will enable more effective use of the valuable donor hearts thus realising the wishes of the donors and their families who have kindly offered their organs for transplantation and the hopes of those patients who are dying from end-stage heart failure in having the chance of a life-saving heart transplant

What 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 number of patients on the heart transplant waiting list has more than doubled in the last 5 years with a number of patients waiting more than 3 years for a heart transplant or unfortunately dying on the waiting list. We hope that we can double the number of hearts used with these strategies as well as improve the outcomes for patients following their heart transplant.

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

Based on our previous experience and other published literature using large animal models we believe that the study groups should have at least 10 animals each. As we have 4 groups including the control group this will mean a minimum of 40 animals being operated on. However, we have accounted for 80 animals to allow for an attrition rate based on our previous experience and the advice of AWERB to ensure that at the end of completion of the study we have sufficient data to allow for meaningful interpretation. Additionally, we have requested for upto 300 animals to have blood sampling to enable the identification of suitable blood donors to obtain cross-matched blood. However, a number of these animals will be destined for re-use as following the blood sampling there will be no lasting distress or physical impairment. We hope to complete all our experiments over a one year period but will be analysing the data and results after each case to ensure that the minimum number of animals are 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 donor animals that will undergo some mild stress of anaesthetic induction but following this no further intervention will be performed until general anaesthesia is established. The animal will continue to be under general anaesthesia which will eventually be terminal with measures taken to monitor the depth of anaesthesia. These animals will not be allowed to recover and all procedures will be done with the animal under general anaesthesia including the removal of the heart. This will ensure that no further distress is experienced by the animal. The animals who are tested for suitable cross-matching will experience some mild distress during the blood sampling but will have no lasting distress or physical impairment. Suitably cross-matched blood donors will under an approved Schedule 1 killing method to collect the donor blood - thereby the animals will not be recovered and any stress to the animal will be at the absolute minimum.

Application of the 3Rs

Replacement

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

Replacement

The porcine heart and anatomy is very similar to the human and has been used reliably as a model to investigate heart transplantation. We hope that the positive findings from this study will allow for further validation in the human setting and the use of a porcine model has previously allowed for the rapid translation into clinical practice with reproducible findings.

This is vital to ensure that no harm is done to potential human heart recipients and that potential transplantable donor organs are not wasted without sound scientific evidence to support our proposed interventions.

Reduction

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

Reduction

Previous work done by our group using large animal models have aimed to use groups of 10. Similarly, other published work which has also translated into clinical practice and further human research has shown us that using groups of 10 will give us sufficient evidence to justify validating any positive findings in a clinical human setting.

We have designed the study such that our control group mimics the current standard of practice, therefore we hope that any positive results will be directly translatable into clinical practice.

All surgical procedures will be performed by cardiothoracic surgeons who have experience with the surgical technique and equipment used to ensure that the minimum number of animals are lost due to technical difficulties.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 pig model as they are very similar in size, anatomy and physiology to the human. Similar methods of resuscitating the heart have been validated in rodent models and therefore a translatable large animal model needs to be validated in order for any positive findings to be then investigated in a human clinical trial. Previous work carried out by our group using similar equipment was also carried out in a pig model, therefore we believe the use of the pig model is the most appropriate to further this work to allow for rapid translation of any positive findings into clinical practice. Our experience with this model will also ensure that the minimum number of animals are lost due to technical errors. We have designed the study to test two interventions such that it minimises the number of pigs required and employed a non-recovery model to minimise suffering and potential complications to the animals. Additionally, the procedures have been designed such that the interventions being studied are all performed following the establishment of general anaesthesia in order to minimise distress to the animal.

Also, final outcome are measured on a device after the animal has expired rather than using a transplant recovery model. This will ensure that the minimum number of animals are used and avoid any potential complications and distress to animals should a recovery model have been opted for.

PROJECT 206

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.

Word limit; 1000 words

Project Title	A murine model of Oculocutaneous Albinism to study the efficacy of drug PTC124 in treating albinism derived visual defects
Key Words	Albinism
Expected duration of the project	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):

The principal project objective is to demonstrate that an eye-drop formulation or systemic treatment of Translational read-through Inducing Drug can improve visual defects in a mouse model of Albinism, with a view to developing the first human treatments for visual disability arising from Albinism.

What 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 aim is to develop a novel treatment for visual disability in patients with oculocutaneous albinism. The project therefore will benefit patients by demonstrating proof of concept and potentially paving the way for human treatment trials.

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

We estimate a total of approximately 300 animals for the project. Exact numbers will depend on the results of the experimental data. Mice were selected as an appropriate genetic model of the human condition of Oculocutaneous albinism as there are genetically relevant albino strains readily available, and they are amenable to eye examination in life. The minimum number of animals will be calculated for each part of the project based on power calculations and statistical estimates. Where possible tissue will be used for additional experiments, and methods employed where multiple experiments can be run on a single sample or tissue.

In the context of what you propose to do to 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 receiving systemic drugs via injection or topical eyedrops will be monitored for any associated weight loss or altered renal function. Severity of adverse effects is expected to be moderate, if any. Eye function tests will be done with minimal animal contact and under general anesthetic. These will be limited in time and frequency. Further examination techniques may include restraint for short periods. Close attention will be made to the health and behaviour of the animals throughout all experiments, and when at rest. Appropriate action will be taken where an animal is thought to be distressed at any stage. Animals will be euthanized at the end of experiment in order that further post mortem studies may be carried out.

Application of the 3Rs

Replacement

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

Replacement

Testing the efficacy of the drug PTC124 in treating Oculocutaneous albinism necessitates its trial in an animal model for ethical reasons, prior to commencing any Human studies. Validating the efficacy of the drug in promoting nonsense readthrough in two human genes provides a vital opportunity to develop treatment for visual disability arising from Albinism. The aim of the study is to develop a suitable model for human treatment trials to be commenced. At this point animal work can be ceased.

Reduction

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

Reduction

The minimum number of animals will be used for each experimental protocol, with a maximum amount of data obtained from each animal. Post mortem studies will also be conducted to build a robust data set. Statistical analysis will be carried out to determine numbers of animals for each treatment group.

Sources of variation within the aimal groups will be closely managed in order to minimise the number of animals necessary for the project, and strengthen reproducability of the 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

We will reduce suffering by minimising the number and frequency of procedures, minimising the stress and suffering to mice during procedures and reducing the number of animals used and combining all *in vivo* studies into a single examination stage prior to culling. For example, for eye examination, animals will need to be restrained. We have established protocols for causing minimal stress and minimising the time of restraint. Strict rules will be applied to the number of procedures or examinations per week and per animal and for the duration of each technique. All procedures will be completed with close attention to animal stress signs and general anaesthetics, local anaesthetics and systemic painkillers will be used as they are when these procedures are carried out in human children. Suffering will also be reduced by accurate statistical calculations allowing us to use the minimum number of animals possible per protocol and by utilising culture thereby reducing animal numbers further per protocol.

PROJECT 207

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.

Word limit; 1000 words

Project Title	The biology of lymphatic filarial nematodes
Key Words	microRNA, in vitro culture, filariae
Expected duration of the project	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;
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):

Nematodes are important pathogens of humans and animals, causing considerable morbidity in human hosts and production losses and welfare issues in animals. There are no modern vaccines available against these parasites and few novel drugs. In part this relates to our inability to grow these animals in vitro and to a lack of up to date genetic and genomic tools. For example, there are currently no cell lines available from any nematode. One of the major aims of this project is to attempt to develop cell lines from filarial nematodes as well as improving the basic culture methods for individual stages of these worms. In addition, we will attempt to improve methods for transfection of adult worms by micro-injection. This procedure will link with the second aim of the project, which is to study the function of small non-coding RNAs in the parasite life cycle.

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

The work described could result in the provision of a filarial cell line, which would reduce the requirement for parasites from infected animals and allow a range of experiments to study aspects of the life cycle such as control of gene expression in addition to basic biochemical studies. Studies on miRNAs will result in an improved understanding of the control of development and reproduction. Longer term-benefits may include a reduction in numbers of animals required for maintaining the parasite life cycle (likely) to better treatments for the control of infection in humans (in the longer term).

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

Approximately 750 adult gerbils over a 5 year period. Gerbils are the only small rodent host for the lymphatic filariae and numbers are based on current usage for maintenance of the life cycle.

In the context of what you propose to do to 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 intra-peritoneally with the infective larvae of Brugia species and will be maintained for period of up to 9 months in groups of 4-5 per cage. All procedures are ‘mild’. We do not anticipate any adverse effects as the parasite is well adapted to the host and rarely results in any pathology. Discomfort during infection is minimised by the use of good restraint. 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

Brugia species are obligate parasites that require an animal host for development. Part of the rationale of this project is to try to develop cell lines from these worms and to optimise methods for culturing parasites in vitro to support development. Only adult animals are used.

Reduction

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

Reduction

There is no re-use of animals on this project. Once infected, animals are maintained until the parasites had developed to the appropriate stage.

In most experiments there are no comparative procedures. In some experiments, we may use infected animals to compare the developmental potential of infective larvae pre-exposed to inhibitors or not. Here power analysis will be undertaken to estimate group sizes 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 gerbil is the only rodent host capable of supporting the whole life cycle of Brugia species. The parasite is very well adapted to this host and infection appears to have minor impact upon the host, causing little pathology and no apparent behavioural changes. Harm is minimised using good restraint during infection and by regular and careful monitoring of infected animals. Animals are maintained in social groups in environmentally enriched caging.

PROJECT 208

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.

Word limit; 1000 words

Project Title	Skin and hair follicle development and regeneration
Key Words	skin, hair follicle, stem cells, regeneration and wound healing, epithelial-mesenchymal interactions and transdifferentiation
Expected duration of the project	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;
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):

Our research focuses on skin, and hair follicles that are skin appendages that come from skin in the embryo. Adult skin and hair follicles both grow very dynamically and we are particularly interested in distinct cell types that are found in skin and hair follicles, and that have important roles in regulating their growth, regeneration and response to injuries such as burns or wounds. These cells have special properties, such as not forming scar tissue after wound injury, or being able to behave like stem cells and turn into different cell types.

The overarching aim of our work is to define new biological properties and discover potential therapeutic capabilities of these cell subpopulations and to make translational use of this information. To this end we hope to:

Identify what cells and molecules are involved in the creation and maintenance of hair follicles and skin fat. We are particularly interested in how cells and tissues signal to each other to control these processes.

Characterise the role of key molecules that distinguish skin and hair follicle cells in the context of wound healing, regeneration, induction of new structures and cell reprogramming.

Use specific cell populations in the creation of new human skin models, replacements and substitutes that are more complex than existing skin replacements (so more like skin as it is found on the body) in particular by incorporating skin appendages.

What 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 tell us about the development of different cell types in skin and hair follicles, and labelling of cells specifically allows us to determine what these particular populations turn into as the skin grows, and whether stem cells are present. One relatively poorly studied tissue is the fat layer in the skin, and discovering what molecules control the development and regeneration of this and other tissues could provide clues for therapeutic interventions of disease and ageing. We will also obtain information about what controls stem cell activity in different layers of the skin. Ultimate benefits could include refinement of isolation of mesenchymal stem cells from skin tissues, and the ability to create new and better human skin replacements and even produce related epithelial structures such as the cornea of the eye, for transplantation.

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

We expect to use around Approximately 5510 mice and 190 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?

Those mice being bred with a harmless marker gene have no expected adverse effects, and the level of severity is mild. Mice and rats used for regeneration/healing studies can have adverse effects due to infection, however the incidence of this is extremely low and the severity level of the operations is considered to be moderate. Mice and rats with an compromised immune system will also be maintained and used for transplantation/grafting work, and they have a greater risk of infection, however the operating conditions and housing are designed to avoid this, and adverse infections are very rare therefore the likely level of severity is moderate. At the end animals will be killed by Schedule 1 methods 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

Where appropriate and possible we routinely use *in vitro* methods to replace *in vivo* animal work. To study stem cells in skin and follicle development and the interaction of different cell and tissue types we have refined established organ culture methods. These allow us to manipulate skin and investigate the role of specific genetic/molecular factors in the development of skin, hair follicles, and the fat layer in skin. However, since these cultures can only be maintained for a limited number of

days transferring them to *animals* is the only way currently of obtaining full information about longer-term outcome. Additionally, since our goal is to better understand the molecular mechanisms that control follicle and fat development, and adult skin homeostasis regenerative responses to injury we need to use genetically modified animal models, because these developmental processes are very complex and involve numerous cell types interacting within the skin. Similarly we have developed models of human skin and hair follicles, but these have limited viability and we are only able to study the interactions involved for longer, and obtain meaningful information, using animals as hosts.

Reduction

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

Reduction

The in-vitro (culture) work that is done in parallel with this research, reduces the number of mice used in our studies.

We have taken into account different aspects of mouse colony management, and will design experiments to minimize numbers of mice needed to maintain mouse strain stocks for surgical and ex vivo studies.

Breeding of the mice will be tightly monitored and controlled depending on the requirements of the user. By virtue of staff having experience and knowledge of the particular line that is being kept, the usage of the animals can be optimised.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 skin and hair follicles that will have relevance to humans and other mammals requires the use of mammals. Mice have been selected for most of this work as they are the species of choice for genetic manipulation, cell labelling and ease of manipulation. For some of the microsurgical studies, however, rats will be used as they are larger and the micromanipulations can be performed with more assuredness of success. Animals will be cared for by dedicated staff in the animal unit with all the requisite training and skills needed to breed and maintain the animals. These individuals will also closely monitor the health of animals that have undergone surgery. In the event that any welfare issues arise they will be addressed at an early stage, and suitable end points will be established by consulting with the

NACWO. Animals incorporating a harmless genetic label will be bred on this licence, at the mild severity level.

PROJECT 209

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.

Word limit; 1000 words

Project Title	AAV gene therapy vector characterisation
Key Words	AAV, Gene therapy, Biodistribution
Expected duration of the project	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):

There are many rare diseases resulting from genetic defects, many of which are untreatable or require an intensive and invasive treatment schedule. AAV gene therapy uses a small, non-harmful viral particle to transfer a therapeutic replacement gene to affected organs or systems within the body. It is the aim of this research to build upon previous work conducted in our laboratory and improve the viral particle to increase the effectiveness and tissue specificity, building the foundations for a new and effective gene 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)?

This project aims to provide several benefits: Firstly, the research is expected to take the first steps in the development of a safe and effective therapy to prevent or treat rare genetic neurological disorders. Secondly, the research is expected to provide information about how certain regions in the virus capsid (‘shell’) affect both how the virus targets different tissues within the body, and how well the virus can transfer the replacement gene, and how the virus can spread throughout the brain, therefore allowing the design of even safer and more effective virus particles. This information will be applied to develop and improve other AAV viruses that can deliver genes to other organs, therefore widening the scope for treatment of different genetic disorders in other organs of the body.

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

Adult mice and rats will be used throughout this research to study how effectively the virus particles can deliver the therapeutic genes to the desired tissues, and how

specific the delivery is. It is estimated that 9 animals will be required per virus for initial studies. Over the course of five years it is estimated that 60 viruses will be tested, and at least two routes of administration tested per virus. In addition, we will breed and maintain genetically altered mice in order to test the efficacy of our treatment in specific disease models. In total, approximately 4000 mice and 1400 rats are expected to be required for the studies we have outlined.

In the context of what you propose to do to 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 housed in social groups and their health monitored daily. Surgical procedures such as intracranial and intraspinal injection are defined as 'moderately' severe, and are commonly carried out in rodents; as such the risk of adverse effects is low. Other administration routes such as intraperitoneal carry a low risk of adverse effects. Adverse effects as a result of the virus administration are uncommon and unexpected, however animals will be closely monitored both before and after administration with all possible steps taken to minimise and prevent discomfort. Animals will be monitored for signs of ill health such as loss of weight and lack of grooming, and those showing signs will be monitored carefully. At the end of the experiment 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

Tissues are complex organs composed of multiple cell types and structural features. When testing new gene therapies for effectiveness it is important to see how far through the tissue the virus can spread, and to see if the virus infects tissues other than the ones targeted. The complexity of tissues cannot be replicated in the laboratory by cell cultures, and so animal experiments have to be conducted.

Reduction

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

Reduction

Statistical power estimations have been carried out to estimate the minimum number of animals required to generate statistically significant results. It should be noted that some analysis methods use tissue prepared by the same methods, therefore this tissue will be shared between analyses where possible to minimise experimental numbers

Where possible animals will be bought rather than bred to reduce the possibility of unneeded 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

Rodent physiology is well understood and provides a complex living system in which to study how efficient the viruses are in a live organism. This cannot be replicated in the lab.

Trained and competent staff will carry out all procedures under sterile conditions to minimise the risk of infection. Painkillers and antibiotics will be administered when necessary to minimise discomfort and risk of infection.

PROJECT 210

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.

Word limit; 1000 words

Project Title	Oral immunotherapy as a treatment to desensitise horses with insect bite hypersensitivity to Culicoides spp. (Sweet Itch).
Key Words	Equine, Allergy, Immunotherapy
Expected duration of the project	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:
Yes	(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):

All animals have a sophisticated immune system which reacts against foreign threats like bacteria and viruses to provide protection from infectious diseases; but simultaneously remains tolerant to non-threatening foreign materials like food. When blood-sucking midges bite, they inject saliva containing several proteins that prevent the blood from clotting. Most horses develop a tolerant immune response to midge saliva, but in approximately 6%, the immune system mistakenly reacts as it would to an invading parasite, triggering a severe “allergic” reaction with intense itching and the development of raw sores known ad insect bite hypersensitivity (IBH). Although stabling, rugs and insect repellents help alleviate clinical signs there is no cure for this disease.

Allergies like IBH can in principle be cured by re-programming the immune system to make a tolerant response as it would to a food.

Now we intend to conduct a pilot trial to investigate whether oral immunotherapy can be used to treat allergy in horses. We will prepare a sticky gel containing the midge salivary proteins which we have made in the laboratory. Small regular doses of gel will be placed under the horse’s tongue allowing the allergens to be slowly released in the horse’s mouth then detected by the immune system in the same way as food. Over the following months, we will monitor the effect of the treatment on each horses’ immune response and the severity of their clinical signs to determine if their allergy has improved. We will also monitor the horse carefully for adverse reactions to this form of treatment to enable us to decide if a wide scale trial of this method of treatment would be justified.

What 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 seeks to use the scientific understanding of the how immune system functions in equine insect bite hypersensitivity to develop a better treatment for the disease that will directly benefit affected horses.

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

We expect that up to 40 allergic horses will be involved in this pilot trial of immune therapy for insect bite hypersensitivity. An additional number (approximately 20) normal horses will be recruited to act as baseline controls. In each horse the trial will last up to 1 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?

Although all immunotherapy carries some risk of an adverse reaction to treatment oral immunotherapy has proved to be very safe compared to allergen injections. In human trials, side effects of oral immunotherapy are usually limited to a transient inflammation of the mouth which occurs immediately after treatment and such reactions become less common as the immunotherapy course progresses . Overall this trial is unlikely to cause any lasting harm or suffering to the animals involved and will provide a first step on the way to developing a treatment that can ultimately benefit horses with IBH.

Application of the 3Rs

Replacement

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

Replacement

IBH is a naturally occuring condition affecting horses. There is no alternative means of carrying out a clinical trial to treat a naturally occurring condition without using subjects that have the condition.

Reduction

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

Reduction

The study is designed to use a minimum number of subjects in order to answer preliminary questions about safety, efficacy and practicalities of immunotherapy in horses.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 is no means of carrying out a clinical trial to evaluate oral immunotherapy as a treatment for insect bite hypersensitivity in horses without using subjects that have the condition. The trial only uses procedures in the mildest category of severity as defined under ASPA. The horses will not be exposed to conditions likely to cause any worsening in their condition and their owners will be free to use any of the common preventive measures to reduce the severity of the natural expression of the condition.

PROJECT 211

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.

Word limit; 1000 words

Project Title	Cell behaviour during neural differentiation
Key Words	Spinal cord development, Neural stem/progenitor cells
Expected duration of the project	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):

Neural stem/progenitor cells give rise to all nerve cells (neurons) and their supporting glial cells in the brain and spinal cord. The aim of our research is to understand how neural stem/progenitor cells make the the spinal cord in the developing embryo and how a small fraction of them is then retained with the potential to make new neurons and glia throughout life. We investigate these processes at multiple scales: from molecules to cells and tissues. This involves first investigating which molecules (or genes) are switched on or off in neural stem/progenitor cells as they become neurons. Changes in the expression of genes inside a cell lead ultimately to changes in cell behaviour, such as cell division or cell migration, that drive the formation and shaping of tissues. To understand how molecular changes direct cell behaviours as the spinal cord forms, we label and manipulate cells and then make movies to monitor changes that take place. These fundamental studies advance our understanding of how genes work together to regulate the development of the spinal cord and inform us of what might go wrong in disease.

Our research into the mechanisms regulating neural stem/progenitor cell behaviour is also uncovering previously unknown structures inside cells that control the cell’s shape and how cells migrate within the spinal cord.). These new insights into fundamental biological processes may provide clues about what may go awry in disease conditions such as cancer, where cancerous cells break away from tissues in an uncontrolled manner.

Our research project also aims to characterise in detail the small fraction of neural stem cells that is maintained in the adult spinal cord. These cells are therapeutically promising because they can be re-activated in response to external cues (e.g. spinal cord injury) to make new cells. We are currently investigating the molecular

properties of this intriguing cell population in the spinal cord of adult mice and how they are set aside during development. In the longer term, this research may uncover targets for stimulating and directing neural stem cell activity to promote spinal cord repair following injury or 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)?

Potential benefits of this research include development of protocols to direct the differentiation of human pluripotent cells to make spinal cord neural progenitors in a culture dish. This may allow the study of the development of neural diseases and may provide a basis for screening drugs for therapeutic effects as well as cell-based therapeutic approaches for the treatment of spinal cord injury or disease. Our investigation of neural stem/progenitor cell behaviour and of the formation of adult neural stem cells will increase our understanding of how the nervous system is made and may have longer term relevance to manipulate neural stem/progenitors cells in the tissue context to enhance spinal cord repair following injury or during disease.

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

10,000 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?

Most procedures will be mild and all animals will be humanely killed at the end. We shall breed and maintain mouse lines that bear specific genetic alterations and harvest early embryos from pregnant females after killing them humanely. In some cases, we will induce the particular effect of the genetic alteration by administering a drug to the female a few days before she is killed. We wish to make use of a new technique in which the genetic alterations are introducued into the embryos direct. This will require a surgical procedure on the mothers, very early in their pregnancies. Any potential post-operative pain following surgery will be minimized by analgesic administration. This would be a procedure of 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 need to use mice for these studies for the following reasons:

1. Assessment of gene function in a physiologically relevant context.

2. Use of available genetically modified mice allow manipulation of genes in specific tissues at specific times. This allows precise assessment of the function of a gene in a defined cell population.
3. This precision allows us to investigate gene function in neural progenitors and adult neural stem cells in their normal environment and opens the way to manipulation of such cell populations for regenerative purposes.
4. Although some of our experiments are carried out chick embryos, gene manipulation in this non-protected embryo does not allow such precise control of gene dosage and targeting of cell populations.
5. Our work in embryos has led to development of protocols for differentiation of pluripotent cells and some of our experiments involving human cells are now carried out in such “in the dish” assays. However, cells in culture are inherently heterogeneous in the cell types they produce, so it is difficult to study regulation of pure cell populations. In addition, any differentiation steps identified in an artificial culture system where cocktails of proteins/small molecules are added and decay at variable rates require validation in the normal developmental context.

Reduction

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

Reduction

We breed our mouse colonies as required for generating mutant embryos for analysis and for maintaining the line. For analysis using wild-type animals, we buy females in and mate them as required to obtain embryos to minimise the animal numbers

A minimum of 4 to 6 mutant embryos showing the same phenotype is current practice in the field for determining changes in gene expression. Where analysis involves more complex procedures such as live imaging, samples are taken from at least 3 embryos for each tissue and comparisons are made between different tissues within these samples.

For live imaging approaches in the developing spinal cord we are developing an in vitro model using human pluripotent cells. This development of a human in vitro model reflects the advent of new genome editing techniques and tissue engineering approaches that allow us to more easily monitor and investigate cell biological mechanisms in human developing neural tissue.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 as a model species because they are the most well characterised genetically tractable model for mammalian development and adult tissue homeostasis and where possible it is important to assess gene function in a physiologically relevant context.

Welfare costs to animals is minimised in a number of ways. To obtain mouse embryos mothers are sacrificed quickly and painlessly and embryos are dissected and preserved rapidly, the majority of which are younger than 10.5 days of development (less than half the gestation period

Animals undergoing the surgical procedures receive anaesthesia and analgesia as advised by the supervising veterinary surgeon.

We have reduced the need for injections when administering inducing agents to pregnant animals by introducing these in food/oral gavage whenever possible.

PROJECT 212

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.

Word limit; 1000 words

Project Title	Treatment and Pathology of Neurological Diseases
Key Words	Therapy, Inflammation, Brain Tumour, Neurological disease
Expected duration of the project	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;
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.

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 purpose of this project is to develop new treatments for neurological disease through better understanding of how they are caused. In the majority of these diseases no adequate treatment is available that treats the brain. We have already identified that inflammation has a significant role in neurological diseases such as metabolic diseases and brain tumours. We intend to study how this influences the progression of the disease and understand the mechanisms that cause it. We will develop new therapies using this knowledge and test the quality, effectiveness and safety profile of them in both mice and sheep. The aims of this project are: i) understanding how blood cells and inflammation interact in normal and diseased brains to assist in treatment development ii) to develop new treatments in mouse models of metabolic and inflammatory brain diseases and brain tumours iii) to improve brain delivery methods for gene therapy in large animals in preparation for clinical trials.

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

The main benefits are that we are developing treatments for diseases in children where no adequate treatments are available. The work will improve how we use bone marrow transplant currently and in combination with other treatment strategies which are most appropriate for therapy in the brain and help to bring them to clinical trial more rapidly. We already have one drug that we developed in a late stage clinical trial in patients. From aim i) we will improve our understanding of how inflammation and other factors influence the development of neurological diseases ii) We aim to develop new and better treatments for neurological diseases using the knowledge gained in aim i, and bring them to the clinic; in particular metabolic

diseases and brain tumours and iii) to improve brain delivery methods in large animals which can be better applied to patients.

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

We will use mainly mice (12,000 over the five year project with 11,500 used in breeding and 4,000 in experiments) and sheep (60 over the five year project). Sample sizes based on previous work were used to estimate the minimum number of animals required 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?

The diseases we are studying are inherited genetic diseases of children so we use a number of mouse models. The animal models and protocols to be used here have been developed by us and our colleagues to give us the best experimental outcomes possible. These mice can replicate the disease seen in humans effectively; however, they can become ill. We will be vigilant to observe such signs and to euthanise them if they do. The severity level of these studies is moderate. Sheep have a brain that is much closer in size to humans and this helps to mimic the scale up problems that typically happen when moving a therapy from a mouse to a human – meaning that clinical trials don't always work as they should. Our aim throughout is to develop new treatments, thus, although some treatment methods can be up to moderate in their delivery, (e.g. bone marrow transplant, injection into the brain); we usually expect to see significant improvements in disease status in both mice and sheep. At the end of the studies animals will be killed humanely. For both mouse and sheep studies, we will collect tissues for biochemical and histopathological analysis.

Application of the 3Rs

Replacement

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

Replacement

Mouse models allow us to understand how these genes work in the context of a whole organism which mirrors human diseases better as cell-based assays may only provide limited results. Genetic diseases often affect a number of different organs; therefore, animal models provide physiological data, such as cell-cell interactions and immune system dysfunction which cannot be achieved in vitro We need to assess that the therapies we develop have the ability to cure all of the affected organs, especially the brain, so computer based assays and cell culture cannot

predict outcomes. There also are no validated methods to avoid the use of animals to assess cell engraftment after bone marrow transplant of the delivery of gene therapy vectors to the brain. Our therapies are assessed with a variety of outcome measures such as behaviour and tissue sampling which can only be achieved with in vivo experiments. For direct brain delivery of therapies it is essential to test in an animal of appropriate size to replicate the delivery technique to be used human patients. This cannot be achieved by any other method therefore it necessitates the use of a large animal.

Reduction

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

Reduction

The number of animals used is kept to a minimum to provide us with meaningful results. We will use statistical calculations to determine the minimum number of animals required for our studies. These calculations are based on our extensive experience of these types of studies. We will breed transgenic animals in a way to maximise the number of animals from each mating that can be used in the project, therefore, minimising the number of unused animals. We will collect data throughout the lifespan of our animals to generate the maximum amount of data. We will also use imaging techniques which will allow us to monitor animals at different timepoints removing the need to sacrifice animals. By applying these methods it will allow us to reduce the number of animals we use. We have a number of collaborators, therefore, maximum use is made of animal tissues across a number of different 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

For most of the project we aim to use mouse models of genetic disease as they can carry genetic defects similar to human disease. Mice allow us to determine to role of specific genes and produce similar biological symptoms associated with human disease, and allow to model and test potential therapies to counteract disease progression. We aim to use a sheep as their brain is a more similar size to a human than a mouse to help us scale therapies up to humans. Where possible, we attempt to prevent onset of disease, rather than treat once disease phenotype has been seen in order to prevent prevents undue suffering. We will use imaging techniques to monitor effectiveness of treatment without needing to sacrifice the animal. Experiments will only be performed by highly trained individuals. Suffering

will be limited by strict monitoring of severity limits and the use of our protocols that do no produce any suffering.

PROJECT 213

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.

Word limit; 1000 words

Project Title	Rodent Imaging for Translational Research
Key Words	Imaging
Expected duration of the project	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;
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):

When researchers test out new drugs in humans (clinical trials), they often want to use different types of specialised imaging to see what the drug is doing to the patient. If the new drug is unlike anything used before, the researchers may not be quite sure what type of specialist scan to use, or how to interpret any changes in the scan after the drug is given. We will use similar types of scan to look at the new drug in mice or other rodents. We will use our scanning results to advise medical researchers how best to scan the human patients. Our scanning results will also help the researchers understand the changes they see in the scans of human patients. This is called “translational research”.

We will use our specialist rodent scanners to understand how such substances distribute around the body, and we will try to improve the scanning techniques

We will also use our specialist rodent scanners to understand how such substances distribute around the body, and we will try to improve the scanning 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)?

• Our imaging studies will play a part in making new medicines available for doctors to pre-scribe • We will come up with ways to make it more convenient and comfortable when patients have to undergo a scan as part of a clinical trial • Sometimes the ideas for new medicines that come out of the lab are just not good enough to make a worthwhile new medicine. We will help stop those projects as soon as we can • We have a lot of experience with rodent scanning, and how to avoid pain and distress. We will work hard to share our methods and ideas with others, so they make the very best use of animal scanning.

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

We expect to use about ~25 rodents, mainly mice, per week, which adds up to 5000 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?

Sometimes the animals will get the new medicine. They will be scanned, sometimes on several occasions. To stop them moving during the scan, we give them an anaesthetic (just as doctors sometimes do when scanning babies). We have to kill the animals at the end, because we need to know how what we see in the scans when the animals get the new medicine relates to what's going on in the body. For cancer medicines we may inject some cells under the skin which grow to form a lump (tumour) under the skin, which we can scan. Although this looks unpleasant it doesn't cause the mice much distress. We don't let the tumour spread to form secondary's (metastases), and we know from humans that cancers are seldom painful until they spread.

Application of the 3Rs

Replacement

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

Replacement

When a completely new type of medicine arrives in the body, it can change the ways the scans look in surprising ways. Because of all the processes that occur in the cell, together with blood flow and motion, we simply can't calculate these without doing an experiment.

So, although it is impossible at the moment to achieve the scientific data necessary to inform human clinical trials without the use of animals we do constantly review the literature for potential non-animal models as well.

Reduction

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

Reduction

We have a lot of experience in designing clever experiments that give a lot of information with small numbers of animals. We work with statisticians to calculate how few we can get away with and still get reliable answers.

Wherever possible we publish in journals which support the ARRIVE guidelines which in turn underpin good experimental design and all the benefits that brings to the reduction in the use 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

We mainly use mice and rats since they have been used a lot in the past by other researchers, and we can build on what they know. It can be unpleasant for the rodents when they come around from anaesthetic and we need to watch them carefully and keep them warm. Some of the potential new medicines might be unpleasant or even harmful and we follow detailed guidelines written by vets to ensure we can pick up if an animal is suffering and step in quickly

After every experiment we will critically appraise what we do to seek out any ways to improve our models to reduce harm to animals.

PROJECT 214

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.

Word limit; 1000 words

Project Title	Pathogenesis mechanisms of bacterial pathogens
Key Words	Macrophages, innate immunity, Listeria, Streptococci, Staphylococci
Expected duration of the project	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;
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):

Infections caused by bacteria are a major health burden worldwide and a threat to society and health systems. New bacterial strains that can cause disease in humans and animals are constantly emerging. Current treatment options are in danger of becoming ineffective as multi-drug resistant bacterial strains emerge. New approaches for prevention and treatment of bacterial infections are thus urgently needed.

This project investigates how cells of the immune system called macrophages recognise and destroy bacteria that invade the body. Macrophages are key cells of host defence that orchestrate a coordinated immune response to any infectious agent. Macrophages recognise and eat bacteria, which triggers inflammation and immune responses. Many of the mechanisms of how they do this are still unknown. We will use mouse models of infection to study the function of genes and molecules that play a role in macrophage host defence. We will also look at mechanisms of how disease-causing bacteria can evade macrophage defence reactions. In our mouse models we will study three major types of bacteria that can cause severe disease in animals and humans. We will use mouse models of *Listeria monocytogenes* infection to study a food-borne disease called listeriosis. It can cause sepsis, severe brain infections, and abortions in susceptible patients and pregnant women, respectively. We will also investigate the interaction of another bacteria called *Streptococcus pyogenes* with macrophages and study how it can induce cell death to evade immune responses. *Streptococci* are also major cause of sepsis in humans when they manage to reach the blood stream. Finally, we are studying how *Staphylococci* bacteria colonise the host and invade the skin to cause abscesses and skin infection. Here we have established a mouse model of canine

skin infection to identify vaccines against the pathogen. Using a chicken model of *Staphylococcus aureus* infection, we are investigating how these bacteria that have originally come from humans, have adapted to avian hosts to cause disease. We are studying how these bacteria can infect the bone of chickens. The resulting inflammation called chondronecrosis is a major cause of lameness and a significant welfare issue in the broiler chicken industry.

What 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 designed to investigate the basic mechanisms of how macrophages recognise and defend against bacteria. We envisage that our research will discover new receptors and signalling molecules that macrophages use to defend against infectious agents and to alert other cells of the immune system to the incoming danger. These new molecules may be useful as new drug targets for improving host defence or to alleviate excessive inflammatory reactions that are associated with severe infectious disease (e.g. sepsis, abscesses, bone infection). Other key benefits of our project are the potential development of new vaccination strategies that will target macrophages as important cells for activating the immune response. Our study of factors that make the bacteria more powerful might lead to new drug and vaccine candidates that can be used to protect against infectious disease. We will also try to improve our understanding of how bacteria adapt to new hosts. We hope to discover mechanisms how Staphylococci bacteria can infect chickens. Such knowledge can potentially be used to improve the welfare of chickens in the poultry industry.

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

Mouse (wildtype and genetically modified). Up to 12400 mice over 5 years. Chicken (wildtype and genetically modified) up to 800 chickens 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?

Potential adverse effects may include the appearance of clinical signs of disease after infection, such as local or systemic infections and tissue inflammation. To minimise adverse effects we are monitoring infected animals closely using standardized protocols. This is important as if we did not monitor them carefully, some of these infections could potentially lead to death. However, we will interfere before this happens and will kill the animals humanely.

Application of the 3Rs

Replacement

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

Replacement

The complex host response to an invading pathogen involves many different tissue and immune cells. Macrophages are very heterogeneous cells that carry out different functions depending on the tissue or organ they are residing in. Currently, we don't have tissue cultures systems for all different types of macrophages available that would allow us to study interactions of the cells with pathogens and other immune cells. Whenever possible we will use cell culture systems and cell lines to analyse particular host defence reactions that don't need a whole animal system. We have recently developed new approaches that allow manipulation of host defence genes in macrophage cultures. These systems will replace and supplement *in vivo* animal experiments to a significant extend. Our aim is to develop these technologies further over the course of this project.

Reduction

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

Reduction

Group sizes of experimental animals are calculated to keep animal numbers to a minimum, whilst ensuring that numbers are used that can provide statistically significant data. Required animal numbers are constantly reviewed based on experience, previous data and statistical advice. Macrophage cell lines and macrophages grown *ex vivo* from the bone marrow will be used to replace *in vivo* infection challenge experiments. As mentioned above we have developed new methods to ablate genes of interest in cultured macrophages which will allow us to study particular aspects of their functions *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

We have chosen to use mice in our studies because the mouse as model systems provides us genetic tools and reagents that we need to dissect functions of immune cells and host defence genes at a whole animal level. The mouse is unique in this regard as a mammalian model system. Chickens are used as an experimental system because the *Staphylococci* bacteria that we study are naturally adapted to avian hosts and there is currently no alternative system available to answer our research questions. Animal suffering is minimised through close and standardised monitoring of health status during infection using a well-established scoring system.

Animals are euthanized when defined human endpoints are reached or when pre-defined clinical endpoints are reached during an infection cause.

PROJECT 215

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.

Word limit; 1000 words

Project Title	Pathogenesis of Protozoan Infections of Livestock
Key Words	Cryptosporidium, Toxoplasma, Neospora, Pathogenesis, Vaccine
Expected duration of the project	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;
No	(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);
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):

The aim of this project is to improve the understanding of disease and infection caused by protozoan parasites on livestock and how these parasites are transmitted between animals and the environment. The project licence focuses on *Cryptosporidium*, *Toxoplasma gondii* and *Neospora caninum*. We aim to find out the mechanisms of how the immune response manages to control these infections and how it prevents disease so that this knowledge can be used to develop vaccines that will prevent disease in livestock species. We also aim to use the immunological understanding gained for the development of improved diagnostic tests that can reliably identify persistently infected animals (especially *Neospora* infected cattle) and that can be used for disease control programmes on farms. We also aim to improve the understanding of how these pathogens are spread from animal to animal and also via the environment in order to develop better disease control 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)?

The short term benefits of this project will be the improved understanding of how the immune response manages to control protozoan infections and how these infections are spread. This knowledge will be shared by presentations and publications. Other potential benefits will be development of improved disease control strategies that are shared with the farming and veterinary community. Other potential more long term benefits arising from this project licence are improved diagnostics and vaccines that will help farmers to manage these infections within their livestock.

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

During the 5 years of this project we will be using up to 120 cattle, 130 sheep, 20 rabbits, 1500 mice and 4 cats.

In the context of what you propose to do to 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 vaccinated and/or infected with protozoan parasites and their health will be closely monitored. The disease symptoms can range from being asymptomatic to having diarrhoea, fever or miscarriage. At the end of the study, animals will be euthanized using a humane method with the exception of the cats (these will be rehomed) and the sheep (they can be returned to the flock).

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 *in vitro* alternatives to study host immune responses and pathogenesis of disease. Currently, there are also no *in vitro* alternatives for the production of some of the life cycle stages of the protozoan parasites that we work with. Unfortunately, this means that we have to use animals to achieve the aims of this project licence. But we are trying to develop *in vitro* culture systems for these pathogens to reduce the need for animal work.

Reduction

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

Reduction

Whenever we design studies involving animals, we work closely with bio-statisticians to help us with the design to make sure that the minimum number of animals will be used to generate 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

Whenever possible we work with the relevant and natural host species of the parasites given direct information about the infections for the host in which disease occurs. Having experience of working with the different infection models has allowed us to refine these models to minimise the disease impact on the animals involved.

PROJECT 216

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.

Word limit; 1000 words

Project Title	The immune basis of Type 1 diabetes
Key Words	
Expected duration of the project	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;
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):

Type 1 diabetes develops due to an imbalance in the immune system that leads to death of the cells in the pancreas that make insulin, a hormone that controls blood glucose and is essential to life. The exact details at a cellular and molecular level of how this arises are not known and are difficult to study in human patients. One of the aims of this programme, therefore, is to shed light on how the immune system kills insulin-producing cells, using appropriate animal models that are modified to represent human disease as closely as possible. A further unknown relates to the best approach to manipulating the immune system to prevent or cure type 1 diabetes. The programme will therefore focus on the development and optimization of immune-based therapies for type 1 diabetes. Finally, there are currently no blood markers or imaging modalities that can be used to assess the degree of damage to insulin-producing cells, or the extent of the inflammation. Therefore this programme will attempt to identify and develop new blood markers and ways to image inflammation in the pancreas.

What 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 disease processes could lead to development of entirely new therapies. There are some immune-based therapies already showing promise but these need to be optimised further for human use. Finally, new ways to image the pancreas and new blood markers could help in drug development, assessing disease risk and monitoring treatment.

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

We expect to need to breed ~10,000 animals over 5 years, and closely study approximately 3000 of these.

In the context of what you propose to do to 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 levels of severity for our protocols are assessed as mild to moderate. The main expected adverse event is development of diabetes which will be managed by giving more fluid to drink and by using insulin treatment if required. At the end of the study, mice will either be designated for breeding or humanely killed.

Application of the 3Rs

Replacement

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

Replacement

Human type 1 diabetes is a highly complex disease involving multiple cells and molecules in the immune system. As such it is impossible to model in test tubes, tissue culture or by computer. Where possible we address as many questions as we can using these approaches and by studying human patients. However, when incisive studies are required, such as developing new therapies, animal models are required.

Reduction

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

Reduction

Before we perform any experiment we design the study carefully to minimize the number of variables, and then use a statistical power calculation to inform us of the correct study size. This guides us to minimize 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

With a complex human disease and challenging therapeutic approach, positioning the model as close to human disease as possible is required. We do this by using transgenically modified to express key human immunological molecules and where

possible we use human immune cells transferred into the mice to obtain data that is as close and as relevant to the human model as possible.

PROJECT 217

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.

Word limit; 1000 words

Project Title	Neural Development in Xenopus and zebrafish
Key Words	Neural development, visual system, embryonic
Expected duration of the project	2 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):

The main aims and objectives of this project are to advance our understanding of how the embryonic brain develops. This is done by the experimental investigation of the mechanisms by which neurons are generated to give rise to neural tissues of the right size and cellular composition, and the mechanisms that are involved in the wiring up of these neurons into functional networks.

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

The potential benefits of this work are largely related to the advancement of science, the brain is the most complex organ known to man, it is also capable of incredibly sophisticated computations. Knowledge of how this organ is built will help neuroscientists understand better how it works. In addition, insights into the developmental mechanisms involved in building a brain may help future medicine aimed at repairing damaged nervous systems.

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

The work in this project focuses entirely on embryonic frogs and fish. In this two year programme of work, we will keep about 300 adult frogs and about 125000 adult fish to produce these 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?

The adult animals are in the case of fish, simply mated with each other to generate embryos. Occasionally, embryos will be genetically modified and grown to adulthood.

We do not expect that this will cause the animals any harm. When the fish get too old to reproduce, they are humanely killed. For frogs, the embryos are produced by hormone-induced ovulation by injection. Again, when the adult females are no longer able to produce eggs for fertilisation, they 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

All of our experimental work is done on very early stages (less than 5 days post fertilization) of development - when the embryos less than two millimeters long and weigh less that a milligram. This is also long before the embryos are first able to ingest food particles. At this stage the embryo is considered to be at such an early stage of development that it does not experience pain and suffering. However, we do use genetically altered adult animals to produce the embryos for our work and this is why the work requires to be licensed. We must use animals for this work because we are studying the biological mechanisms of brain development, which occurs only in animals.

Reduction

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

Reduction

We try to gain the maximum amount of information from each embryo by using high resolution microscopy in three spatial dimensions and also a time dimension (i.e. 4-dimensional microscopic analysis) using multi-coloured transgenic fish to enable us to follow as many different cell types as possible. This allows us to collect a great deal of data from single embryos. The more reliable and better our imaging is the fewer animals we need to use. In the frog work, we use also often use time-lapse analysis with multiple fluorescent markers so that developmental events and perturbations can be monitored and quantitated in single embryos again reducing the number of animals needed per 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

By using species which produce hundred of embryos per mating, we can hugely increase the amount of experimental results that can be obtained from the smallest number of adult animals. The procedures we use are all mild in nature, basically only mating the fish, which is a natural behaviour, or in vitro fertilization of hormone induced egg production in frogs. The use of genetically altered animals in which particular molecules and cells are labelled enables us to obtain the most information from single embryos.

PROJECT 218

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.

Word limit; 1000 words

Project Title	Mechanisms of glia-neuron interactions in the brain
Key Words	neuroscience, synaptic transmission, astrocytes, plasticity and pathology
Expected duration of the project	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;
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):

Neurons are electrically excitable cells in the brain and are the foundation of rapid communication in the nervous system. However, they cohabit the brain with a host of other cells known as glia. This project aims to investigate the contribution of astroglia in the immediate, real-time activity of neural networks, given recent evidence that astroglia engage in rapid, molecular signal exchange with neurons in a variety of circumstances. While these modes of communication have been well characterised in systems of reduced complexity (such as cell cultures and brain slices), there are still some unknowns as to the role of these forms of communication in an intact brain. In particular, there is an imperative to research the role of astroglia in higher brain functions and in neurological disease. This project will explore this conceptual gap using cutting-edge, novel experimental approaches in imaging, electrophysiological recording and genetic tools.

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

The principal benefits of this project relate to new knowledge on fundamental aspects of brain function. In addressing some of the ongoing controversies concerning astroglia in the brain, this project is potentially beneficial to a large community of neuroscientists and neurologists. While the primary impact will be among basic researchers in pre-clinical neuroscience, there are important benefits to clinical practitioners and drug developers, particularly with regard to the possibility of identification of new therapeutic targets associated with astrocytes.

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

This project, at the time of writing, will involve contributions from approximately 10 post-doctoral researchers and 2 Ph.D. candidates, using a maximum of 1500 animals (both rats and 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?

2 of the 4 procedures outlined in this project are non-recovery procedures. In this case, the animals will be terminally anaesthetised and killed while unconscious. Such procedures will involve a minimal amount of distress to the animal. The other 2 procedures are recovery procedures of ‘moderate’ severity. In these cases, there is a small chance for concerns over welfare to manifest. Each procedure will involve a single surgical operation under general anaesthesia and attentive perioperative care. This includes meticulous monitoring of the animal during and after the operation, use of analgesics and antibiotics throughout as appropriate, and continuous consultation with the animal welfare staff in the institution. REDACTED Incidents of significant welfare concern are expected to be very rare (<1%). If such cases arise, the animal will immediately be treated in accordance with advice from the Named Veterinary Surgeon. Such cases are likely to involve poor recovery following the surgery, infection of the incision site or detachment of a surgical implant. As this likely to cause significant distress to the animal, the animal will be humanely killed if they do not respond to the treatment. In cases where such treatment is not possible or not in the best interest of the animal’s welfare, the animal will be immediately killed.

Application of the 3Rs

Replacement

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

Replacement

Our group takes a multifaceted approach to neuroscience research, making use of animals, cell cultures and other in vitro systems, human samples, and computational models to answer fundamental questions of brain function at the cellular level. The complexity of the nervous system necessitates the use of a certain amount of animal research, as isolated systems lack some of the nuanced and emergent properties of cells and networks seen in organised brain tissue. However, the ongoing use computational and in vitro experimentation in this group allows us to continuously optimise our experimental design and make better predictions about cell function, thereby reducing the number of animals used in our experiments. REDACTED

Reduction

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

Reduction

REDACTED We will employ suitable statistical testing to ensure that the minimum number of animals is used without underpowering any given experiment, to allow for robust hypothesis-testing and for valid conclusions to be drawn. Other refinements in experimental design, including tests using repeated measures during time-lapse imaging and repeated recordings from individual animals over the course of weeks or months, will allow us to get as rich a dataset as possible from each animal, to further reduce the overall 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 use of rats and mice is suitable for various reasons. As mammals, findings of fundamental importance made in these species have been relevant to human biology and health and many of the key physiological concepts in brain communication have been established through experiments with these species. Additionally, the molecular biology tools (including targeted genetic modifications) and experimental techniques that are relevant to this proposal have been developed and validated in mice and rats, removing the need for additional experiments in setting up our methodology.

Procedural refinement, particularly for Protocol 2, has been implemented throughout our previous project license, and we will continue to refine procedures with the aim of minimising harm to the animal. As this protocol involves several stages of habituation and training, animal welfare will be closely monitored and habituation protocols will be refined in line with measures of animal health made routinely during husbandry. For instance, parts or mimics of the imaging and restraint apparatus may be used as environmental enrichments for the animal's home cage to allow to animal to familiarise itself with equipment. Experimenter's will also be in regular handling contact with the animal prior to experiments. These steps, in addition to other refinements, should help reduce distress that the animal may experience during the project.

PROJECT 219

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.

Word limit; 1000 words

Project Title	C3, a master regulator of the immune system: – exploration of adjuvant and immune cell effects due to complement activation
Key Words	Vaccine, Adjuvant, Complement (C3), Immune-response
Expected duration of the project	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.
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):

Complement component 3 (C3) is a protein found in the blood of all mammals, which plays an important role in the immune system. We wish to to harness the natural function of C3 to improve vaccination responses and develop new vaccine formulations that can be stored/transported at room temperature before use.

We have previously established that a bacterial protein can function as an aide to vaccination. Therefore, we wish to investigate whether this protein will generate an appropriate vaccine response in both young and aged mice. We also need confirmation that the protein only acts through the actions of C3 and C3 alone. We will also check that C3 activation is safe and functions in individuals with over active C3 or under active C3.

Proteins are affected by heat and dehydration and so often need to be transported and maintained in fridges (a.k.a the ‘cold chain’). This cold chain makes delivery of protein-based vaccines expensive and very difficult in the third world. If we do not need to keep vaccines cold, we can significantly increase the ability to use these effectively worldwide and reduce the cost of all vaccines of this nature. Therefore, we will also investigate whether bacterial protein based vaccines can be placed in tiny protective cages (ensilicated), survive being heat treated, and then released from the cage and work as a vaccine as if no caging had been undertaken. Alternatively, use of DNA versions of the vaccines which are resistant to heat, will be tested as these are also resistant to heat or dehydration. All these scenarios will establish the ability

of vaccines to be stored and shipped at room temperature, even if that room is in the Sahara.

Immune systems fail with age and we do not know why. Over stimulation of the cells that make up the immune system might be a driver for the changes we see. C3 activates and stimulates the immune system, yet the mechanisms behind this remains unknown. Our analysis of Sbi vaccine function, when carried out across the life span of mice will provide essential data not only about the effectiveness of the vaccines but the role of C3 in the aging immune system.

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

This research could provide an important step towards improving the function of vaccines as well as understanding the fundamental mechanisms that associate with ageing and poorer responses to vaccines. This research will also provide significant detail on how the immune system changes with age and the ability of certain bacterial derived proteins to be used to potentiate vaccines. It will suggest where defects occur in the aging response and the mechanisms that drive the immune system to respond to particular stimulus. We will also investigate the potential to use ‘tiny cages’ (ensilication technology) to protect vaccines during transport, which may have significant impact on reducing the cost of vaccination projects around the planet. Development of new methods to improve the delivery and efficiency of existing vaccines could help prevent the deaths of some of the 2.5 million children who die annually from vaccine preventable diseases (WHO). If the research confirms the functionality of the bacterial protein we have identified, its vaccine enhancing ability could feasibly be extended to work with any disease or target.

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

We will use adult and genetically modified mice. We will breed up to 2000 mice across the five years. Most will be humanely killed to provide cells and tissues for laboratory experiments. Several hundred will be used in immune response experiments lasting around 6 weeks or for testing the long lasting effects of vaccines (up to a year). We may also use up to 10 rabbits for the production of key research reagents.

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

These experiments have a low likelihood of causing harm i.e. will be of mild severity. Immunisation with antigens commonly used in human are likely to be safe in mice and rabbits. However, certain mice might experience some discomfort of moderate severity in response to particular formulations in vaccination or due to normal ageing maladies, which would be closely monitored. This could occur if vaccination triggers

unexpected outcomes but control measures are in place to ensure these are recognised and dealt with quickly. Mice will be humanely killed (with the majority providing tissue or blood products for the study). Rabbits may experience mild local tissue inflammation because of adjuvant use. These will be monitored for closely and advice sought from Vet as required. Rabbits may be released from the act and re-homed on advice from the vet.

Application of the 3Rs

Replacement

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

Replacement

Human tissue or cells can be used to answer simple questions/ outcomes but vaccination/immune responses are complex, involving multiple cell types and pathways; therefore, it cannot be modelled in the laboratory and animal studies are necessary to fully dissect
mechanisms. Standard laboratory based methods (ELISA) for the determination of v accine potency cannot be used for the research proposed herein

as bespoke antigen and vaccine formulations are being used. Therefore, we dont ha ve any standard to assess potency against, we do not known the linearity of composi tion to outcome as yet.

Computer based modeling approaches can also offer valuable insight but again thes e have not be sufficiently refined to account for all the variarables at play and so it is essential that animals are used to model and test the effect of each new vaccine co mposition or storage process.

GM mice allow us to find out if changes in normal protein expression in the body will have a major effect on vaccine function. We have included the option to generate unique monoclonal antibodies (through immunisation and subsequent laboratory work). These may be replaced by new laboratory technologies (phage display) and where at all practical we will use such technologies. We will always check commercial sources and thoroughly investigate the scientific literature for available antibodies to fulfil our plans before using mice for monoclonal or polyclonal antibody production. We have included a provision for polyclonal antibody production in rabbits but will endeavour not to use this provision by extensive review of commercial sources of such reagents.

Reduction

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

Reduction

Statistical techniques have been used to ensure we use only the minimum number of mice for each stage of the experimental procedure. We will use power analysis based all available data to determine the minimum number of experimental animals needed for the first experiments. This may require small pilot experiments with 1 or 2 animals per antigen to establish efficacy followed by only the animals required after power calculations have been adjusted according to the data achieved from the pilot experiment. Where ever possible human tissue or cells will be used to minimise animal use (but as noted above this will be limited to simple questions).

Where possible tissues collected from previous experiments in mice (serum, spleens and lymph nodes etc.) will be used before new animals are used 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

The mouse is a well-established model for experimental evaluation of the immune response, and there is a large body of published data in this species. In respect to the immune system, it is well characterized in the mouse and is highly similar to humans. Although there are differences between human and rodent immune responses, these have only minor effects when studying responses to most antigens/vaccines/delivery technology.

Genetically altered mice for which a specific gene is either deleted (knockouts) or reintroduced (transgenic) are very useful tools for the study of gene function. Use of genetically altered mice with specific genetic alterations believed to affect complement function will allow us to identify and determine the influences of specific proteins in normal immune responses in both the young and aged.

Minimum severity: Protocols to be carried out adhere to “best practice” and current guidelines to cause minimum distress to the animals used. All protocols allow for appropriate supportive care in consultation with experienced technical and veterinary staff. Mice will be group housed and will be provided with cage enrichments (shelters and nesting material).

Production of polyclonal antibodies in rabbits is routine and provides essential tools to fully understand complement/immune protein expression and function. Extensive searchers for commercial sources of polyclonal antibodies will always be carried out before the decisions to generate such reagents is taken. They are included with this

project as a backup or insurance policy against the risk that reagents cannot be acquired by any other means.

PROJECT 220

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.

Word limit; 1000 words

Project Title	Mechanisms of memory destabilisation to facilitate reconsolidation impairment
Key Words	Memory, Fear, Drug addiction, Reconsolidation;
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic r 219 esearch;
	(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):

The retrieval of a memory can lead to the opportunity for it to be disrupted or updated to reduce its impact upon behaviour. In this project, we aim to explore whether disruption of a retrieved memory can effectively reduce the expression of fearful behaviour and reward-seeking behaviour. We will also aim to determine the mechanisms by which retrieval leads to this opportunity for memory impairment, opening the possibility to enhancing the disruptive potential

What 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 of impairing fearful and reward-seeking behaviours has implications in two areas: First, we are studying fundamental processes in long-term memory, and the project outcomes will increase our understanding of these processes. More widely, the ability to reduce memory expression with experimental treatment has potential benefits in the treatment of conditions such as posttraumatic stress disorder, drug addiction and compulsive food seeking. If fear responses and reward-seeking behaviour can be shown to be reduced in experimental animal models of PTSD and drug addiction, the same intervention may be pursued beneficially in the clinic.

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

We plan to use rats. Our preliminary experiments have been conducted in rats. We anticipate using up to 6000 rats over the course of 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?

The majority of the experiments will be purely behavioural in nature. These will involve initial memory training, followed by retrieval and drug and/or behavioural intervention. The behavioural study will culminate in a single memory test, or a series of tests. The fear/trauma element of the project will involve exposure to mild electric footshocks, which cause transient pain. The drug-seeking experiments require the implantation of intravenous lines, through which rats can self-administer the drug. For some experiments, we will target the brain mechanisms of the process, which will involve drug administration, either as a systemic injection or by infusions directly into the brain via a cannula previously implanted under anaesthesia. We will also use genetically-engineered viruses to insert modified molecules into the nerve cells of rats so that brain activity can be modulated acutely. The expected level of severity is moderate for the footshock experiments and those involving surgical treatment. Some experiments involve the seeking of food reward and no surgical preparation. These experiments are expected to be mild in severity. At the end of the experiments, the rats and mice will normally be humanely killed. Should we need to analyse brain tissue post-mortem, this may require preparation of the brain under terminal anaesthesia followed by humane killing.

Application of the 3Rs

Replacement

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

Replacement

The key measurement in the project is behavioural memory performance, which cannot be modelled or replicated in non-animal alternatives. Moreover, given that the outcomes of the project are anticipated to have translational relevance to human memory, non-protected animal alternatives are not of sufficient relevance to human neuroanatomy, neurophysiology and behaviour.

There is the possibility that computational models of the brain will progress sufficiently to be able to mimic real, variable, behaviour. Such developments will be monitored on an ongoing basis to evaluate if and when progress is sufficient to address the project objectives without the use of animals.

Reduction

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

Reduction

We will design experiments carefully, using appropriate group sizes and control conditions in order to draw valid conclusions from our data. In order to minimise variability in behavioural performance, we will control environmental and experimenter conditions carefully (e.g. testing at the same time of day).

We ensure the minimum numbers of animals are used by using good experimental design, seeking appropriate statistical advice and we 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

Rodents are sufficiently similar to humans in their biology and behaviour that the outcomes of the project will have relevance to the understanding of human memory processes.

Declarative memory testing causes no lasting harm or suffering, and we will use the minimum intensity of footshock to motivate robust behavioural outcomes. We will continue to use best practice in surgical procedures and will use the earliest age at which memory decline is robustly observable in order to minimise age-related health welfare costs

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.

PROJECT 221

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.

Word limit; 1000 words

Project Title	Chemistry and Biology of Novel Bone Graft Substitute Materials
Key Words	Osteoporosis, Bone graft substitute, Ovariectomy
Expected duration of the project	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;
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 main objective of this study is to use a rat model to study the effectiveness of a new bone graft substitute (BGS). BGS are natural or man-made materials that are used to replace bone in patients with diseased or damaged bone. The project license will focus on osteoporosis, a disease that is characterised by bone loss in women due to oestrogen deficiency following the menopause. The bone loss that is seen in osteoporosis substantially increases the risk of fracture in these women. Using a rat model of bone loss following surgical removal of the ovaries (ovariectomy), we will determine whether new BGS materials can be used to replace the bone that is lost in osteoporosis.

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

The data from this study will support an application for regulatory approval for human clinical trials of a new bone graft substitute for use in women with bone loss and skeletal fragility caused by post-menopausal osteoporosis. Although the BGS materials to be tested have been shown to be effective in patients with normal bone, their effectiveness when used in diseased bone is untested. One of the key questions that regulators will need to see answered is how the material behaves in osteoporotic bone. It would be extremely difficult to secure ethics approval to undertake a clinical trial to inject AGN1 (or other BGS) in patients with osteoporosis because the injection procedure in humans will require a surgical procedure; without some evidence of efficacy in osteoporotic bone, it is very unlikely that ethical review board approval could be secured. This rat study will form the foundation of the preclinical data that will be needed to confirm efficacy in osteoporotic bone, paving the way for subsequent human clinical trials. Additionally, since most women with

osteoporosis are now treated with drugs called bisphosphonates that partially block bone loss, it is important that our rat work includes a comparison between the effects of the BGS materials in untreated osteoporotic bone and in bone that has been treated with bisphosphonates.

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

The first study under this license will evaluate a calcium sulphate bone graft material, and this work will require 108 skeletally mature female rats over the next two years. Additional studies, using other bone graft substitutes alone or in combination with cells, are anticipated in the future. Some of these experiments may focus on combining bone-forming cells with the BGS materials; others will make use of stem cells that will be collected from the patient, grown in the laboratory (to increase the number of cells available for transplantation) and then mixed with BGS and implanted in the bone defect in the rat. These additional studies are under planning and can only be initiated when additional grant funding has been secured. Each subsequent study on a BGS material is expected to require approximately 100 rats and we anticipate testing at least three additional variants of BGS (or GBS plus cells) during the 5-year lifetime of this project license. In total, we would expect to use a total of up to 400 rats under 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?

This study will use surgically prepared female rats that have undergone surgical removal of both ovaries (ovariectomy, or OVX), performed by the commercial supplier of the animals. 4 to 6 weeks later, when animals have fully recovered from surgery, the rats will be delivered to our institution, where they will begin weekly treatment with subcutaneous doses of either alendronate (a drug used to treat osteoporosis in women) or a placebo (saline). 12 weeks after OVX, when the bone loss has been fully established, rats will undergo surgery to create drill hole defects in the bottom end of the left and right femurs. The defects will be filled with the new BGS material and the animals followed for times ranging from 2 hours up to 6 weeks. At each time point, groups of animals from each treatment group will be killed and tissue samples collected for analysis of (a) the chemical changes in the BGS over time, and (b) the bone response to the BGS over time. In some animals, bone markers (fluorescent labels that bind to bone, providing a way of quantifying new bone formation in the living animal) will be injected into the animal in order to allow quantitative assessment of bone turnover through histological evaluation of bone specimens collected at the time of euthanasia. These labels are well tolerated by animals and will allow us to determine how much new bone forms in and around the BGS materials. The most significant potential adverse effects are expected to be post-operative pain, lameness and an increased risk of fracture due to the creation of bone defects. Post-operative pain and lameness would be considered moderate

severity so drugs that will provide pain relief will be administered. Fractures are a potential complication of any surgery involving bone, but the risk will be minimised through the use of careful technique. If a fracture occurs during surgery when the animal is anaesthetised, the animal will be killed without being allowed to recover consciousness.

Application of the 3Rs

Replacement

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

Replacement

We make use of non-animal alternatives wherever possible, including cell culture models for studying the effects of bone graft substitutes on isolated bone cells, as well as for evaluating the effects of simulated body fluids on the chemistry of the biomaterial. However, none of these laboratory systems recapitulates the interactions between the implant, bone, bone marrow and immune system that develop in the living animal.

Reduction

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

Reduction

This is a pilot study to determine the time course of changes in implant chemistry and bone microstructure following implantation of bone graft substitute materials. Sample numbers are based on best available data from the literature. Within each experiment, we maximise data collection from individual animals by using serial non-invasive imaging and blood tests, allowing us to obtain a number of data readings from the same animal rather than using one animal per measurement, thus reducing the number of animals 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

The rat is recommended by the Food and Drug Administration, the governmental agency that regulates the approval of new drugs and treatments in people in the United States as the preferred preclinical model for studying therapies for osteoporosis. The focus of this study is to better define early changes in implant

chemistry and relate them to the bone response around the implant. The surgery involves the lower part of the thigh bone and from previous work in rats we expect that animals will tolerate the procedure well. Pain relief will be provided to all animals during and after surgery and thus any pain effects are minimised as far as possible. The study is also limited to 6 weeks since this is the time period during which we anticipate seeing the most scientifically informative changes in chemistry and biology in and around the implanted material.

PROJECT 222

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.

Word limit; 1000 words

Project Title	Models of skin disease
Key Words	Dermatology, genetic disease, epidermis, skin
Expected duration of the project	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;
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):

This project is aimed at the development of therapies for a group of highly debilitating inherited skin disorders that affect some tens of thousands of people in the UK alone and that are currently untreatable and incurable. We have developed two broad types of experimental medicines aimed at these diseases. One of these is a type of molecule that can “switch off’ the defective gene to treat one group of conditions. Another type of drug is aimed at reactivating “silent” genes in a different group of genetic conditions. Through the work we have done over the last decade in this area, we are now close to planning clinical trials for these devastating skin conditions.

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

This project is likely to lead to development of new personalised medicines aimed at treating incurable human skin diseases that cause lifelong pain and suffering. Worldwide, these conditions affect many tens of thousands or low hundreds of thousands of people.

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

We expect to use a few thousand genetically modified mice over the course of the 5 year project.

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

We will use these animals in two ways. Firstly, we have introduced human disease-causing mutations into the corresponding mouse genes to produce mouse models of human skin disorders under study. These genetic modifications have been designed to limit the area of the skin affected in the animals to small regions, such as the footpads. This greatly limits the effects of the mutation and so the severity is mild/moderate. Secondly, we have introduced bioluminescent genes into the mouse genome in such a way that small regions of the skin, typically the footpad epidermis, emit light. These genetic modifications are completely harmless. These animals allow us to test the skin delivery, efficiency and safety of therapies aimed at silencing or activating genes in the skin using live animal imaging. Live animal imaging greatly reduces the numbers of animals required for this type of therapy testing (typically by a factor of ten).

Application of the 3Rs

Replacement

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

Replacement

The tissue we study, the epidermis, has many cell layers and in cultured cells, we cannot fully model these layers, which only form properly in the context of living animal skin. In particular, the delivery of therapy molecules must be tested using intact fully functional skin. More than two thirds of my research is acheived through the use of *in vitro* or *ex vivo* systems such as primary cells, established cell lines as well as whole skin explants and biopsies obtained either from human donors or post-mortem material from other species such as pigs.

Reduction

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

Reduction

We routinely carry out power calculations to determine the minimal numbers of animals required to obtain statistically robust 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

REDACTED Specifically, we make use of gene promoters that limit the affected area of the skin to the footpad epidermis (as opposed to affecting all of the mouse's skin). This leads to very mild effects.

Furthermore, we also make use of bioluminescent (light-emitting) genes in the skin of genetically modified animals, that have no harmful effects but that can be used as a surrogate for the disease-causing genes we study. Use of these animals, in conjunction with live-animal imaging techniques, greatly reduces the numbers of mice required to study delivery and effectiveness of new therapies, particularly when studying the effects of therapies over time.

PROJECT 223

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.

Word limit; 1000 words

Project Title	Specialist Aging, Support and Supply Service for Previously Approved Neuroscience Projects
Key Words	Neuroscience, Support, Service
Expected duration of the project	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):

To assist in the provision of aged rodents for the discovery of new neurological medicines, and, where medications are already available, to provide more effective treatments with less side 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)?

This project will provide naturally aged animals as models for neuroscience research including research into disorders associated with aging. This project will assist projects in the sourcing Genetically Altered Animals with either spontaneously occurring phenotypes, or experimentally induced phenotypes which are relevant to aging conditions.

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

If every protocol on this licence were used to its maximum, then this project will use up to 17,100 rats and mice. A more realistic figure would be 4000 animals, but this depends on the demands of the customer as they receive results of ongoing animal studies and clinical trials which may influence their demands accordingly in the future.

In the context of what you propose to do to 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 allowed to age naturally and the effects will be those of a naturally aged animal. At the end of the study, the animals will either be humanely

killed, and their tissues harvested, or they will be supplied to other projects where they take part in studies to look at the effect of ageing on neurological systems.

Application of the 3Rs

Replacement

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

Replacement

Naturally aged tissues come from animals that have been through the ageing process and provide samples that cannot be produced in a laboratory.

Genetically Altered Animals will also help us obtain this tissue, and understand the effect of the presence and activity that a gene may have in the ageing process.

None of this would be measurable in non-animal alternatives.

Reduction

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

Reduction

Studies are designed using the advice of an experienced statistician, to ensure that the number of animals used is kept to a minimum, while providing the data outcome required.

By ageing animals in a barriered environment, and managing their access to feed, the number of animals are kept to a minimum as more will survive into old age allowing the study of ageing.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 used in the Project will go on to support other Neuroscience Projects. In these projects rats and mice may be easily trained to perform behavioural tasks which can be used to look at the effects of ageing. The animal's performance can easily be recorded, making them good models for this work.

The animal’s access to feed will be controlled ensuring they are less prone to being over-weight, and reducing the incidence of tumours, circulatory dis-orders and cases of arthritic diseases.

Whenever possible animals will be kept in social groups, unless they prove incompatible, or social housing would null the value of the experiment. Animals will be provided with environmental enrichment and nesting material to provide more stimulation in their environment.

Genetically altered animals can be used to study the effects of a single chemical in the body, and its relevance in ageing diseases, or interactions with the medications being developed.

Each animal will have its own lifetime history, which will be supplied to the scientist for future reference in their experiments.

Animals will be aged in a facility specialising in the care of aging rodents, by Technicians who are specialised or can specialise in their specific care as they age.

PROJECT 224

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.

Word limit; 1000 words

Project Title	Safety Evaluation of Industrial and Agricultural Chemicals
Key Words	
Expected duration of the project	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:
Yes	(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.

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 primary purpose of this licence is to establish toxicological and safety data in animals following exposure to industrial, agricultural or veterinary products that Man may be exposed to. The studies performed will ensure compliance with regulatory requirements and support successful market authorisation.

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

Chemicals play an important role in daily life and their use is increasing. It is therefore, essential that their safety for Man, other animals and the environment is carefully assessed. By establishing sufficient toxicological and other safety data in animals, safe handling precautions may be determined thus protecting the health and welfare of hundreds (e.g. for a site limited industrial chemical intermediate with limited potential for human exposure) to millions (e.g. industrial or agricultural with worldwide market) of humans and animal species which may contact the materials concerned and facilitate the worldwide marketing and safe use of products. The projects performed under this licence provides safety data to facilitate sound regulatory decisions worldwide that protect the public and the environment from possible hazards. The regulated products have the potential to improve and enhance the health, well-being and quality of life of people and animals. For example, improved crop protection increases food security, while development of safer chemicals and chemicals with reduced environmental impact is clearly beneficial to humans and animal health and in environmental protection. The project undertaken use methodologies that are well established and known to produce accurate and reliable results that can be used in regulatory risk assessment. Furthermore, the studies can rapidly identify any overt toxicity which would cease the development of

the test item and therefore, enable the Sponsor to make a decision at the earliest opportunity to cease production: reducing the risk of possible human exposure and avoiding unnecessary expenditure and use of resources. The work performed under this licence will be undertaken in a GLP compliant laboratory thereby, ensuring data integrity and accuracy.

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

The species selected is considered on a study-by-study basis, but is typically for reasons of regulatory requirement and/or pharmacokinetic and species sensitivity to the test material. The majority of studies performed will use rodents; primarily the rat, but the mouse may also be used. There will be occasions when studies need to be performed in a non-rodent species and on such occasions the rabbit or minipig will be considered. There will be occasions where the rodent, rabbit and minipig are not suitable for generating the safety data required and it is on these occasions that the beagle dog will be considered. In compliance with legislation, the dog will only be used when species of a lower sensitivity would be unsuitable. It is estimated that up to 17000 rats, 10000 mice, 2000 rabbits, 900 minipigs and 900 dogs may be used during the five years this licence is active.

In the context of what you propose to do to 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 performed under the authority of this licence will cause transient discomfort only to the animals. The procedures include administering the test material to animals using a route which replicates the expected route of exposure; typically dermal application and oral gavage (administered in the animals diet of given by intubation). Blood and urine samples will be taken at predefined intervals during the course of the study. There will be times when reactions to treatment are observed. In the majority of occasions these reaction will be mild – a slight reduction in food intake leading to minimal weight loss or a reduction in weight gain, to moderate – a greater weight loss which will be monitored closely. Other reactions considered to be of moderate severity include pale extremities, subdued behaviour and hunched posture. The objectives of all studies performed do not have death as an objective or endpoint. All animal on test will be closely monitored by experienced Animal Technologists and any animal giving concern with regards to it clinical health will be appropriately documented and reported. The frequency of observations will be increased if considered necessary in order that the affected animals do not suffer unduly. Twenty four hour veterinary cover is available at all times. In almost all cases the animals will be euthanised humanely at the end of the study and undergo a post mortem. The tissues are viewed microscopically and changes in cell morphology and other toxicological findings can be assessed at this stage. In a few cases, a post mortem at the end of a study is not required and providing that the animal is proven to be in good clinical condition (dictated by a detailed inspection by a veterinary

surgeon), these animals may be considered suitable for use on another study. By re-using animals in this way we can reduce the overall numbers of animals used in research.

Application of the 3Rs

Replacement

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

Replacement

The way in which a material interacts with, or is metabolised and distributed by a living mammalian body has a significant effect on its potential toxicity. Unfortunately at this time, effects on complex interacting biological systems such as the cardiovascular and reproductive systems cannot yet be replicated by the sole use of cells, computers and other research methods that do not involve the use of animals, and as a result, the use of animals remains a necessity.

However, the use of in-vitro techniques still remains an important part of safety assessment and whilst they cannot completely replace animal use, they do have a significant part to play in the safety evaluation of chemicals. Alternative methods such as the *in-vitro* techniques discussed above will be used as much as practicable to supplement the work involving protected animals which are a legal requirement of worldwide regulatory authorities.

In essence, animals will only be used where there are no validated in-vitro alternatives available to us.

Reduction

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

Reduction

All studies performed will use the minimum number of animals necessary to achieve their objectives, recognising the fact that reduction is not achieved by using too few animals if the objectives are not realised as a consequence.

The majority of studies will be performed in compliance with internationally accepted guidelines. These guidelines provide the number of animals necessary to generate statistically viable data.

It is a regulatory requirement that the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; Core study designs are based on international guidelines where these exist and in most case the OECD guidelines and other similar regulatory guidelines

specify the group sizes required. Otherwise reference is made to internal guidance on study designs to provide the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. Project specific variations are used as required. The core study designs have been used extensively under previous project licences and in other establishments and we have a track record of successful submissions and ability to eliminate unsuitable compounds. They are generally in line with those used throughout the industry.

Refinement

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

Refinement

Animals will be housed in conditions compliant with the Home Office Code of Practice.

Legislation requires that the animals subjected to regulated procedures be of the lowest neurophysiological sensitivity practicable of achieving the objectives of the study. Scientific considerations should also dictate the choice of species for toxicity tests; in particular the species sensitivity and metabolism and the availability of background data. In the majority of cases the rat will be the species of choice, but there will be occasions when the mouse, rabbit or minipig will be more suitable. When legislation dictates testing in a non-rodent species and the pig and rabbit have been confirmed as not to being suitable then the beagle dog will be considered

Range finding studies using minimum number of animals (approximately 60) are first used to establish dose levels that are suitable for use on longer term regulatory toxicity studies. By adopting this approach we are able to screen out unsuitable dose levels thereby, preventing a larger number of animals from potentially being exposed to unnecessarily high dose levels.

The procedures performed e.g. the administration of test materials and the taking of blood and urine samples will be fully validated techniques and will cause minimal discomfort only to the animals. We will however, continue to seek refinement of our procedures in an attempt to reduce any associated discomfort further.

Response to observed effects will depend on the objective of the study. Trained animal care staff will inspect the health of our animals and will take remedial action to avoid any unnecessary suffering. In some cases this may include cessation of dosing or removing animals from study.

Animals will be provided with an environment suitable for the species and their and their physiological and psychological needs. Environmental enrichment will be

included in their cage/pen which allows play and natural behavioural activities like gnawing and foraging. In the majority of instances, animals will be housed in groups of the same sex and treatment. Animals will only be housed individually when it is a scientific necessity e.g. the route of test item exposure is dermal and ingestion from cage mates should be avoided, or in times of animal welfare.

The well-being of animals is of paramount importance. Steps will always be taken to minimise any pain or suffering caused by the performance of procedures and treatments given. Preliminary studies will first be performed using few animals to establish suitable dose levels before performing main studies involving larger groups of animals. On occasions, and when it is felt to be in the best interest of the animals, habituation to procedures will be performed.

Twenty-four hour veterinary cover is available seven days each week, with Named Animal Welfare Officers with vast experience of laboratory animal care in place to ensure that animals are cared for in the correct manner and that advice is available whenever needed.

PROJECT 225

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.

Word limit; 1000 words

Project Title	Mental health disorders: mechanisms and treatments
Key Words	Mental illness, animal modelling, behaviour
Expected duration of the project	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;
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):

To better understand mental illness and the potential routes available to alleviate both the range and severity of symptoms associated with these mental illnesses

What 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 appreciation of the cause and outcomes of mental disorders and the potential development of pharmaceuticals to address these outcomes.

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

Mice and rats, ~2000-3000 mice over a 5 year period ~200-300 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?

We expect to see reduced cognitive functionality in animals and or changes in a standard behavioural profile that may be corrected by pharmaceutical intervention. We expect moderate adverse effects across the animal's research period. All animals are to be culled at the end of the experimental period

Application of the 3Rs

Replacement

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

Replacement

The complex nature of the cognitive deficits investigated cannot at the present time be adequately modelled in anything other than an animal model with rats and mice as lowest vertebrate groups that can be used to produce the neurobiological and behavioural deficits of schizophrenia and other mental health disorders.

Reduction

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

Reduction

By using modern statistical analysis to extract the maximum level of information from each individual experiment, multiple levels of behavioural testing for individual groups of animals to maximise information 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

Mice and rats are translationally close to human cognitive function and represent the best model of genetic alteration identified in association with human mental illness.

High levels of adverse welfare costs can adversely alter the results from these animals and as such each step of the experimental process is examined with an air to minimising welfare costs, frequent communication with both vetinary care and home office liaison will be sought for additional advice where an obvious route to minimal welfare costs is not apparent.

PROJECT 226

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.

Word limit; 1000 words

Project Title	Lymphocyte Development and Activation
Key Words	Immunity, Lymphocytes, Cancer, Vaccines, Transgenic
Expected duration of the project	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):

The immune system has evolved to provide an adaptable defence against infectious disease. Vaccines boost immunity against diseases without the need to be infected, but the development of new vaccines is proving challenging because we do not yet fully understand the basis for what makes a successful vaccine. Moreover, successful vaccination at different ages is likely to require different approaches because the immune system changes as we age. It is now clear that as we age we become more susceptible to autoimmune and inflammatory diseases. This reflects a loss of the ability of the immune system to self-regulate. Remarkably, the cells and genes involved in controlling autoimmunity appear to overlap with those that inhibit anti-tumour immunity. Despite this insight understanding the cellular and molecular basis of immune cell development and function is a work in progress. Our studies using mouse models of immune cell development and responses will enhance our understanding of the cell types involved and their interactions with each other for normal and abnormal immunity. They will shed light on how the immune system becomes less functional as we age. At the molecular level they will contribute to an understanding of how cells signal and use those signals to bring about changes in gene expression and cell fate.

What 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 of fundamental biological interest to understand how the cells of the immune system develop and function. This requires knowledge of the cells, their origins and molecular components as well as a clear view of how they interact with each other. The outputs from this project identify new pathways and interactions that can

manipulate the immune system to control immunity, e.g. for vaccination, or anti-tumour responses,or limitation of inflammatory and autoimmune disease.

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

About 88,000 mice will be used over a five-year period. The mice that we use will be genetically altered to allow tissue-specific control of genes that we hypothesise are important for development or function of the immune system. The majority of mice that we will use will be on inbred genetic backgrounds maintained in a specific pathogen free facility.

In the context of what you propose to do to 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 be genetically altered and will be killed humanely and their tissues used for experiments in the lab. After development of a robust hypothesis, some mice will be subjected to immunological challenge e.g. by vaccines, microorganisms (e.g. influenza virus, bacteria) or tumours or allowed to develop autoimmunity. These exposures will elicit immune responses and the mice will develop symptoms of disease. Exposures will be calibrated to elicit measurable and informative responses but to minimise pain, suffering, distress or lasting harm. Humane endpoints have been carefully considered and are continually revised 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 remain necessary because our proposed studies are to investigate the systemic properties of immunity and/or cell types that cannot yet be generated in vitro. Features such as the distribution of lymphoid organs throughout the body and the intrinsic properties of lymphocyte recirculation to tissues such as the lung make investigations in the whole animal context essential.

Reduction

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

Reduction

We will implement methodological innovations that reduce the number of animals used. this will include methods for the generation of GM mice as well as breeding

and management strategies. We will seek to improve experimental design with careful consideration for the least number of animals required to test a hypothesis.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 limited by the fact that in evolutionary terms adaptive immunity is largely a vertebrate invention and ‘less sentient’ species are not informative. Importantly, many cellular and molecular mechanisms remain conserved between mouse and man. The use of tissue-specific and temporal regulation of gene expression in genetically modified animals allows the testing of mechanism in a way that cannot be achieved with any other approach. The use of infections will test features such as the distribution of pathogen and immune cells throughout the body.

A small number of mutant mice will experience severe consequences. Given the impact on the well-being of the animals, studies of this type are classified as severe and the numbers of mice involved are carefully limited. Careful consideration of what measurements are required for meaningful results and review of experimental results and endpoints allow ongoing refinements.

Animals will be housed in social groups when possible with the provision of environmental enrichment. Experienced and highly skilled staff will perform the experimental proceedures and provide care for the animals.

PROJECT 227

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Study of vertebrate skeletal myogenesis
Key Words	Skeletal Muscle, Regeneration, Stem cell, Muscular Dystrophy, Cell signalling
Expected duration of the project	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):

Regeneration of skeletal muscles following injury or in patients with muscular dystrophies relies on the activity of muscle-specific stem cells called satellite cells. Satellite cells need to operate within a special cellular environment that provides support and regulates satellite cell activity. Previous work, including from our lab, has identified that communication between cells, the external network of specialised proteins, and also cellular structures cooperate to control satellite cells and muscle regeneration. However, much remain to be done in order to decipher the exact nature of the function these players have in muscle regeneration. This project focuses on these particular issues, and uses a combination of in vitro cell culture and ex-vivo approaches, as well as in vivo studies on genetically-modified mice and mouse models of muscular dystrophy, to unravel the mechanisms underlying the action of these players in muscle regeneration.

What 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 recent advances, the natural process by which our muscles repair remains partially understood. Our project will provide additional knowledge of the mechanisms controlling satellite cell activity and muscle regeneration. It is likely that a better understanding of satellite cell function would benefit research and clinical studies in muscle aging (sarcopenia) and in muscular dystrophies.

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

We will use approximately 6000 mice for 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?

All procedures described in this project do not exceed ‘moderate’ severity limit, with 80% of mice subject to procedures that do not exceed ‘mild’ severity limit. All experiments proposed are well-established protocols and will be carried out by trained individuals. Animals will be closely monitored, and culled by a Schedule 1 method at the end of the protocol.

Application of the 3Rs

Replacement

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

Replacement

Although we already use alternative methodologies to investigate satellite cells (such as primary cell cultures and muscle fibre ex-vivo cultures), these methods are currently not capable of reproducing faithfully the normal environment of satellite cells in vivo. For this reason, we use also animal models to investigate how the environment affects satellite cell activity and muscle regeneration. The mouse is the animal of choice because it is closely related to humans from the evolutionary point of view making translation of our findings to humans more relevant. The mouse also provides a large choice of lines already established to monitor and target specifically satellite cells.

Reduction

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

Reduction

REDACTED For instance, where possible animals are bred as homozygous to minimise the number of mice generated with an irrelevant genotype. Power calculations and statistical analyses have allowed us to determine precisely the number of animals required for a specific analysis. Finally, we also operate a policy of sharing animals whenever possible with other scientists to maximise the output 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

We use the mouse as animal model because the process of muscle regeneration in the mouse resembles that in humans, a large number of transgenic and knock-out lines are available for the study of skeletal muscles, and animal models of muscular dystrophy and reagents for studying satellite cells (antibodies) are also available.

Good animal husbandry practice, and close monitoring and the use of analgesics are in place to minimise distress and pain. Although all protocols used are well established, we endeavour to review and refine them regularly.

PROJECT 228

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.

Word limit; 1000 words

Project Title	Analysing the genetic basis of ADHD, autism and aggression
Key Words	Zebrafish, ADHD, Autism, Aggression, Behaviour
Expected duration of the project	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;
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 aim of this research is to study genes that are connected to ADHD, autism and aggression in human patients. We create zebrafish lines that lack the function of genes linked to these diseases and then measure their behaviour focussing on aggression, hyperactivity and sociality. We will examine the alterations to brain function that lead to these behavioural changes. We will also identify novel drugs that can reduce aggression as a first step towards improving treatments for human patients who express high aggression levels. This research will enable us to better understand the basic biology of ADHD, aggression and 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)?

This research has the potential to benefit human patients who suffer from ADHD or autism. We will identify brain areas that are important for these disease. We can use our zebrafish models to identify biomarkers permitting faster diagnosis of these diseases. We can also screen for novel drug treatments for ADHD and autism, by testing chemical compounds that can rescue the behavioural changes in our zebrafish lines.

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

This research will be carried out using zebrafish. We will create groups of fish that lack the function of ADHD or ASD-linked genes. We expect to use about 33,888 zebrafish 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?

During this project we will maintain breeding stocks of zebrafish as well as measuring behaviour at both larval (6 day old) and adult (3 month) stages. Breeding and measuring both adult and larval behaviour are mild procedures. Adult fish will be killed using a Schedule 1 procedure at the end of the project. Larval fish will be killed by an overdose of anaesthetic followed by immersion in a suitable fixative. The possible adverse effects of this research include a toxicity effect of drug application. We will reduce these adverse effects by conducting a toxicity test before applying drugs to many animals. A single larval fish will be immersed in the highest concentration of drug for one hour and it behaviour monitored. Any sign of distress, such as loss of balance of decreased locomotion will cause the experiment to be stopped and a lower concentration of drug used. This should decrease the potential of harm to large numbers of zebrafish. In some cases, we will inject drugs into adult zebrafish intraperitoneally. We will reduce the potential harm caused by injected by treating fish with anaesthetic. We will then monitor their recovery from injection by placing them into a separate tank and looking at their behaviour (gill movements, swimming ability) every ten minutes during a thirty minute period.

Application of the 3Rs

Replacement

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

Replacement

The research described in this proposal uses behavioural analyses of zebrafish to examine genes that cause attention-deficit/hyperactivity disorder, autism spectrum disorder and aggression in humans. Since it is difficult, if not impossible, to study behaviour in cell lines or organ cultures it is necessary to use animals for this research. I have looked at the FRAME website and the NC3Rs website for possible replacement protocols but have not found suitable alternatives.

Reduction

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

Reduction

All of the experiments proposed here are based upon data from our laboratory and other published research. We have designed our experiments using validated experimental design tools such as the Experimental Design Tool (EDA) on the NC3Rs website. This permitted us to choose the number of animals to use in each experiment and select the appropriate statistical analysis. For example, 12 adult

zebrafish are required in each group of a behavioural experiment to a significant difference in aggression, boldness or exploration. 20 larval zebrafish are required in each group of a behavioural experiment to detect a significant difference in locomotion. We will use 6 larval or adult zebrafish to accurately determine where genes are active in brain, since this will permit us to get a reproducible staining pattern.

The majority of research covered by this project license will be based upon genes that have been identified in studies of human patients. This will reduce the number of animals used in this research since the genes have already been identified before starting this research.

For the drug screen we will calculate the correct concentration of drug to be administered to animals based upon previous work, pilot experiments, online resources and manufacturer's recommendations.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 have chosen to use zebrafish for this research because they are easy to maintain in the laboratory and we have already established many techniques to measure behaviour and assess brain function. We will house adult zebrafish 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 density in environment-enriched tanks.

We will handle fish as little as possible during this project and we will only use anaesthesia when necessary. We will use pilot experiments to calculate the concentrations of drugs to be used in behavioural experiments. Many of the drugs used in this research have already been approved for use in humans or other model animals. In the case of unexpected adverse effects caused by drug application (e.g. a reduction of larval swimming caused by sedation), we will terminate the experiment by washing off the drug and allowing the fish to recover. The Named Veterinary Surgeon will be contacted for advice before using the drug again.

PROJECT 229

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.

Word limit; 1000 words

Project Title	The effect of periodontal dysbiosis and resolution mediators in pregnant mice
Key Words	gum disease, ligatures, pregnancy, resolution
Expected duration of the project	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):

We aim to describe step-by-step the changes in bacteria and inflammation in pregnant mice with induced gum disease. For this purpose we will create a mouse model of adverse pregnancy outcome as a result of the oral disease. This is when the pregnancy ends up on small offspring, stillbirths or pups unsuitainable to life. This will be later followed by recording the events when a local therapy to control the inflammation in the mouth is used to prevent the systemic effect.

Results from previous animal and human studies, suggested that inhabitant bacteria of the mouth can travel through the bloodstream and cause a pregnancy complication. However the mechanisms of how this happen is not well understood. Normally bacteria originating from the mother's mouth can commonly travel through the bloodstream without causing any trouble. In many cases it can colonise the amniotic fluid in healthy pregnancies. We remain to know when this transfer of pathogens could become a problem and require action.

In this project, we would like to test the hypothesis that the immune reaction occuring as a result of advanced gum disease is the main reason for the consequences occurring in the womb, irrespective of the bacterial transfer. As the bacteria variations occuring in mouse may differ from that in humans, we still need to elucidate which pathogens increase in disease in gum disease in mice and which ones are able to translocate. The first part of this project will be therefore to describe the patters of bacteria in the gums, blood, organs and amniotic cavity in the model.

In the second part of the proposal we will apply a local therapy to the mouth using a molecule that can reverse the inflammation to explore if this could prevent the systemic adverse outcome. This study will support the need to rethink the treatment of advance gum disease particularly with the aim to reduce systemic effects. Particularly it will contribute with an understanding of the processes and to the design of appropriate clinical 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 proposal will serve to elucidate the mechanisms occurring when gum disease affects pregnancy causing complications such as stillbirths, low birth weights, prematurity and illness. This information should provide the foundation to rethink the designs of clinical studies by providing a better understanding of the processes linking the two diseases. It will clarify if the inflammation is the most likely cause of the effect and not necessarily the bacteria as currently believed. It will also provide support to the use of products that helps regulating the inflammation to arrest the disease in the mouth as well as prevent the pregnancy complications.

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

The number of mice expected to be used range between 800-1000. The type of mice that will be used is called C57BL/6J mice, which is the most widely type used as model of human disease and therefore broadly available. Up to 500 mice will be purchased for the study, predominantly females. Offspring that survives past third trimester are also accounted in the total number of animals that we will used in the study to a maximun of 1000. The study will be performed over the next 5 years with different stages of work being implemented during short periods of 3-4 month once or twice 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?

The mice in this study will serve to develop a pregnant model in which we induce gum disease by placement of ligatures around molar teeth. This intervention will cause an inflammatory reaction that will impact a later pregnancy causing adverse outcomes. Placing the ligatures will be performed using general anaesthesia after which animals should recover without complications. In previous work the mice has not shown any changes in behaviour or reduction of eating capabilities due to the ligatures, however they will be closely monitored for the occurrence of any issues such as reduced appetite, extreme loss of weight, change in behaviour or hair loss specially as the inflammation progresses. Pregnancy complications such as offspring born prematurely, low birth weight, reduced number of offspring or pups born with severe illness. Severe illness is not expected in more than 1% of cases. This is because the new technique is meant to induce a low-grade inflammation as oppose

to an acute infection. If an acute infection is observed, the mice will be treated as required by removal of ligatures, pain killers, anti-inflammatories or even antibiotics if required. Animals will be killed at the end of the experiments as a requirement to perform the anatomical, histological and molecular assessment as explained in the programme of work. Pups will be normally also killed within 24 hours of delivery for histological analysis, except for a small number which will be followed for up to 28 days to evaluate early development. These pups will also be subjected to close monitoring to observe for any signs of distress that may require intervention.

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 this project was concluded after exploring the publications that have been done in both clinical samples from humans as well as animals. There is a lack of information that explains how chronic gum disease can impact the outcome of a pregnancy despite evidence of an association have been seen in several studies. The purpose of this project is to explain these processes and to explore two possible options as the main cause: the bacteria translocating to the amniotic fluid and causing the inflammation, or the effect of the systemic inflammation causing the bacterial changes. We are aware of alternative ex-vivo systems to study the effect of gum disease locally, however this won't allowed us to understand the complex systemic effect that occurs distant to the initial infection/inflammation in the mouth. In order to understand these mechanisms in a step-by-step process, we will require a controlled experimental work in animals in a time point study. Understanding of these processes will support the design of clinical studies in the future.

Reduction

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

Reduction

A well planned study will allow us to use the minimal number of animals in the study. Initial experiments will serve to test the new model before moving into the time point experiments. There is also clear explanation of the objectives that are expected at each stage before being able to move to a next one and alternative procedures have also been defined. The sample size has also been calculated based on previous animal studies to ensure there are sufficient numbers to observe the effects of the intervention compared to our control. Also, the staged-process will allow to increase the numbers by including additional cases, particularly in pregnancy observations when outcomes may be more variable than expected.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 strain of mice selected for this study are C57BL6 Black mice which are broadly used to understand human diseases and therefore broadly available. The severity of the interventions is maintained due to the implementation of a more sophisticated technique to mimic a low but constant inflammatory reaction similar to the one occurring in humans. The techniques used will avoid acute infection or inflammatory reaction and should cause less harm to the pregnant mice and offspring.

PROJECT 230

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.

Word limit; 1000 words

Project Title	The role of proteases in neuronal function and dysfunction
Key Words	proteases stress anxiety fear depression
Expected duration of the project	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):

Adaptation of the brain to the surrounding environment is fundamental for animal's (and human) well-being and survival. While exposure to short term, mild stress can be beneficial, long-term exposure to stressful stimuli often damages the brain in a way that results in the development of anxiety and depression. What are the mechanisms the brain uses to protect itself from such a damage? Among other mechanisms, the brain produces proteases (proteins that cut other proteins) to help the neural tissue to adapt, and in this way minimize the likelihood of developing psychiatric disorders. However**,** our understanding of how proteases work to control anxiety and depression is still inadequate. First, it is not known what brain regions and cell types are regulated by specific proteases. Second, it is not known how neuronal cells use proteases to communicate with each other to ensure adequate expression of emotions. This project aims to fill these gaps by methodically investigating the role of proteases and proteins they interact with in the development of anxiety and depression.

Objective 1: To determine which brain functional units (regions, cells, projections), related to anxiety, fear or depression, are regulated by proteases

Objective 2. To determine how protease-regulated brain functional units (regions, cells, projections) interact with each other to control anxiety, fear or depression-like states.

Objective 3. To determine the role and the mechanism of action of proteases and their molecular partners in neuronal signalling relevant to anxiety, fear or depression.

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

Anxiety disorders are the most common psychiatric diseases, that affect up to ~25% of the population. They often co-exist with depression and drug abuse, in combination representing the third leading cause of disease. Anxiety and depression affect 180 million people worldwide, leaving more than 120 million disabled. Current predictions suggest that the negative impact (economic and social) of anxiety and depression will increase further. Despite significant efforts in developing drugs to combat these disorders, the available therapies are often ineffective. The improved understanding of anxiety and depression is therefore an important goal of psychiatry and neuroscience. Our project aims to offer the following benefits: 1. New knowledge generated here will help us to precisely determine which brain areas, cell types and neuronal connections should be targeted in order to combat the above mentioned psychiatric conditions. Thus, achieving this aim will be beneficial to the neuroscience community in the short, medium and long term. Also, in the medium- and long-term the research will be beneficial to the biopharma industry. 2. We aim to develop methodologies to reduce anxiety and will verify the validity of novel therapies for anxiety and depression that could potentially be translated into the clinic (in the long-term). Thus, in addition to immediate benefits for basic research, achieving this aim has a long-term translational potential to guide clinical studies. 3. We aim to provide novel information on the basic mechanisms associated with anxiety and depression and identify novel potential drug targets for the development of new therapies against these disorders. The beneficiaries include the scientific community, but also in the long term the biopharma companies, clinicians and patients, as well as the wider society.

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

Approx. 4100 mice over 5 years. The project will be conducted on wild-type mice and approx. 5-7 genetically modified strains of mice. This represents less than 150 mice per strain a year to be used for biochemical, morphological, electrophysiological and behavioural 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 overarching aim of this project is to decipher neural mechanisms leading to anxiety, fear and depression using mouse models of the above neuropsychiatric states. In particular, the role of proteases and their molecular partners will be investigated using a broad array of methodologies (molecular biology, cell biology, genetics, optogenetics, pharmacology, behavioural studies). This strategy will allow us to decode the role of proteases and protease-related mechanisms at different levels of brain complexity – from molecules to whole animal behaviour. These studies are expected to fall under a moderate severity category. The whole

procedure (maximum experience) may result in stress which may manifest itself as slower than normal weight gain, decreased levels of motor activity, and increased anxiety. Animals will be inspected daily by the Animal Facility staff and, during experiments, will be under constant care of the responsible personal licence holder. Any animal will be humanely killed (Schedule 1 method) if it shows evidence of suffering that is greater than minor and transient (e.g. weight loss >20%, evidence of infection, changes in posture or activity longer than transient) or in any way compromises its expected behavioural repertoire. Animals exhibiting any unexpected harmful phenotypes will be killed (schedule 1), or in the case of individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector. At the end of the studies, the animals will be killed and tissues will be taken to advance the scientific aims 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 study of emotions involve different components such as subjective experience, cognitive processes, expressive behaviour, psychophysiological changes, and instrumental behaviour. The acquisition and expression of emotions requires the presence of highly complex brain structures which can only be studied and understood when whole animals are used. Although invertebrate models (such as Drosophila melanogaster) have been used as a surrogate to study emotions, scientists agree that they only express “emotion primitives” that may be evolutionary antecedents to emotional states in vertebrates. Mice are the lowest vertebrates for which procedures to study emotions have been well-established.

Other alternatives, such as computer simulations, studies in fish or cell lines have been considered but are not compatible with the above stated aims of our study.

Reduction

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

Reduction

All the molecules and genetic constructs we use are first verified in vitro for their utility and efficiency and animal studies are only performed when we are satisfied that the compounds work as desired.

When the changes to be observed are anticipated to be small we will undertake pilot studies and then use this information to carry out statistical power analyses to inform

us of the minimum number of animals that would be required to achieve statistical significance.

When justified by the research aims we will use genetically modified mice to target the activity of proteins in specific cell types in the brain, which will enable precise control of cellular activity. This methodology will reduce the amount of variables within our studies and allow us to reduce further the number of animals used.

Strategies for breeding genetically altered animals will be carefully designed to minimize the amount of breeding necessary to generate the number of animals needed for experiments, thus reducing 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 are the mostly commonly used research model for the study of anxiety and depression; therefore, the vast majority of the existing research literature comes from this model. As such, the mouse as model to study anxiety and depression has already been extensively validated by the scientific community and anxiolytic / antidepressant drugs are often validated in mice in order to be approved for clinical trials. Unlike flies or fish, mice have similar brain circuits of anxiety to humans and therefore the results of these studies may be applicable to psychiatric patients.

Mice are the only mammalian species easily amenable to genetic modification and can be used for identification of critical molecular pathways important for the development of anxiety and depression. Furthermore, the ability to create cell-type specific and drug-inducible genetic modifications in mice allows for further refinement and specificity in the targeting of molecular pathways *in vivo*.

To minimise the welfare cost to all animals undergoing surgical procedures (e.g. stereotaxic injections or implantations) aseptic technique will be used and perioperative analgesia administered.

The methods to induce anxiety (restraint stress, electric foot-shock) are the most refined to produce anxiety without long-lasting harm to the animals.

The behavioural tests to be used in these studies are non-invasive and involve observation of mouse behaviour in various types of arenas and mazes (elevated-plus maze, open field, light-dark box).

PROJECT 231

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.

Word limit; 1000 words

Project Title	Pathophysiology of cardio-renal disease.
Key Words	Cardiovascular, Heart, Kidney, Diabetes
Expected duration of the project	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):

Our research programme aims at understanding the mechanisms of heart and renal disease in animal models with the goal to find new treatments for these devastating diseases that could subsequently be applied to humans.

Specifically, our work will focus on novel factors involved in organ repair in diseases. We plan to alter the levels of these factors in animals and subsequently study their role in normal and disease conditions.

If these factors demonstrate a positive role in treating a disease of the heart or kidney, future work will aim at developing new medicines that could be utilised to treat/cure diseases 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)?

Cardiac and renal diseases are a serious illness and, at times, life threatening. A suboptimal heart/renal function (closely linked together) leads to illness, and ultimately death. Although people can be treated with drug helping heart/renal function the prevention of these devastating diseases is crucial for the well-being of patients. Diabetes and hypertension are the major causes of cardiac/renal disease. Diabetes and hypertension affect approximately 600 million people worldwide. High blood glucose and hypertension activates drive diseases that lead to cardiac/renal damage. In treating cardiac/renal disease and its causes (e.g. diabetes, hypertension), health care professionals are engaged in finding new therapeutic approaches for preventing cardiac and kidney failure. Our studies aim at understanding the mechanisms of cardiac/renal disease, which will help the assessment of new therapies, which hopefully will be of benefit to patients. In our

experiments, we will study specifically the heart and the kidney with novel techniques (e.g. techniques that allow to investigate specific tasks of the heart and the kidney), which will allow us to study their function in physiology and disease.

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

Mice. Approximately 2500 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?

In this project mice may become diabetic and/or develop high blood pressure (hypertension), either by the injection of a substance that inactivates the insulin production in the pancreas, or by diets rich in fat (or combination of the two), or by using mice that have a genetic alteration, which makes them susceptible to developing diabetes/hypertension naturally. Both are well defined and characterised animal models of diabetes/hypertension. Animals will be monitored for progression of disease. This will involve taking small blood and urine samples. Some animals might undergo surgery to implant devices for the measurement of blood pressure. After surgery animals will receive painkillers and will be monitored closely during their recovery. The housing and care of animals will be adjusted when animals become diabetic, for example by providing extra water and bedding to keep them comfortable. In our experiments, we will study specifically the heart and the kidney using non-invasive imaging techniques in anaesthetised animals in which will allow us to study the function of the heart and kidney in health and disease. The presence of diabetes should only cause minimal impairment of heart/renal function and we do not expect any significant impediment from repeated anaesthesia or use of agents utilised to visualise tissues. To study mechanisms involved in blood pressure regulation animals may be exposed to diet with altered (e.g. increased or reduced) salt content, or to brief periods (less than 1 day) with deprivation of water. At the end of the study animals will be humanely killed and tissues 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

Our laboratory has been involved in cardiovascular and renal research for many years and we have been utilising cells grown in laboratories to answer our questions with a closer approximation of human physiology and pathology and to avoid, wherever possible, research on animals. The physiology and pathophysiology of the cardiovascular/renal system is regulated by a myriad of factors that function as

links between different cells especially in complex organs such as the kidney and the heart. We have been favouring research models that, wherever possible, reduce the number of animals used but, to study whole organ physiology we feel that we need to complement our research with experiments in animals.

In our clinical practice, we study human patients with cardiac and renal disease and we are limited to the nature of studies we can perform; patients will often have been receiving treatments for some years and it is rarely possible to study patients before they develop disease or in the early phase of the disease itself. As a result, we need to use animal models of disease that allow us to study the mechanisms of disease from its earliest stages and during disease progression; this will allow us to monitor the effect of specific molecules, in a controlled and standardised setting. Our plan of work clearly reflects the need to complement the laboratory based research which will, where possible, always replace animal use in research.

Reduction

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

Reduction

We established methods to reduce the number of animals studied and enhanced animal well-being. Good planning of experiments and regular interaction with colleagues and statisticians significantly helps towards a reduction in the number of animals utilised in our research. We have set up experimental techniques that maximise the acquisition of experimental information from the same animal without increasing harm. We will continuously refine our techniques and keep the number of animals needed to a minimum to answer our 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

We propose to study mice as these are least sentient animal that can be used to representatively model the human disease conditions and can be used to investigate specific genetic manipulations of relevance to cardiac and renal physiology and pathophysiology of diseases. Any contingent suffering will be minimised by careful planning of experiments. The induction of diabetes may be induced with injection of a substance called streptozotocin. Reports suggest that multiple low-dose injections of streptozotocin induce a delayed but progressively increasing state of hyperglycaemia in mice, which does not completely damage the insulin producing cells of the pancreas and usually does not warrant insulin administration (injections),

nor lead to dramatic weight loss. This “moderate” diabetes allows longer survival of the animal allowing multiple determinations of parameters (e.g. time course for blood sugar levels and blood pressure) which will allow better and solid information to aid answering the scientific questions posed. Tissues and blood is collected under terminal anaesthesia so the animal is unaware of procedures as it is killed at the end of the study and is not allowed to wake up.

PROJECT 232

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.

Word limit; 1000 words

Project Title	INFECTION AND IMMUNITY OF POULTRY VIRUSES
Key Words	avian influenza virus, food security, zoonosis, vaccines, diagnostics
Expected duration of the project	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.

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):

Aims: The major aim of the research is to improve control measures against avian influenza viruses (AIV) infecting poultry. We will investigate underlying mechanisms that define how avian influenza virus infections arise, evolve and spread in poultry populations, how the viruses cross species barriers and how vaccines and diagnostics loses effectiveness. Co-infection in the field of poultry with AIV and other common poultry viruses such as; Marek's Disease virus (MDV), Infectious Bronchitis Virus (IBV), Newcastle Disease Virus (NDV), Chicken Anaemia Virus (CAV), Infectious Bursal Disease Virus (IBDV) and Duck Enteritis Virus (DEV), Avian Metapneumovirus (aMPV), are frequent and can exacerbate morbidity and mortality. Therefore our secondary aim is to understand the relationship between viruses during co-infection as well as improving vaccines so they can target multiple poultry viruses to enable better protection of birds and improved vaccination efficiency for the poultry industry.

Objectives: AIV naturally circulates in birds and continually evolves gaining genetic changes that influence infectivity, pathogenicity, transmission and persistence in different animal species including poultry and humans. This exceptionally rapid evolution of AIV and the interconnectedness of wild bird migratory pathways mean that global populations remain continually vulnerable to sudden changes in virus infectivity, transmissibility, virulence and antigenic diversity. Thus, in order to develop effective control measures, there is need to increase fundamental understanding of viral and host factors that increase virus fitness and pathogenicity in different host species, overcome vaccine effectiveness and species barriers to infect humans.

This research will investigate;

- how viruses evolve to increase pathogenicity, improve spread and virulence in both poultry and humans;
- how viruses escape from vaccine effectiveness
- How advanced technologies can be used to improve vaccines, diagnostics and antiviral therapeutics effectiveness.
- The effect of co-infection of AIV and other poultry viruses

Whether vaccines targeting multiple poultry viruses offers cost-benefit to the poultry industry.

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

The research will generate and deliver knowledge that will directly impact on AIV disease control systems globally. The research will advance the fundamental understanding of influenza evolution, spread and virulence enabling development of more effective control measures including better vaccines and improved and more rapid diagnostics. The specific research outcomes will be • an increased assessment of risks of evolutionary changes on virus pathobiology including infectivity, replication, transmissibility and virulence in both poultry and humans; • an increased understanding of the immunological basis for vaccine failure in poultry; • an availability of improved poultry vaccines that not only protect poultry against AIV but also provide protection against other poultry pathogens such as MDV, NDV, DEV, IBV, IBDV, aMPV and CAV through a single vaccine dose, delivered by mass vaccination methods; • an availability of new antiviral therapeutics which are highly potent against influenza viruses infecting both humans and animals. The availability of better disease control tools will aid in the reduction of poultry production losses and thus be important for global food security and improved animal welfare whilst also reducing possible zoonotic transmission to humans. Thus, the proposed research will provide direct benefits to farming communities as well as substantial indirect economic, public health, environmental and social benefits to wider community at national and global scale.

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

It is estimated that approximately 1900 embryonated eggs, 1900 chicks, 650 ducks, 650 turkeys, quail 650 and 250 mice will be used over the 5 year period.

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

The majority of the expected adverse effects will be classed as mild to moderate. For example the administration of a vaccine to a chicken and the subsequent testing of its effectiveness by infection with a low pathogenicity avian influenza virus could result in some mild local inflammation at the administration site, dependent upon the route, and moderate clinical signs, (red and watering eyes and/ or mild diarrhoea) if

the vaccine is not effective. There will be the occasional protocol that is classed as severe since we will be analysing protection against circulating highly pathogenic influenza viruses as well, which as is indicated by their name can cause morbidity and mortality within 24-48 hours after exposure. We will minimise suffering in such severe protocol experiments by frequent monitoring (every 2 hours in some cases) and euthanise by an appropriate schedule one technique when initial humane end points are observed.

Application of the 3Rs

Replacement

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

Replacement

Where appropriate, cell culture and other relevant in vitro techniques will always be used as the initial methods for assessing the virus infectivity and replication efficiency. To define the pathogenesis and transmission of virus within a specified host it can only generally be studied effectively and qualitatively within that same host in order to provide scientifically sound data. This is particularly important for the groups of viruses being researched here, where biological and antigenic variation is often related to, and dependent on the host of origin. Since the information from such studies contributes greatly to disease definition, host species compatibility is important.

Reduction

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

Reduction

REDACTED Where control groups are required we will perform as many concurrent experiments as is practically and scientifically possible so that the same control groups can be utilised, reducing the number of birds or embryonated eggs required over the programme of work.

Refinement

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

Refinement

This project benefits from the expertise of scientists who have spent many years working with influenza viruses and animal models. We have the experience to

understand what humane end points are appropriate for protocols and how to use the experience of collaborators and the published literature to determine the appropriate end points and timings of these end points. Wherever possible we will carry out maximal observations of welfare for both birds and embryonated embryos and these will be modified in line with the assessed risk for harm. REDACTED

The birds used in this research will be housed in isolators which are designed to protect personnel and the environment from cross contamination. Whilst isolators present inherent challenges, we are committed to providing high standards of animal welfare. REDACTED Birds are social animals and so they are housed in groups to allow for normal social interaction. Where possible, they are also afforded more space than legally required within the Home Office Code of Practice.

Foraging, scratching and pecking are all important behaviours to chickens and so we provide our birds with substrate on the floor to allow foraging and dustbathing and toys to enable them to express their species specific behaviour.

The animal facilities are managed by our Animal Technicians who are experienced specialists in the care of animals. They are all trained in daily animal handling, husbandry.

PROJECT 233

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.

Word limit; 1000 words

Project Title	Utility, metabolism and toxicity of compounds
Key Words	Toxicology, Drug Discovery, Drug Development, Risk assessment
Expected duration of the project	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):

The process of drug discovery and development is a complex, multistage process. Only a small proportion of compounds achieve their therapeutic potential, the large majority failing at the pre-clinical and early clinical stages of development. Many drugs fail because of poor efficacy, frequently due to poor bioavailability, or unacceptable toxicity. There are pressing needs for information on the efficacy and potential hazards of existing and novel molecules. Much of this information can be gathered using existing methodologies; however, novel more sophisticated technologies are required to accelerate these processes and to enhance the quality of data to be used in decision making.

What 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 comprises two closely related aspects. Firstly, the determination of the likely utility of candidate drugs (including novel nanoparticle formulations for drug delivery) and secondly, the investigation of the metabolism and toxicology of chemicals (including environmental and engineered nanoparticles) in order to estimate the risk they pose to human health and safety following occupational or environmental exposure. It is envisaged that the work carried out within this project will: • Assist in the development of new, more efficacious, safer drugs, food additives, pesticides, etc. • Enhance hazard identification and risk assessment • Further the understanding of mechanisms of toxicity and the basis of species differences in response. • Utilise new models that will use fewer, but “more relevant”, animals. • Validate and refine existing or novel methodologies and procedures in order to use fewer animals while maintaining scientific relevance. Data produced under the present licence will contribute also to establish databases that will be used world-

wide to allow the founding of the predictive (or “virtual”) toxicology, whereby a newly synthesized substance will be compared with existing substances to identify its potential safety and health risk before animal and laboratory testing, with the ultimate goal of performing such tests only with the minority of compounds that will pass this initial screening.

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

We expect to use rodents (preferentially mice and rats, but also guinea pigs and hamsters for limited applications) for an approximate total of 35,500 rats, 33,000 mice, 750 guinea pigs, and 750 hamsters, distributed over 7 lines of investigation and over 5 years of duration of the licence.

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

Rodents (preferentially mice and rats) will be exposed to various compounds (pharmacological and non-pharmacological) via various routes of exposure and the adsorption, distribution, metabolism, excretion and potential toxicity (ADMET) of the compound measured in specific target tissues and body fluids. In the studies carried under this licence, the vast majority (more than 95%) of the animals will experience a mild level of severity; in our experience, only a minority (less than 5%) of the animals experience moderate adverse effects; these effects will vary depending on the substance being administered to the animals and the typology of the study, but will mostly be of a transient nature and reverting to a mild discomfort upon withdrawal of the dosed substance(s). Animals will be closely monitored for the duration of the studies, and those approaching a moderate level of severity (assessed in consultation with the named veterinary surgeon) will be humanely terminated if the signs will not be resolved or the animals will not show signs of improvement after a set period of time. Analgesia for surgical procedures will always be administered, unless interfering specifically with the scientific end-points. In all cases, the NVS approval will be sought before the study starts. The animals will be terminated at the end of the studies, as organs and tissues will be routinely collected for further analysis and they will not be re-usable due to the nature of the studies conducted.

Application of the 3Rs

Replacement

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

Replacement

We are highly focused on minimising animal numbers and discomfort, and wherever possible, we aim to and already replace animal studies with *in vitro* studies; however,

due to the complexity of the biological response to chemical substances and drugs, it is not possible to completely avoid animal research, as in many cases the data obtained *in vitro* are not completely comparable with those obtained *in vivo*.

Importantly, wherever possible, during this programme of work *in vitro* systems such as cell culture, enzyme and human tissue assays will be utilised, with the aim to reduce the number of animals used to the absolute minimum required, or even replace them when feasible.

Reduction

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

Reduction

Our studies are conducted using the minimum number of animals that can provide meaningful and useful data; in particular, we always use a number of animals that can allow us to conduct statistical analysis of data, and thus avoid the necessity for repeat 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

We use rats and mice as they are the gold standard in toxicology and have a considerable database in these species that is an invaluable support to our ongoing studies.

We increasingly make use of animals that have been genetically modified to make their drug metabolism pathways more closely akin to those in humans, with the expectation that the predictive value of these studies will continue to rise.

We constantly refine our procedures and methodologies to reduce harm to animals.

PROJECT 234

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.

Word limit; 1000 words

Project Title	VALIDATION OF AN IN VITRO INTRAMUSCULAR INJECTION MODEL
Key Words	INTRAMUSCULAR INJECTION MODEL
Expected duration of the project	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;
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):

We aim to validate an *in vitro* tool that can be used by scientists at pharmaceutical companies to screen early stage formulations designed for administration via intramuscular (IM) injection. The objectives of this programme function is to characterize the IM injection site and to identify formulation parameters affecting drug disposition at the IM injection site that can be modelled *in vitro*.

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

The major benefit of this programme will be to establish a model for the faster development of IM-injectable preparations using fewer animals over a reduced time frame. We will also analyse parameters at the injection site (eg acidity or pressure) to aid in the future formulation of new compounds.

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

Rats; 267 as a maximum.

In the context of what you propose to do to 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 IV injections, the animals are maintained under anaesthesia throughout the whole procedure and then killed and will not feel any pain; this has a low severity rating termed “non-recovery”. For IM injections, the animals are recovered after injection. The compounds are already in use for human patients and the injection volumes are analogous to those used in the clinic and so adverse effects from these are not expected. Any serious effects from the anaesthesia would manifest as a

breathing or heart problem and, should this occur, the experiment would be terminated and the animal euthanized. Injection and bleeding could induce local scarring at the puncture site which has the potential to lead to ulceration. To limit the potential for serious events developing, animals will be observed regularly and, in the unlikely event of a serious complication, the animal will be euthanized. At the end of the experiment all animals will be killed using an approved humane (“Schedule 1”) method. Injection and multiple blood sampling of the same animals gives this protocol a “Moderate” severity rating. .

Application of the 3Rs

Replacement

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

Replacement

We are developing an *in vitro* model that would, hopefully, replace *in vivo* animal models for the screening of biopharmaceutical formulations designed for IM injection. Unfortunately, validation of this *in vitro* model as a replacement for *in vivo* testing will require demonstration of its predictive value in an *in vivo* setting. The aim of the studies proposed in this application will be to obtain data that demonstrate this predictive capability. Thus, we must use animals to obtain this information.

Reduction

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

Reduction

We will use quality by design (QbD) methods to minimize the number of animals used. This approach uses a matrix of parameters that might have effects on IM injection outcomes. By examining a selected group of parameter combinations within the matrix, we will be able to exclude entire regions of the matrix possibilities that translates to fewer animals being used.

We have independant statistical advice to ensure that our experimental design is robust and meaningful data can be obtianed from our experiments. This means we keep animal use to the minumum required for effective scientifc output. We also 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

Rats are the smallest species that can be used for IM injection volumes required for these studies. Animals will be housed together to provide these social animals with other animals. We will provide food and water ad lib throughout all studies. The majority of the studies will be performed as non-recovery procedures to minimize the possibility of pain and suffering. All studies involving long term assessments following IM injection will be performed using drug formulations previously tested in humans and determined to not be unacceptably painful.

In addition we critically review our experiements and look for ways to mitigate any harms we observe.

PROJECT 235

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.

Word limit; 1000 words

Project Title	Molecular mechanisms of stroke
Key Words	stroke, neuroprotection, inflammation, blood flow
Expected duration of the project	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):

Stroke is a major health issue. The most common type of stroke is caused by a blood clot which blocks one of the major blood vessels supplying the brain. Someone experiences a stroke for the first time somewhere in the world ever 2 seconds. Of these stroke patients 1 in 8 die within a month, 1 in 4 die within a year. At the moment we can only treat stroke by breaking down or removing the clot from the brain. However, this has to be done within 4 hours of the onset of the stroke, otherwise it can cause more harm than good. After the stroke has occurred, a significant proportion of patients will die from an infection, because their immune system becomes faulty. With this in mind stroke research is important for a number of key reasons. Firstly, it is important to find out what happens to the brain after 4 hours, to try and find out if we can extend this time to give more patients a chance of treatment. Secondly, it is important to find out how the brain talks to the immune system, to discover why so many patients get infections after a stroke. By understanding more about what happens to the brain and the rest of the body after a stroke we may be able to develop new drugs.

The brain undergoes a huge variety of changes after a stroke. The area of the brain directly supplied by the blocked blood vessel dies extremely rapidly. However, there is an area of surrounding tissue which is not yet dead, but is in danger of dying. Some of the processes which can cause this area to die are 1) changes in blood flow, 2) inflammation within the brain and 3) inflammation in the rest of the body.

1) adequate blood supply to the brain is vital to survival, this is why stroke is so damaging. By removing the clot which blocks the artery we can help restore blood supply to the brain, however, this is not always possible. In addition, even when we do remove the clot, sometimes the blood does not flow back to the brain properly

because the blood vessels are constricted. By understanding more about what makes these blood vessels constrict, and more about how blood flow is rerouted around the area of dead brain after a stroke, we may be able to develop new drugs which are capable of improving treatment.

2) inflammation in the brain is an important part of the recovery process. A stroke generates a large area of dead tissue and this needs to be 'cleaned up' by cells that live in the brain. However, as part of the 'clean up' process, these cells often release chemicals which can damage nearby cells. This means that inflammation in the brain is a double edged sword. Necessary for the removal of dead tissue, but also the cause of 'chemical friendly fire' death of neighbouring cells. However, it is a very complex process involving a number of different types of cell and a number of different chemicals. By understanding the exact ways in which these cells behave and which chemicals are responsible for the 'clean up' and which are responsible for the 'friendly fire' we may be able to prevent deterioration after a stroke.

3) the immune system becomes complicated after a stroke. Whilst a large proportion of patients will die from an infection (usually pneumonia) after a stroke, suggesting their immune system doesn't work properly, some will die because an infection has increased their immune system response, suggesting their immune system is over reactive. We still do not understand how the brain, which is physically separated from the rest of the body by vessel barriers, tells the immune system that it is injured, or what the purpose of this communication is. Again, by understanding more about these processes, we may be able to more effectively treat stroke patients.

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

The long term benefits of understanding more about the underlying processes occurring after a stroke include the development of novel therapeutic agents, as well as new techniques for studying stroke in humans. Our significant body of previously published work has demonstrated that the processes occurring after a brain injury are complex and multi-faceted. By understanding more about how these work in the diseased brain, we will be able to apply our knowledge to these processes in the healthy brain. This research will therefore further our understanding of both how a stroke affects the brain and the body, but also more fundamental information about how the body works.

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

We will use both mice and rats over the 5 years of the project licence, with approximately 3500 mice and 2000 rats. This is based on usage from our previous licence and from our experience of these 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?

Approximately 50% of the animals used under this licence will have the equivalent of some form of stroke. Of the remaining approximately 25% will have sham surgery or will be used for breeding purposes and 25% will be used to investigate the effects of stroke mediators on the rest of the body. Animals undergoing surgery for a stroke will receive all appropriate pain relief, and will suffer the symptoms of a stroke (some weakness on one side of the body), this is an expected outcome and will be tolerated under the licence providing it does not significantly interfere with the animals' ability to behave normally (eat, drink, move around). 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

Our work involves studying a complex brain injury, which results in damage to many different cell populations within the brain. Whilst it may be possible to study these individual populations in culture, it requires them to all be functioning as a unit to find out not only how they act together, but also how they then affect the animal. We are particularly interested in functional outcomes, how the stroke affects specific behaviours like walking, complex co-ordination and learned behaviours which requires whole animals capable of performing such tasks. Rodents cover these requirements well and have a similar brain structure and immune system to a human. In addition, our interest in how the brain communicates injury to the rest of the body involves sets of molecules only expressed in mammals.

In addition to our work in rodents we also carry out work in isolated cells in culture. As part of this we are working in collaboration with other researchers to develop 3D models of groups of cells within the brain. For example, in the brain there is a neurovascular unit - this is made up of the main brain cell type (neuron), and some supporting cells (astrocytes, endothelial cells, pericytes). We currently look at all of these cells in isolation, but our aim is to build them up in a 3D format to make a neurovascular unit in culture. This will enable us to model some of our effects without using animals.

Finally we also use human tissue to study the effects of stroke. We have access to blood from stroke patients, as well as brain tissue, so we can study how some things change after a stroke in the human tissue. This is key to validating whether our animal work is properly mimicking the human disease.

Reduction

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

Reduction

Wherever possible we aim to run small pilot experiments, to prevent excessive use of animals when we are unsure of the outcome. For all animals we aim to acquire as much data as possible, taking behavioural readings and tissue from the same animals to compare functional outcomes with biological outcomes. For larger experiments where we aim to run pre-clinical tests of drugs in concert with other stroke labs, we use power analysis to find out the appropriate number of animals to use for a designated outcome.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 involves using a number of different techniques to model stroke which helps us to define exactly what we need to look at. For example, to model stroke which occurs in the most common area of the brain (the territory of the middle cerebral artery) we can use a model where we occlude the middle cerebral artery, which effectively mimics the human condition. For strokes where the blood supply is blocked somewhere very small, we can use a local injection of something which constricts the blood supply in that area, to mimic a small local stroke. Choosing the correct model is key to having good outcomes.

In these models we aim to minimize harm to the animals in a number of ways:

Before surgery: we aim to handle animals and acclimatize them to their environment for up to a week prior to surgery. We find that handling makes the animals more calm and they recover better after the surgery. If animals are going to be pre-treated with drugs, the most appropriate route of administration is chosen. For example, if a drug needs to be given by injection three times a day for a week, we instead implant the animal with a small device which gradually releases the drug over time. We feel this results in less stress and discomfort than repeated injections.

During surgery: all efforts are made to undertake surgery in a sterile environment, to minimize the chances of an infection during recovery. In addition all efforts are made to minimize pain and suffering during and after surgery. All surgery is carried out under appropriate anaesthetic and pain relief drugs are given in the food afterwards, and to local areas such as wound sites at the end of the surgery. To prevent dehydration and hypothermia, animals are given fluids throughout the surgery and

kept at all times on a blanket which regulates their body temperature and keeps it at 37ºC.

After surgery: animals are kept in groups after surgery. Rodents are social animals and isolating them after surgery often causes distress. Group housed animals often huddle together, providing warmth for each other which helps with recovery. We provide pain relief drugs in sweet foods (usually nutella) immediately after surgery and provide easily palatable and easily digestible food (wet mash and jelly) for up to 3 days after surgery.

Throughout the protocols, animals are monitored for pain, suffering and distress and we have a number of specific points which we have designated as unacceptable for animal welfare.

PROJECT 236

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.

Word limit; 1000 words

Project Title	Immunology and pathogenesis of pneumoviruses
Key Words	vaccine, Pneumoviruses, pathogenesis, immunity
Expected duration of the project	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.

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 aims of the project are:

1. To identify the role of viral protein in the pathogenesis of pneumovirus infections

2. To establish methods for the delivery of pneumovirus antigens to induce protective immunity

3. To evaluate improved phophylactic and/or therapeutic immunological strategies and/or antiviral compounds against RS viruses.

4. To determine the mechanisms by which viruses predispose to bacterial infections in the bovine respiratory tract

What 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 provide an understanding of the pathogenesis of pneumovirus infections and to contribute to the identification of strategies that result in the development of safe and effective pneumovirus vaccines, and safe and effective anti-viral prophylaxis or therapies. Studies to determine the mechanisms by which viruses predispose to bacterial infections in the respiratory tract, will be informed by in vitro studies of viral and bacterial co-infections in bovine airway epithelial cell cultures. The severity of clinical disease in studies on viral and bacterial co-infections will be minimised by limiting the duration of the studies to no more than 72 hours after bacterial infection, and a pilot study will be undertaken to determine the minimum dose of bacteria that results in adherence to airway epithelial cells in the bovine respiratory tract. The lowest dose of bacteria that results in adherence to airway epithelial cells will then be

used to determine the effects of viral infection on bacterial colonization in subsequent studies.

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

Cattle: 310 in 5 years Mice: 900 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?

In the context of pneumovirus infections, we propose to vaccinate mice and calves to drive immune responses and then challenge with pathogenic strains of pneumoviruses to show protection from disease. Severity level: Moderate for both mice and calves Expected adverse effects. Mice: weight loss, ruffle fur, reluctance to move. Calves: high temperature, coughing, nasal and ocular discharge. At the end of the studies, all animals will be killed and disposed.

Application of the 3Rs

Replacement

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

Replacement

A fundamental understanding of the immune system, the pathogenesis of respiratory infections and the testing of vaccine and prophylactic approaches requires the use of living animals.

Reduction

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

Reduction

Statistical advice will always be sought. Pilot experiments will always be carried out before undertaking a new area of research. The EDA tool will be used as required to plan experimental designs.

Refinement

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

Refinement

Bovine RSV & other pathogens associated with bovine respiratory disease, only infect calves, so only cattle can be used. During studies using calves, all animals will be housed in open barns, with fresh straw bedding, with continuous access to food and sterile water and in groups of 2-5/pen, depending on age and size. Colostrum-deprived calves are provided with blankets to maintain their temperature and human contact to reduce and prevent stress, and reared in a clean and dry environment, with imposition of biosecurity mea sures.

Experimental infection of mice with HRSV is used as a model of HRSV infection in m an. During sudies in mice, animals have freedom of movement, continuous access to food and water and are housed in groups of 3-5 depending of age and size. Mice will be housed in clean cages and with environmental enrichment.

PROJECT 237

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.

Word limit; 1000 words

Project Title	Rat posterolateral spinal fusion model
Key Words	Spinal fusion, Growth factors, Bone, Tissue engineering, Bio-regenerative scaffolds
Expected duration of the project	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;
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):

Osteoinductive molecules promote bone formation and are found naturally in the body. This is a useful quality in tissue engineering to help bones heal or to cause a fusion (to stop movement and pain). Currently, there are osteoinductive molecules available to use in clinical practice to enhance spinal fusions and to aid severe fractured bones in the leg to heal. However, there have been some side effects reported, such as swelling, which has caused concerns. The adverse side effects are thought to be because the dose of the osteoinductive molecules used is much higher than the levels found naturally in the body.

Our research group has found a way to combine osetoinductive molecules in a bio-regenerative scaffold to localise the effect of the osteoinductive molecules to one area, and as such, much lower and safer doses can be used. This has been proven in cell culture experiments and also in mouse studies investigating new bone formation. However, to progress this novel technique we aim to replicate the experiments performed by leaders in the industry by using a rat spine fusion model to demonstrate that the dose of osteoinductive molecules can be lowered when used in conjunction with a bio-regenerative scaffold and still result in a solid fusion. This work is an essential step towards adopting this novel technique 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 potential benefits of this study are to make the delivery of osteoinductive molecules safer and more effective, so that bone formation can be promoted without side effects. This has a direct link to clinical practice to improve patient care.

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

Approximately 40 adult rats will be used in each completed study, aged 3-6 months old for a duration of 6-8 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?

Minimal adverse effects are anticipated. The expected severity is moderate. Administration of anaesthetic – anticipated adverse effects should be rare as long as appropriate dosages are used. Anaesthesia will only be administered by personnel trained in the required techniques and all animals will be treated as would be post-operative animals (e.g. kept warm and closely observed until fully recovered). Surgical risk – surgery will be performed using aseptic techniques to minimise the risk of infection. The surgeon (if necessary)will wear magnifying eyepieces to minimise the risk of injury to neurological structures. Osteoinductive molecules/bio-regenerative scaffolds – these materials have been used in animal models (mice, rabbits and sheep) by our group with no adverse effects and therefore, are not anticipated to affect the well-being of the animal. Postoperative recovery from the procedure – following surgery, the rats will be closely monitored for assessment of motor and sensory dysfunction. Infection of the surgical site would require euthanasia of the animal. Wound dehiscence – wounds that open up will be assessed for suturing. Animals will be anaesthetised and wound aseptically cleaned and re-sutured. If wound dehiscence occurs a second time, then the animal will be euthanised using a Schedule 1 method. Animal suffering will be minimised by, 1. Administration of analgesic agent to relieve pain and distress. 2. Use of aseptic techniques throughout to minimise any risk of infection, which will be closely monitored. 3. If the wounds open, animals will be anaesthetised and wounds aseptically cleaned and resutured. If the wounds open again then the animals will be euthanised using humane Home Office-approved methods. If adverse effects due to implantation of materials into the spine are detected, the principal investigator, licence holder and animal technologists will determine whether or not the animal has reached the humane endpoint determined in the licence and therefore should be killed using humane a Home Office-approved method. If there is any doubt the Named Veterinary Surgeon will be consulted.

Application of the 3Rs

Replacement

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

Replacement

In vitro experiments have been performed with encouraging results that require further exploration in an animal model to reproduce the complex biomechanical and physiological environment that cannot be simulated in tissue culture. *Ex vivo* experiments have also been performed to ensure biocompatibility of osteoinductive molecules and bio-regenerative scaffolds. Currently the use of some of these materials are not licenced for use on humans and this study is an essential step towards clinical translation.

Reduction

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

Reduction

We are conducting this study in accordance to previously published standards, which decreases the numbers of animals required.

The use of *in vivo* µCT scanning (i.e. the SkyScan 1176 high resolution *in vivo* micro CT scanner) during the lifetime of this licence will further reduce the number of animals needed for each procedure and maximise the amount of information that can be extracted from each animal. The *in vivo* µCT machine will allow us to analyse the bone formation within the animals at various time points without having to sacrifice the animals at these set time points.REDACTED

Refinement

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

Refinement

Appropriate anaesthesia and analgesia will be administered to the rats under full aseptic conditions to minimise any risk of infection. Anaesthesia will only be administered by personnel trained in the required techniques and all animals will be treated as would be post-operative animals (e.g. kept warm and closely observed until fully recovered). Analgesia will be continued for as long as required, as assessed by signs of distress and pain. Infections will be closely monitored over 24-48 hours to ensure animals are not at risk of pain or suffering.

Following surgery, the rats will be closely monitored for assessment of motor and sensory dysfunction suggestive of nerve injury. Researchers and trained animal technologists will check all animals daily and should adverse effects be noted due to implantation of materials into the defect site, the PI and PPL holder REDACTED will determine initially whether or not the animal has reached the humane endpoint (as stated in Protocol 1) determined in the licence and therefore should be killed using a

Schedule 1 method. If there is any doubt the Named Veterinary Surgeon will be consulted to determine whether or not the animal has reached the humane endpoint. Distress will be assessed using LASA Working Party Guidelines on the control of severity (Laboratory Animals (1990) 24:97-130).

All animals will be assessed daily for signs of distress or ill health. Any animals exhibiting signs of distress and/or pain refractory to standard care will be killed by a Schedule 1 method. Handling will be minimised to routine husbandry and procedures required for the project.

PROJECT 238

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.

Word limit; 1000 words

Project Title	Production of polyclonal and monoclonal antibodies
Key Words	Monoclonal, polyclonal, antibody
Expected duration of the project	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):

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.

PROJECT 239

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.

Word limit; 1000 words

Project Title	Use of Arbovirus and Robovirus Disease Models for Transmission and Intervention Testing
Key Words	Arbovirus; robovirus; modelling; transmission.
Expected duration of the project	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;
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.

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 aim of this project is to test susceptibilities of animals to arboviruses and roboviruses. These experiments will culminate in testing new vaccines or therapeutics in disease models to examine whether they confer protection and also be used to inform on pathogenesis determinants which may have an impact on public 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)?

There are multiple potential benefits from this project. This project will test the ability of viruses to infect foetuses or neonates, either by in utero infection or via suckling an infected mother. This project could also help inform whether by treating the mother the foetus or the suckling pup is also protected. It will also test whether novel therapeutics can protect animals against the disease, which will contribute to human trials. This project will also look as to whether new and emerging viruses could be transmitted by UK vectors and become established within the UK.

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

Wild type and genetically-altered mice will be used. Up to 5050 mice, 500 rats, 600 guinea pigs and 425 ferrets could be used over the five year life of this project.

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

The project can be broadly divided into four levels of severity and procedures. Firstly, animals will be bred to generate a mixed genotypic profile of mice which are slightly

less resistant to viruses then wild-type mice, but not totally susceptible to viruses. This should not have any adverse side effects and is a mild procedure. These animals may then proceed to assist in the subsequent procedures. Secondly, animals will be given therapeutics or vaccines to test whether they cause any adverse effects and the immune system responds to them (in the case of vaccines). We will also investigate the properties of the therapeutics; how long they last in the body and if they reach all the biological areas they need to. This is a moderate procedure as the animals should not exhibit any severe side effects. However, some of the therapeutics and vaccines used may cause some moderate discomfort. Thirdly, the animals will be infected with a virus. This is a severe procedure as, depending on the virus, the type/strain of animal and the route of administration may result in the animal developing disease. Once the animal has reached its humane end point (once it has been determined that the animal will not recover) the animal will be culled to prevent further pain and distress. Lastly, the vaccines or therapeutics will be tested by measuring their ability to protect against or remedy disease. This again is a severe procedure. Animals will be given either; (i) vaccines to test whether they induce immunity and subsequently prevent disease developing in the animal model subsequent to an otherwise disease causing challenge dose of virus or (ii) therapeutics to test whether they remedy the disease process in the animal model before or after they have been challenged with an otherwise disease causing challenge dose of virus. If an animal reaches its humane end point (once it has been determined that the animal will not recover) the animal will be culled to prevent further pain and distress. Although this application has several severe procotols within it, the animals will be monitored with increasing frequency when clinical signs are observed. It is expected that with this increased frequency of observations, if the animal meets its defined humane end point then it will be culled to prevent further pain and distress and will only have undergone moderate severity.

Application of the 3Rs

Replacement

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

Replacement

Mice and other rodent species and ferrets are required to be used in this project as there is no alternative available. Tissue culture or computer modelling is not sufficient to provide the complexity of, not only a whole organ but multiple systems within the body and the biological and physiological interactions that occur therein.

Reduction

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

Reduction

We aim to use the most appropriate stains of animals to minimise the number of animals required to achieve a successful result. We will perform appropriate pilot studies to inform us on the numbers of mice required to achieve a statically significant result with the lowest numbers of mice yet still confer statistical significance and confidence in the generated 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 most appropriate animal species will be used to ensure the most relevant results are obtained. In addition, genetically-altered mice will be used, which will reduce the risk of experimental failure and allow smaller group sizes to be used. The candidate vaccine or therapeutic will be evaluated in a step wise program, so that only interventions that show promise will continued to be used. Biological samples will be taken to minimise the number of animals being used. The animals being utilised will be monitored frequently, with checking frequencies increased during critical phases of the studies to ensure humane endpoints are captured and limit any potential suffering.

Anaesthesia: the following precautions will be taken to ensure that no potential adverse effects of anaesthesia will occur. Where parenteral anaesthetics are used, a dose appropriate for the animal's weight will be used. Once anaesthesia has been induced and where appropriate, steps will be taken to minimise hypothermia and dehydration. Animals will be observed post-anaesthesia until appropriate recovery is confirmed. Most commonly animals will be anaesthetised with a gaseous agent (e.g. isoflurane) and again steps will be taken to minimise side effects and the animals will be observed until appropriate recovery is confirmed.

PROJECT 240

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.

Word limit; 1000 words

Project Title	Functional investigation of a genetic region involved in risk of type 1 diabetes and autoimmune disease.
Key Words	Diabetes mellitus, Autoimmunity, Genetics, Microbiome
Expected duration of the project	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;
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 purpose of this project is to investigate the way in which genes influence the immune system, particularly in the development of type 1 diabetes, where the body destroys normal pancreas tissue. Many autoimmune diseases have no cure, and can only be managed with treatments which are inconvenient (e.g. insulin in the case of type 1 diabetes), do not work completely, or have significant side effects. These illnesses are debilitating and often impact on the quality of life and life expectancy of the sufferer. The research in this project will study part of the genome which is known to affect the risk of type 1 diabetes in humans. This work will help to identify new targets for treating and preventing autoimmune diseases. At present we are studying one particular gene of interest, which we have proven previously to be involved in protection from diabetes.

We now seek to understand the mechanism by which this gene can protect from diabetes. The long-term aim of our work is to target the pathways that this particular gene uses to prevent or treat the development of autoimmune disease.

We are also interested in the interaction between this gene and the environment, and in particular the micro-organisms that live in the gut, known as the microbiome. We are investigating whether it might be possible to replicate the protective effects of this gene in type 1 diabetes by making changes to the diet or 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)?

- Increased understanding of how genetic variations can protect against autoimmune diseases like type 1 diabetes - Potential discovery of a new pathway in the development of diabetes, which could be a target for future interventions to prevent diabetes or other autoimmune diseases. - Improved understanding of whether changing the type and number of micro-organisms in the gut may help to prevent diabetes - New interventions based on the biology of this new gene and / or its interaction with the microbiome which may act to reduce the development of type 1 diabetes in genetically susceptible children

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

The project will last for 5 years and is estimated to use up to 3000 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?

Many of the studies will involve the observation of genetically modified animals for the development of autoimmune disease, such as diabetes. Previous work has shown that these genetic modifications do not lead to any major changes in the mice. Some studies will involve animals being dosed with test substances (either by mouth or by injection) followed by observations such as development of diabetes (diagnosed with a urine sample), analysis of a blood sample or analysis of a sample of faeces. Animals may also be killed humanely to provide tissue samples. These animals will usually experience nothing more than minor discomfort from dosing and bleeding procedures. We will also change the number or type of micro-organisms in the gut in some animals, for example using substances such as antibiotics or probiotics. In some studies, a small number of animals may undergo a bone marrow transplant to assess the effect of this procedure on the immune system and particularly on the risk of developing diabetes. These mice will be checked very regularly for side-effects and if the animals reach their humane end points they will be killed. We will regularly check the urine of the mice for glucose, which will tell us if they have become diabetic. Any mouse found to have diabetes will be humanely killed as soon as it is diagnosed. All animals used under this licence will be humanely killed at the end of procedures.

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 the development of autoimmunity is the result of many genetic variations, combined with a wide range of environmental

triggers in a whole organism. Diabetes is a complex disease affecting the whole animal and cannot be replicated in a test tube. Other organisms such as fish and worms do not have diabetes nor do they have the genes being studied, so it is not possible to do these studies on lower species.

We also collaborate with laboratories studying the human immune system, so wherever possible we will translate findings to human studies as early as is feasible.

Reduction

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

Reduction

All the proposed experiments will be carefully designed to achieve the scientific objectives using the minimum numbers of animals.

We have used new technology for gene editing to generate the mice for study, allowing us to minimise the numbers of mice required for our breeding programme. We have also generated and stored frozen embryos from the lines we have generated so that we are only breeding and studying a small number of lines while the rest are preserved for future work if necessary.

We will use the scientific literature and pilot studies to work out the lowest number of mice possible for this work and we will consult statisticians for advice on analysis. We are committed to using the minimum number of animals to achieve our scientific purpose.

When animals are culled, we will keep extra samples wherever possible (e.g. blood, tissues, faeces), which may be used for future work, thus avoiding breeding more mice at a later date when 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

Mice will be the only species on which work will be conducted under this licence. This species is considered to be of the lowest neurophysiological sensitivity available with achieving the study aims and novel genetics tools are available to allow targeted genome manipulation. .

The limits on clinical signs to which the animals are allowed to display are defined in this licence, and when or before they are reached, the animals will be humanely killed.

Where bone marrow transplantation is undertaken, we will use antibiotics where necessary to reduce the risk of infection in immunosuppressed mice. We will also consider using naturally immunosuppressed mice (NOD-SCID) rather than irradiating mice as bone marrow recipients wherever possible.

We will use the most refined route of administration where substances (e.g. antibiotics) are being administered and we will use pilot cohorts with close monitoring to minimise harm.

We also have a procedure in place for extra monitoring and care of aged animals and a prospective monitoring arrangement (urine glucose testing) to diagnose diabetes and cull mice prior to the onset of clinical signs where mice are genetically susceptible.

PROJECT 241

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.

Word limit; 1000 words

Project Title	Pre-clinical Pharmacology of Inflammatory Disease
Key Words	New drugs, Inflammatory disease, Translational
Expected duration of the project	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;
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.

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

PROJECT 242

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.

Word limit; 1000 words

Project Title	Making skeletal muscle less prone to disease mediated damage and ageing induced damage
Key Words	skeletal, muscle
Expected duration of the project	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;
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):

At present we have very little knowledge about the genes that control muscle growth and about those that make muscle work properly.

Our work is focused to address this lack of knowledge by:

1. Breeding mice that lack certain genes to determine their normal role.

2. Identify molecules that enlarge muscle.

3. We will test the muscle that we have induced to grow and determine whether it can repair itself like normal muscle.

4. We will determine whether the muscle that we have induced to grow responds like normal muscle when the diet is changed.

5. We will determine whether the muscle that we have induced to grow allows the mouse to exercise like normal 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)?

The key benefits of this project are expected to be in the treatment of ill health and the treatment of skeletal muscle that fails to work properly in humans. Results from this project will be of immediate benefit to doctors as they are focused on human diseases, drug companies as well as researchers. Very importantly we believe that our results could give immediate hope to patients who have previously been thought of as untreatable. Treatment of ill health will include patients with Duchenne Muscular Dystrophy which affects 1 in 5000 boys and leads to death at about 25

years of age. Skeletal muscle loss is found in many kidney diseases, cancers and HIV infection. However we all will experience muscle wasting as it is the key feature of ageing which leads to it not only decreasing its size but also its ability to carry out its normal function. This process, called sarcopenia leads to a reduced quality of life and has huge costs associated with it to the economy. Therefore advances in developing therapies that reverse age-related muscle wasting are likely to be beneficial not only to those who suffer from it but also all of society since we all pay for their treatment through taxes. Money that can be saved based on our studies can be used to help others.

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

2000 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 most severe protocol has been rated at moderate. We have provided details of adverse effects which should be all controllable should they arise at all. Muscle wasting and damage protocols are the most severe in this application and rated at moderate. These will induce an initial lameness in the mice which disappears within 6-7 days. Mice will be given medication to minimise pain where necessary. Giving mice chemicals that enhance the uptake by cells of small DNA molecules can induce pain as well as diarrhoea in less than 1% of cases. Mice will be given medication to minimise pain where necessary. Mice with diarrhoea will be treated by giving them water in their food and carefully monitored by our staff. All animals will be killed in a humane manner at the end of a relevant study or if advised by trained staff due to welfare concerns.

Application of the 3Rs

Replacement

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

Replacement

Skeletal muscle is a complex tissue made of not only muscle fibres but also blood vessels and nerves. These tissues all respond to changes in their surroundings, for example the food that we eat, in a way that we are only beginning to understand.

Although we can grow cells in a test tube, these do not behave in the same way as muscle in an animal. This is why it is still necessary for work on mice that is outlined in this proposal.

Reduction

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

Reduction

Information from experimental research conducted under a previous licence has led to a vast reduction in the number of mice needed for this project. Furthermore we have developed experiments that can be carried out in test-tubes using new technology that allows us to extract more data from each mouse experiments which again leads to the need for fewer animals.

To further reduced the number of animals used for anyone experiment, we turn to a statistician who is able to tell us the minimum number of animals we are likely to need to produce meaningful results.

We will also use a protocol in our regeneration studies which allows us to get much more information from a single experiment than was possible before. This again means that we will use fewer animals in our 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

Mouse muscle uses the same molecular and cellular processes during muscle development and regeneration as humans. Other experimental models, *e.g.* fruit flies, nematode worms and fish do not share these characteristics and hence we need to carry our experiments in rodents.

We will always conduct experiments in using non-animal protocols (for example with cells in test-tubes) before carrying out ones in mice. We will be mindful for the development of new techniques which may allow us to further reduce harm caused to mice. This approach is highlighted by two examples of our working practice.

With regards muscle regeneration we have conducted our work in such a way that by damaging a muscle that is made of two different parts, we can get two lots of data from a single experiment. This means that a mouse does not need to be injected twice.

Secondly we use a means of making mice exercise that involves us tickling the back of mouse with a paint brush while it is on a treadmill. Previous protocols would have used and mild electrical shock for the same outcome.

PROJECT 243

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.

Word limit; 1000 words

Project Title	Experimental necrotising enterocolitis
Key Words	Remote Ischaemic Conditioning, Necrotising enterocolitis
Expected duration of the project	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;
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):

Necrotising enterocolitis (NEC) is a devastating disease of premature babies. Up to a third sadly die and many of the survivors are left with life-long disability. There are around 3000 cases of NEC in the UK each year.

The causes are complicated but one key aspect of NEC is what is known as ‘ischaemic-reperfusion injury.’ Whenever any organ of the body does not have enough blood flow this is called ‘ischaemia’ which results in tissue damage. When the blood supply is restored, further damage occurs and this is called ‘reperfusion injury’

Ischaemic-reperfusion injuries are part of several common diseases such as heart attacks and stroke

Experimental work has shown that the use of ‘Remote Ischaemic Conditioning’ (RIC) can reduce significantly the damage done by a heart attack or other ischaemic-reperfusion injury.

Remote Ischaemic Conditioning means giving a small ischaemic ‘hit’ to some other part of the body. This does not cause any lasting injury or damage. The easiest way to do this is to use a blood-pressure cuff inflated for a period of a few minutes. This conditions the body so that the damage done from the major ischaemic-reperfusion injury is massively reduced

Our aim is to establish if RIC can be used as a treatment for NEC in premature 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)?

Our hope is to be able to use a blood pressure-cuff based treatment to treat human babies with NEC. We will also use samples from the animals to study the mechanisms by which RIC works in order to look for other treatments as well. We have previous experience of translating potential treatments from experimental animal research into clinical trials.

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

Rat pups Around 150 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 expected adverse effects are mild. Inflating the blood pressure cuff is well tolerated and has no long lasting effects. Prior to the surgery, animals will be housed in suitable cages, with species-appropriate environmental enrichment, within a well-resourced, well equipped, modern facilityThese are maintained at the ideal temperature and humidity for the rats. Appropriate food and water are provided at all times. If, at any time, there are concerns about any animals, advice will be sought from the NVS/NACWO and appropriate steps taken to ensure animal welfare including humane killing if necessary. The model of necrotising enterocolitis involves surgery on the abdomen and a temporary interruption to the blood supply to the bowel. At the end of the experiment, multiple samples are taken from each animal to assess the effect of the model and the possible benefit of the intervention. This whole process will be performed under terminal anaesthesia. The animals will not be recovered from the anaesthetic. All animals will be closely monitored under anaesthetic to ensure sufficient depth of anaesthesia and a humane end-point.

Application of the 3Rs

Replacement

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

Replacement

Necrotising enterocolitis is a complex disease affecting not just the intestine but the entire baby. We have developed a model of this disease which closely reflects the human disease. The complex interactions between various parts of the whole animal are necessary for the model to closely replicate the human disease. Furthermore remote ischaemic conditioning must be applied at a site away from the intestine. Such mechanisms can only work in whole animal systems.

Reduction

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

Reduction

Through close attention to experimental design and statistical advice we will use the minimum numbers of animals necessary to gain statistically and clinically meaningful data. We will gain as much information as we can from each experimental animal (i.e. both physiological monitoring and analysis of several organs/fluids) in order to reduce experimental numbers

As we progress we will continuously review the results to ensure that we minimise confounding factors and do not use more animals that are absolutely necessary whilst maintain 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

We will use the rat since the physiology of the rat intestine closely resembles that of the newborn human and it is an appropriate size for the program of work (smaller animals are too small).

To minimise harms, all invasive procedures will be performed under terminal anaesthesia (i.e. without recovery).

PROJECT 244

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.

Word limit; 1000 words

Project Title	Mechanistic, inflammatory and arthritis models.
Key Words	Inflammation, Immunity, Arthritis, Rheumatoid arthritis
Expected duration of the project	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;
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 overall objective of this project licence is to identify new medicines for the treatment of chronic inflammatory and autoimmune diseases such as rheumatoid arthritis (RA).

RA is a disease of the joints and the cause is unknown. Patients have inflamed painful joints, cartilage and bone destruction, fatigue and a reduced life expectancy. Current treatments are associated with side effects and do not completely stop disease progression and not all patients respond to treatment. There is still a clear unmet clinical need for better treatments which will give patients a better quality of 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)?

This project is expected to identify novel medicines and processes involved in diseases such as RA which will lead to new treatments and improved quality of life for patients.

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

Rodents (mice, rats) and rabbits will be used in this project. It is expected that an average of 1,600 animals could be used annually in this project and the majority of these will be 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 animals will undergo procedures that may involve injections and they may experience moderate discomfort as they respond to inflammatory stimuli and develop symptoms of disease, such as swollen paws. Overall the level of severity for procedures in this project is moderate. At the end of studies 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

Inflammatory, immune and disease responses in humans are complex and involve interactions between tissues and various cell types. This complexity cannot be mimicked *in vitro* hence the need to assess the effect of new medicines in animal models.

Reduction

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

Reduction

To ensure the fewest number of animals are used, only the most effective drugs that have been pre-screened for activity in vitro will be examined in animals. Due to the nature of disease models incidence of disease may not be 100%. 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 number of animals 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

Rodents are the mammalian species of lowest neurophysiological sensitivity in which these inflammatory models have been developed.

A number of the animal models in this project are well established both in-house and within the literature and have been shown to model different aspects of human

disease. However, no single model accurately reflects human disease and it is therefore necessary to study different models that model different components of human disease.

All procedures have been ethically reviewed and all animals undergoing procedures are monitored closely by trained staff that work closely with a veterinary surgeon. In addition, distress scoring sheets are used to monitor disease severity and these are under constant review to ensure the correct level of disease scoring is achieved with minimum stress to animals. Humane endpoints are employed to limit suffering and disease burden.

REDACTED

PROJECT 245

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.

Word limit; 1000 words

Project Title	Pre-clinical Pharmacology of Idiopathic Pulmonary Fibrosis
Key Words	Incurable, Respiratroy Disease, New drugs
Expected duration of the project	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;
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.

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):

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.

PROJECT 246

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.

Word limit; 1000 words

Project Title	The role of ion channels in platelet and megakaryocyte function
Key Words	Platelets, megakaryocytes, thrombosis, ion channels, blood clotting
Expected duration of the project	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):

Platelets are the smallest cellular element of blood and essential for arrest of bleeding. However, unnecessary stimulation of platelets can often occur, leading to thrombosis and thus diseases such as heart attacks and stroke. There is a clear clinical need for anti-thrombotic medication that inhibits platelet activation without causing excessive bleeding. The project aims to identify the role of membrane ion channels in platelet responses and thus the possibility that they be 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)?

We know relatively little about the role of ion channels in platelets and their precursor the megakaryocyte. This project will examine the role of several different classes of ion channels in platelet and megakaryocyte responses such as bleeding, thrombosis and maintaining adequate numbers of circulating platelets. This will identify whether their inhibition could be suitable for preventing thrombosis

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

Rats and mice will be used, up to 2500 mice and 800 rats. The numbers are based upon the study of up to 10 ion channel-related genes. Mice are an accepted model for studies of human platelet function and a wide range of genetically modified mouse strains, including these lacking specific ion channels, have already been developed. Certain strains of rat are of interest due to their altered megakaryocyte phenotype.

In the context of what you propose to do to 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 the genetic modifications to the mice within this project, and natural mutations within certain strains of rats also used, will result in mild defects to blood clotting and that the animals are essentially healthy. Some mice will receive an altered diet to modify their phenotype in a manner that mimics the normal progression of human cardiovascular disease but again this is not expected to have a major consequence for the overall well-being of the animal. The progression of the cardiovascular disease will be monitored by a non-harmful procedure such as magnetic resonance imaging on a limited number of occasions during which the animal is anaesthetised and thus will not experience any stress. In other tests, mice or rats will receive an injection of a non-harmful marker of blood cells, followed by sampling of blood over a limited time period to assess platelet turnover. The amount of blood taken on each occasion, and also in total from each animal, has a strict limit in order to prevent harm and discomfort and thus this protocol has a mild severity limit. Some mice or rats will receive drugs that affect platelet function prior to experiments, or will be allowed to recover following analysis of bleeding from a superficial blood vessel. These procedures also have a mild severity level as the drugs will only have mild effects on blood clotting and the assays of bleeding will be carried out under anaesthesia with a limited duration to avoid excessive loss of fluid. Any animal that suffers in a manner deemed to exceed the mild severity levels for the above protocols will be killed humanely as soon as possible. Some mice will be exposed to radiation followed by receipt of bone marrow cells from a donor mouse to replace the marrow stem cells or receive a platelet-depleting reagent followed by a donation of platelets isolated from a donor mouse. In these experiments, the donor mouse is humanely killed. For the mice receiving the radiation or platelet depletion followed by marrow cell or platelet transfer, these protocols have a moderate level of severity. They will be studied very closely, and should they fail to thrive, show evidence of isolation, distress, bleeding or are generally unwell, they will be killed humanely as soon as possible. Some experiments have are non-recovery as they are performed under general anaesthesia and the mouse or rat is humanely killed at the end of the procedure without regaining consciousness. Therefore, in these experiments, the animal will not feel anything and will not wake up after the experiment. These non-recovery procedures include 1. the removal of large amounts of blood to assess platelet function outside the animal; 2. tests of thrombosis in the circulation or 3. imaging of platelet production from their precursor cell the megakaryocyte. Each animal will only be subjected to one of these non-recovery procedures.

Application of the 3Rs

Replacement

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

Replacement

No selective inhibitors are available for inhibition of the specific ion channels to be studied, however mice lacking each channel can be generated by transgenic methodology. Furthermore, important in vivo experiments can be carried out that are not possible in humans. Experiments are also exploring use of donated human marrow and culture techniques that can generate megakaryocytes and platelets in the laboratory.

Reduction

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

Reduction

Tissue can be obtained from animals euthanized for other purposes. Rigorous statistical approaches will ensure that sufficient but not excessive numbers of animals 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 small rodent (mouse or rat) is a well-established model for the study of platelet and megakaryocyte responses. Health and well being of the animals will be carefully monitored. Experiments will have strict defined limits to reduce adverse effects, such as excessive bleeding. Invasive in vivo experiments will be carried out under deep anaesthesia followed by euthanasia without recovery of consciousness. Procedure refinements will be constantly under consideration to reduce any observed suffering, or the likelihood of suffering. Examples are microsampling (https://www.nc3rs.org.uk/microsampling) to reduce the volume of blood taken, or restraint tubes during measurement of blood pressure (https://www.nc3rs.org.uk/handling-and-restraint)

PROJECT 247

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.

Word limit; 1000 words

Project Title	Investigation of the genetic drivers and therapeutic vulnerabilities of myeloid leukaemia and related cancers
Key Words	Leukaemia, Ageing, Mutation, Treatment, Blood disorders
Expected duration of the project	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;
No	(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):

Acute myeloid leukaemia (AML) is an aggressive cancer of the blood that rapidly leads to death if untreated. If AML is treated in time with intensive chemotherapy, this can lead to complete remission of the cancer (i.e. clinical control of the disease). However, in most cases the AML returns in a more aggressive form leading to the death of most patients, although some can be rescued by bone marrow transplantation. Overall less than 30% of AML patients survive long-term, a statistic that has improved only very modestly over the last 20 years, highlighting an urgent unmet clinical need. Additionally, other cancers related to AML, including the myelodysplastic syndromes (MDS) and the myeloproliferative disorders (MPD) whilst less aggressive, also remain incurable and lead to the demise of most sufferers. Also, these diseases are much commoner in older people for whom intensive chemotherapy is inappropriate.

The reason for this lack of progress has been our poor understanding of how AML and related cancers develop and what their treatment vulnerabilities are. Over the last few years, the development of DNA sequencing techniques has enabled us to identify the genetic changes that drive AML, understand how it develops and to help develop 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)?

The proposed project aims to directly address an unmet clinical need and help develop new treatments against AML and related cancers by: i) improving the understanding of how genetic changes cause AML, ii) understand the role of ageing in the development of these diseases and iii) identify potential therapies. It is hoped that the proposed work will identify and validate new treatment targets against AML and related cancers. Our aspiration is to help develop new treatments that can be tested in patients within the next decade. Also, we hope to identify factors related to ageing that affect leukaemia development in order to prevent the disease from developing. 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 the study of leukaemia and other cancers.

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

We plan to use up to 27,000 mice, most of which will be used for breeding. Animals used for experiments will be: i) mice with genetic changes to introduce and study human leukaemia mutations, ii) mice aged to investigate the impact of ageing on leukaemia development, and iii) mice used to identify or test the validity of new treatment approaches.

In the context of what you propose to do to 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 individually ventilated cages with a maximum of 5 mice per cage with food and water always available. Genetically modified (GM) mice with human leukaemia-causing mutations will be kept for up to 20 weeks and then killed to obtain cells for study. Others will be kept for longer to determine if they develop leukaemia. Some will be given injections, including into the thigh bone and under anaesthesia, to give them mutated or cancerous/pre-cancerous cells or to activate mutations. Injections into the thigh bone are needed because certain types of leukaemia do not grow if injected elsewhere. Some mice will receive prior irradiation to enable injected cells to grow and not be rejected by the immune system. Some GM mice will be aged to test if they develop leukaemia and some will undergo non-invasive imaging to quantify leukaemic cell numbers/volume. Mice will be monitored daily when at risk, so they are detected early and killed humanely when unwell. Mice used for ageing studies can develop the normal effects of getting older (e.g. reduced mobility, weight gain, reduced activity), but if they exhibit significant suffering they will be killed humanely. Mice used to study drugs and therapeutic interventions, will either be those developing leukaemia naturally or others injected with leukaemia cells. These mice will be given treatments used in mice before aimed to reduce, slow or stop leukaemia development. Treatments used here will be given at doses expected to cause only mild to moderate side-effects. However, if significant side-effects are

observed, mice will be killed humanely. At the end of procedures or ageing studies, all mice 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

Most of our research does not use animals and relies on studying primary human samples and leukaemia cells in culture. However, we need to use mice to study the effects of leukaemia-causing gene changes in the body and appreciate how leukaemia develops in a living animal in order to understand how to reverse this process. Also, animals are required to study how getting old affects disease and to determine if particular treatments are not only effective for killing cancer cells in culture, but also do the same in a living animal, which is an essential step for progressing treatments towards clinical development.

Reduction

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

Reduction

To reduce the numbers of animals we use we have adapted the breeding strategies to set up matings generating mostly animals with the genetic make-up of interest. Additionally, we draw on our expertise to calculate the minimum number of animals required for our experiments. Also, we recently developed a genome-editing technique for generating blood stem cells with leukaemia-causing changes, which can be transplanted and studied in mice, significantly reducing the number of mice required for creating genetically altered mice.

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 so that some experiments using animals do not have to be repeated.

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

To reduce the suffering of our mice, we will be: 1) designing genotyping (DNA) tests so that they can be performed from ear punch biopsies, limiting the need for tail biopsies which cause more pain, 2) increasing expertise for better assessment of the welfare of animals at risk of developing tumours/leukaemias so that ill mice are identified early, 3) having a process whereby sick animals are identified as quickly as possible and culled if necessary, 4) developing or importing mice that can be used in transplantation experiments without the need for irradiation, 5) using a mouse strain that enables the transplantation of genetically changed into mice to avoid the need to develop/breed new mouse strains with the genetic alterations, 6) allocating technically difficult procedures to the best-trained team members to ensure they maintain their skills and minimise operator issues with procedures.

Surgery

Mice will be kept warm following surgery to ensure they remain warm.

Analgesia

All animals may experience some post-operative pain or discomfort following surgery. Pre-, peri- (during) and post-operative pain killers will be given and maintained after surgery for as long as is necessary to alleviate pain.

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’.

We use a sophisticated animal tracking system to ensure welfare data on all animals can be readily accessed/analysed.

We will comply with [best practice guidelines, e.g., the Home Office Minimum Standards for Aseptic Surgery and the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery, and Guidelines for the Welfare and Use of Animals in Cancer Research (British Journal of Cancer (2010) 102, 1555-77).

PROJECT 248

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.

Word limit; 1000 words

Project Title	Genetics of Hearing and Deafness
Key Words	Hearing, Genetics, Deafness, Ageing
Expected duration of the project	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):

Hearing impairment is the most common sensory deficit in the human population. Our studies aim to identify the genes and disease processes that underlie this condition. To do this we will investigate mouse models of early- and late-onset hearing loss and otitis media.

Currently, only around two-thirds of the genes for human non-syndromic deafness (deafness as the only clinical condition) have been identified. In addition, the vast majority of genes underlying human deafness syndromes (deafness in association with other clinical conditions) are unknown. Furthermore, 31% of people 60-69, and 63% of those over 70 suffer a significant age-related hearing loss (ARHL), making ARHL the most common sensory deficit experienced by the elderly. However, very little is known about the genes and disease processes associated with this prevalent condition.

In addition, we are keen to continue to advance our understanding of the genes and molecular mechanisms involved with chronic otitis media (OM). Chronic OM with effusion (COME) or “glue ear” is the most common cause of hearing impairment in children, causing language delays, learning and behavioural problems. Ventilation of the middle ear with tympanostomy tubes remains the best available treatment, and is the most common hospital operation for children in the UK requiring general anaesthesia. In the US the annual health care costs associated with this condition are estimated at $4 billion. However, while we know there is a very significant genetic component to chronic OM, currently we know very little of the underlying genes or pathways involved.

What 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 bring many benefits including: a better understanding of the process of hearing; knowledge of genes involved with both sensorineural hearing loss and middle ear disease; insight to the processes causing the age-related decline of hearing; and, the opportunity for better diagnosis and counseling of patients with non-syndromic and syndromic hearing loss. In the longer-term, we hope our project will identify targets for therapeutic intervention, or replacement therapy, to ameliorate hearing loss.

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

Approximately 52,500 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 with hearing loss will be sensory deprived so we avoid individual housing as this may increase stress. However, these mice can otherwise be healthy, with no additional welfare concerns. Moreover, the tests we use to assess hearing and associated traits (e.g. balance) are generally non-invasive and well tolerated. Where appropriate we use general anaesthetics, but we limit the number and frequency of these. Given that we are breeding mice that are expected to be sensory deprived, and that some of our tests require the animal to be under sedation, the expected level of severity is moderate. By this we mean the animals may experience short-term moderate pain, suffering or distress, or a moderate impairment of the animals wellbeing or general condition. At the end of the experiment mice will be humanely killed and, when appropriate, tissues taken to allow assessment of: cellular structures; protein localization/levels; and/or, gene transcript abundance, which will help further characterize the condition.

Application of the 3Rs

Replacement

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

Replacement

The mammalian auditory system is highly complex, having neuronal connections that need to be maintained from the cochlea to the brain. Given the complexity of the auditory apparatus, with its myriad of interacting cell types, it is currently impossible to reproduce and study this biological system using non-animal alternatives. In particular, the study of the ageing cochlea and the chronic middle ear inflammation seen in otitis media is not possible in an *in vitro* or *ex vivo* system.

However, as we begin to investigate our new models at the molecular level we may be able to make use of newly developing techniques. For example, a group has reported a 3D culture system that can generate large numbers of functional inner ear cells. However, these ‘organoid’ cells resemble vestibular hair cells rather than auditory cochlear hair cells.

Moreover, while non-mammalian model organisms (e.g. fly, worm and fish) are unsuitable for many auditory studies they can be employed for preliminary expression analyses to help prioritise genes for further investigation. Indeed, we have set up a collaboration with a team in the United States to assess the expression of novel hearing loss genes arising from our mouse studies in the fly.

Over the duration of this licence we will continue to monitor the development of new techniques that offer the potential to replace the use of animals, and if they become available we will implement these.

Reduction

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

Reduction

We will regularly analyse our data using statistical methods to ensure that the lowest possible number of animals are used per test group, while still giving reliable and robust data. Indeed, before embarking on new projects we use our prior knowledge and experience to estimate the lowest number of test mice required to generate robust data, and as such can manage our breeding strategies accordingly so as not to over-breed. In addition, where possible we maximise the data obtained from each mouse by combining tests in the same animal, which allows the interpretation of data to be correlated directly rather than inferred.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 continues to be the predominant model organism for hearing research in part due to the close similarities in the structure and physiology of their auditory apparatus when compared with that of the human ear. Additional strengths of the mouse as a model for studying the functional genomics of the mammalian auditory system include: the close evolutionary relationship of their genome to humans; short lifespan; genetic standardization; and, the genetic toolkit available for the manipulation of their genome. Indeed, mouse genetics has played an important role

in our understanding of the development and functioning of the mammalian auditory system. Many deaf mouse mutants have been identified and characterization of these have enabled genes critical for hearing function to be elucidated. In addition, mouse mutants continue to help elaborate the molecular mechanisms and disease processes associated human hearing impairment.

Importantly, while non-mammalian organisms (e.g. fly, worm and fish) can be used to model certain cell types found within the mammalian cochlea, they cannot be used to study the entire process of sound acquisition and processing.

We will minimize harm to animals by applying the highest possible welfare standards and monitoring, so that problems are identified, remedied quickly, and used to inform future practice. We will collect data by the least invasive and most efficient route, and when pain is likely to occur (e.g. in a procedure such as blood sampling) we will employ anaesthetic. Through prior planning, our aim is always to try and anticipate potential problems so that they can be avoided or managed appropriately, such as putting in place adequate monitoring and treatment protocols (e.g. when breeding a new mouse model for the first time, or carrying out a new procedure).

In addition, we will continue to make use of new technologies, such as genome editing, to generate mouse lines that lack confounding characteristics, which will allow us to more easily identify affected from non-affected animals. Moreover, it will also mean that the wildtype and heterozygous littermate controls will not harbour these confounding characteristics, e.g. age-related hearing loss, thus reducing suffering in these mice.

PROJECT 249

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Novel therapies for Parkinson's disease
Key Words	dopaminergic neurons, stem cells, novel drugs, microglia, alpha-synuclein
Expected duration of the project	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;
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 overall aim of this project is to investigate novel therapies for Parkinson's disease. Currently the available drugs treat the symptoms of Parkinson's, but do not slow or reverse the disease. A major aim of this project is to develop a cell transplantation therapy for Parkinson's that restores the dopamine-producing nerve cellst that die in this condition. The second major aim is to test the most promising drugs with potential to halt the progression of this disease. These drugs will first be tested using non-animal technologies, and only the most effective and potent drugs will be tested in a Parkinson's animal 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 benefits include an increased understanding of the disease processes of Parkinson's, and importantly the discovery of disease-modifying therapies that could slow, stop or reverse this condition. This work could led to new therapies that improve the quality of life of people diagnosed with Parkinson's. The knock-on benefits include a reduced burden on the healthcare system and increased economic productivity.

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

The rat will be used to model Parkinson's and as a platform for testing potential therapeutics. Over this 5 year project we expect to use about 600 genetically-modified rats and an additional 600 wild-type rats for the 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?

Some of the rats will be allowed to age naturally and subjected to mild behavioural tests and tissue collected. A small number of animals will be subjected to medical imaging, such as PET and MRI, and this requires general anaesthesia, which is a procedure of moderate severity. Other animals will be subjected to surgery to induce neurodegeneration or deliver cell therapies to the brain and other agents. This also requires general anaesthesia and is a moderate severity procedure. In the end all animals will be sacrificed by a humane method to collect tissue for molecular and/or pathological analysis.

Application of the 3Rs

Replacement

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

Replacement

The pre-clinical testing of novel therapies for complex neurodegenerative disorders like Parkinson's disease (PD) requires a whole animal system. The complicated architecture of the brain and complex interactions between neurons, astrocytes, and oligodendrocytes is very difficult to model in cell culture. High-throughput screens and initial drug-testing will be performed in patient-derived neuronal cultures, but getting a drug to a human clinical trial, and eventually to patients will require a demonstration of efficacy and safety in an animal model. Only the most promising drugs, based on data on human cultured neurons, will be tested in the rat models. In terms of cell-based therapies, an animal model is required to demonstrate that transplanted cells can re-build a neuronal network *in vivo*. There does not exist a surrogate assay for this type of therapy.

Reduction

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

Reduction

Rat experiments will only be conducted for novel drugs when there is compelling molecular and cell culture evidence that the therapy has a high likelihood of being efficacious. Potential therapies will be prioritized based on all available evidence, and the top candidates will move ahead into rat experiments.

For each experiment, power calculations will be used to ensure that (i) statistically significant results can be obtained, and (ii) the number of rats is kept to a minimum to achieve these 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

Before commencing with any animal experiments, compounds will be extensively tested in human neuronal cultures for efficacy against Parkinson's-like defects. Only drugs that have a strong body of experimental evidence to reduce or prevent Parkinson's pathology will be tested in rat model of Parkinson's.

We use rats instead of mice to model PD for several reasons: (i) rats have more complex behaviour than mice, which allows for more human-like behavioural tests to be performed, including important cognitive assays, (ii) rats have larger brains, which allows for PET and MRI imaging and easier cell transplantation, and (iii) rats are more susceptible than mice to Parkinson's symptoms caused by a protein called alpha-synuclein. This protein is critically involved in progressing Parkinson's in humans, and therapies that work to stop this protein in rats will stand a higher chance of working in humans.

Animal welfare and well-being will be carefully considered and monitored throughout this project. All rats will be normally group-housed and provided with enrichment material, unless an experiment requires otherwise. Rats undergoing a surgical procedure, such as stereotactic injection, with given general anaesthesia, and treated with appropriate analgesia and care. Rats undergoing behavioural tests will be carefully habituated by the handler to minimize undue stress. Our animal facility maintains very high welfare standards, and when necessary will seek advice from the Named Animal Care & Welfare Officer (NACWO), the veterinarian, or other qualified staff to minimize any suffering.

PROJECT 250

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	The genetics of Wilms' tumours and normal kidney development
Key Words	kidney development, Wilms' tumour, stem cells, kidney regenerative medicine
Expected duration of the project	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;
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):

Many kidney diseases are due to things going wrong when the kidneys develop, or are caused by the processes that are important during development getting active again later in life. To treat kidney diseases, it is therefore important to understand how the tissues where the disease is found develop before birth. In addition, for many kidney patients the only options for treatment are dialysis, which has an enormous negative impact on the quality of life, or a kidney transplant, for which there are not enough donor kidneys available. An alternative for this would be kidney regenerative medicine, in which new kidney tissue specific for the patient is made in the lab and used to replace the damaged kidneys. However, before this is possible again much more needs to be known about normal kidney development.

In this project we will study the earliest stages of normal kidney development in mice, by studying the genes that are mutated kidney cancers in young children, known as Wilms' tumours. These are the result of normal kidney development going wrong, when the stem cells which normally form the actual filtering units of the kidney (nephrons), lose their control, giving rise to tumours instead. Therefore, understanding what goes wrong in these tumours will give important information on how these cells are normally controlled.

In this project we will use mouse models with mutations in the same genes as found mutated in Wilms' tumours. We will study how this disrupts the normal development of these kidneys and from that we can understand how the normal kidney 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)?

This project will teach us much about normal kidney development, how this is disrupted in disease and how we can maybe use this information in the future to make new kidneys tissue in the lab. In particular we will get much more information about Wilms' tumours. This will help us to develop new therapies for these patients. The mutant mouse models we will use in the project could be used to test these therapies.

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

I estimate we will use 13,050 mice over 5 years. Importantly, the embryos that we will use for most of the experiments will be of such a young age that they are not yet covered by the law, so these are not included in this number. REDACTED I expect that approximately 85% (just over 11,000) of these animal will be born during the breeding of the transgenic animals but not have the desired combination of mutations, and will not be used for further experiments, or will only be used for further breeding to generate the embryos for the actual experiments. Where possible we will use statistical tools, like power calculation, to determine the lowest number of animals we need to answer our questions.

In the context of what you propose to do to 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 only be used for breeding and to generate embryos to study the kidneys from. In most cases we will take the embryonic kidneys and let these develop further in a dish, and use an automated microscope to follow what happens over several days. A small number of mutant animals may be born to study which could develop Wilms' tumours. We will carefully follow these animals for any signs of illness, for instance losing weight or scruffy fur. If this happen they will be humanely culled and we will study the kidneys for tumours or other problems. We will also conduct surgery on a small number of mice to place cells for example, under the skin or next to the kidney to test how they grow and whether they start to look like tumours.

Application of the 3Rs

Replacement

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

Replacement

Kidney development is dependent on three different cell types working together, 'talking' to each other via chemical signals and responding to each other. At present it is not yet possible to study the complexity of this at the level we need for this work using only cells in a dish. Moreover, many of the kidney cells used in cell culture

experiments are cancer cells (but from a completely different form of kidney cancer than Wilms' tumours) and are therefore not useful for our work. We have however included experiments in this project that will help us to develop new cell systems to study some aspects of kidney development. I hope over the course of this project this will lead to more possibilities to use cells in dishes instead of animals. However, more work requiring animals will be needed to develop and test these systems first.

Reduction

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

Reduction

In our experiments we use combinations of different mutations in mice. Our main way of reducing animal numbers is by crossing these different models in such a way that as many animals as possible will have combinations of mutations that we need in our experiments.

Second, we reduce animal numbers by analysing as many kidneys as possible in kidney organ cultures, in which we take the kidneys from a very early embryo and let these kidneys develop further in a dish. Where possible we will use an automated microscope system with which we can follow the development of mutant and normal kidneys at the same time for up to 7 days. This way, we get information throughout this time period from the same kidneys, instead of needed different kidneys for different developmental stages. Again this greatly reduces the number of animals we will need.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 the same type of kidneys as we have. In fact, most that we know about human kidney development was originally discovered in mice and has recently been confirmed in human embryonic kidneys. Animals that are not mammals, eg fish, have very different kidneys to man and would not be suitable.

We will make sure that we know as quickly as possible which animals we need for our experiments, so we don't keep animals unnecessarily long. Animals that might get sick will be followed very closely and humanely culled and studied as soon as we see any signs of illness.

PROJECT 251

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.

Word limit; 1000 words

Project Title	Protein kinases and phosphatases in health and disease
Key Words	Parkinson's Disease, Huntington's Disease
Expected duration of the project	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):

Parkinson's disease and Huntingdon's disease are serious conditions,causing progressive loss of brain function and muscle control. The underlying causes of the most common form of Parkinson's disease are unknown, but we are beginning to understand the genetic basis of the rarer inherited forms. About 20 clear-cut genes have been identified in which mutations are associated with the death of defined sets of nerve cells (neurons) and the symptoms associated with Parkinson's disease.This suggests that the products of these genes are involved in similar or the same molecular mechanisms as operate in the more common sporadicform of the disease. Our research involves making defined changes in the equivalent genes in mice, investigating what the molecular consequences are and determining how they may cooperate in causing signs of disease. We are already studying two genes, called LRRK2 and alpha-synuclein,each of which has been implicated in Parkinon's disease. Specific inhibitors of LRRK2 may prove to be new medicines for the disease, but it is still unclear how LRRK2 interacts with alpha-synuclein or with the products of any of the other genes implicated in the disease.

The genetic basis of Huntingdon's disease is known, but the molecular events that lead to symptoms are not well-characterised and there are as yet no effective medicines. REDACTED

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

Our basic research is aimed at understanding the molecular mechanisms that underlie the initiation and development of serious neurological disorders such as

Parkinson's disease and Huntington's disease. We anticipate that this work may lead to the identification of new targets or new medicines for these devastating disorders.

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

We expect to breed and maintain a number of mouse lines in which specific genes have been altered. We expect to use up to 18,000 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?

In our experience, mouse models of these human diseases exhibit very few signs of adverse welfare, though tissues analysed after the animals have been humanely killed demonstrate informative molecular changes. We not require animals, if they do show any signs, to progress beyond early signs of disease. Animals may be administered chemicals that are believed to be potential new medicines, in order to measure their effects on the molecular mechanisms and on the development of any outward signs of disease. These chemicals are not, in themselves,expected to cause any harm. We will conduct some tests of animals' memory and motor skills, but these too are not expected to cause harm.

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 define the role Parkinson’s and Huntington’s genes have in the biology of the different types of regions and neuronal cells brain tissues, a mouse model is needed. Also, to establish whether treatment with LRRK2 inhibitors will delay early deficits seen in mouse models of Parkinson’s disease is also best addressed in a mouse model. The genes suspected of involvement in the development and progression of Parkinson’s disease and Huntington’s disease may have important activities in several distinct areas of the brain (and, in fact, inother organs of the body too). Their actions therefore cannot yet be modelled sufficiently well without using intact animals. We will also culture primary neurons from mice and make use of brain tissue to undertake in vitro studies of the signalling pathways. In parallel we are devoting significant effort into in vitro investigation of the genes we are studying in mice. The knowledge gained from both the in vitro and in vivo work is expected to provide new fundamental novel information on how genetic manipulations that mimic disease causing mutations influence signalling systems and biology in neurons and the brain. This would be expected to lead to follow up studies that could be undertaken in an in vitro system rather than in animal models.

Reduction

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

Reduction

We will manage our breeding colonies so as to produce the right amount of tissues for laboratory analysis. When we are testing novel chemicals for their potential as medicines, we shall use statistical power calculations in order to design experiments that will give robust answers while not wasting animals. We will undertake power calculations to ensure that we use the lowest number of mice to get scientifically rigorous results. REDACTED This should help reduce the number of mice needed assess impact of mutations on LRRK2 pathway activity.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 ease with which specific genetic alterations can be introduced into mice, and the molecular similarities between their nervous systems and our own make them the ideal species in which to investigate the basic mechanisms of neurological disease. Our studies will not require animals to exhibit signs of advanced disease, indeed the majority of the animals will live apparently normal lives and will be killed humanely before tissues are harvested for detailed laboratory studies. All mice will be very carefully monitored to minimise welfare costs including monitoring signs of reduced weight loss, neglect of grooming, reduced ambulation, early signs of movement impairment and resistance to passive movements.

PROJECT 252

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.

Word limit; 1000 words

Project Title	Investigation of anti-insulin receptor antibodies as a potential therapy for extreme insulin resistance
Key Words	Insulin resistance, antibodies, Donohue Syndrome, Rabson Mendenhall Syndrome, Insulin receptor
Expected duration of the project	3 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;
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 hormone insulin is critical to life and works together with the insulin receptor on the surface of cells to drive energy storage after nutrient consumption. Rare people with genetically damaged or “mutant” insulin receptors have an uncommon form of diabetes with extreme resistance to insulin that is very difficult to treat using currently available therapy. A great deal of information is available about the structure of the insulin receptor, and the consequences of such mutations, but to date, there has been no reliable way to directly bypass the blocking effect of the receptor mutations in affected people. As a result, death occurs in infancy or in the second decade of life for less extreme cases. Our work aims to use the power of specially created antibodies that bind and activate the insulin receptor in a different way to insulin, to overcome the lethal consequences of insulin receptor mutations. By investigating how these antibodies are able to activate damaged receptors, and by testing whether they can lower blood glucose in mice with the same mutations that we see in human patients, we aim to take a major step towards developing these antibodies as potential life-saving new treatments for this group of conditions.

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

This research aims to test mouse antibodies as a potential novel treatment of severe, life-threatening forms of diabetes caused by mutated forms of the insulin receptor. The results of these studies will be a key step in helping to decide whether the antibodies should be developed further and humanised to enable their use in people. If the antibodies show promise, a further potential benefit may even be that

they are developed for use in a wider range of clinical settings and more common forms of diabetes A secondary potential benefit will be increased knowledge of the molecular mechanisms by which some insulin receptor mutations that don't have obvious functional defects when studied in vitro cell models cause severe disease in humans. Understanding of these mechanisms may potentially identify further ways to overcome the effects of these mutations leading to other therapeutic strategies.

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

Over 3 years, approximately 770 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?

These mice will be genetically altered such that the normal insulin receptor can be removed from their livers with a single injection. Mutant insulin receptors will then shortly afterwards be 'added back' to the liver with a second injection. Re-introduction of the mutant insulin receptors will permit the degree of impact that each mutation has on insulin receptor function and glucose metabolism to be tested, enabling a baseline to be established before the antibody treatments are tested. The most invasive procedure in living mice will be injections into veins, or blood sampling from veins. This 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. We are expecting to see high insulin and maybe high blood glucose levels once the insulin receptor has been knocked out of the liver, however prior studies of liver-specific insulin receptor knockout suggest that diabetes will not be a threat to wellbeing. Hypoglycaemia due to antibody administration is unlikely at low doses. At higher doses of antibody, sucrose can be included in the injection to avoid any transient hypoglycaemia associated with anti-insulin receptor antibody injection. At the end of the study, and to ensure the maximum information is gained from every mouse and each experiment, animals will be humanely sacrificed so their livers and other key tissues can be examined.

Application of the 3Rs

Replacement

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

Replacement

REDACTEDTogether with the observation that some mutant receptors don't have obvious impairments when studied in cell in vitro models but do cause severe disease in humans, confirms that cellular studies are incomplete surrogates for assessing in vivo insulin action as they cannot represent a whole-body system which

includes variable, pulsatile insulin exposure, and involves extensive receptor turnover and recycling. Thus we are at a point where studying the glucose-lowering effects of the antibodies in a living creature is an essential next step. This cannot be done in rare, very unwell babies without extensive prior in vivo testing, and because we require the study of specific mutant insulin receptors, the animals used have to be amenable to genetic manipulation. Thus, genetically modified mice represent the only practical in vivo model.

Reduction

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

Reduction

We shall minimise the number of animals first with good experimental design that relies on injections of genes rather than requiring extensive breeding of new animals. This approach substantially reduces the amount of breeding and numbers of animals required and avoids problems due to the primary genetic defect. REDACTED Because we have a good understanding of the variability in our measurements, we can be precise in using power analysis software in calculating exactly how many animals we need to show a clinically significant effect size. Together with the refinement of the experimental model, techniques and animal environment (outlined below) this will prevent unnecessary experiments and therefore 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

Along with the use of appropriate anaesthetics and analgesics, animal suffering will also be minimised by having tests performed by experienced staff familiar with the protocols which will further reduce pain and stress to the animals. Separate to this, as much as possible the cage environment will be enriched as to encourage normal mouse behaviour, thereby reducing stress of the housed animals. Our experimental design of injecting genes will avoid the liver pathologies reported previously with congenital insulin receptor knockout mouse models thereby minimising any potential adverse effects. Furthermore, utilising this approach, the time frame from inducing

liver knockout of the insulin receptor and performing the experiments will be minimised thus reducing the time the mice will display a diabetic phenotype.

PROJECT 253

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.

Word limit; 1000 words

Project Title	Developing new treatments for congenital and acquired disorders of the urinary tract
Key Words	kidney disease, therapy, bladder disorders, congenital abnormalities, glomerular disease
Expected duration of the project	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;
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):

This research aims to develop new treatments for chronic kidney disease. Our specific aims are to:

(i) work out the pathophysiological and molecular processes these therapies act on;

(ii) test the treatments in pre-clinical models, examine their safety profile and attempt to target the treatments specifically to the urinary tract;

(iii) use genetic data from renal patients to find new therapeutic targets to prevent kidney disease;

(iv) establish new interventions for kidney disease using stem cells;

(v) perform high-throughput screening to identify new drugs which may treat chronic kidney 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 work in this project licence has the potential to improve patient care; the incidence of chronic kidney disease has doubled in the last ten years in the UK to 108/million population and is rising. There is currently no cure for CKD and patients require either transplantation or dialysis, interventions which are costly to health services and place a high burden on patients. Therefore, there is an urgent need to design new therapies to improve the life of patients with kidney damage.

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

Less than 1,500 postnatal rodents/year will be used. 200 adult zebrafish/year 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 in this project will be used for: production of timed rodent pregnancies to obtain embryos for gene expression analyse and obtain embryonic kidneys for transplantation into kidneys of neonatal and adult hosts; breeding and genetic typing of mutant rodents carrying genes which cause potentially harmful renal defects; induction and treatment of renal diseases in adult and neonatal rodents and adult zebrafish. Some of the animals have genetic alterations and others have induced renal disease. Animals will have appropriate anaesthetics and pain relief to minimise discomfort, but any becoming overly distressed will be humanely killed. Occasional blood sampling, collection of tail-tips for genetic testing and injections will be required. These animals should experience only handling discomfort and transient pain at the time of the procedures. In the cases where the genetic or acquired defect might affect postnatal lifespan, some of the procedures are designed to treat their underlying condition using either surgery or chemical therapies, but we will kill the animals by a human method if they show signs of distress. The severity limit of the procedures proposed is no more than moderate and all necessary measurements will be taken to minimise the sufferings of the animals during the whole procedure.

Application of the 3Rs

Replacement

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

Replacement

Animal studies are required because to urinary tract disorders are complex processes involving not only interactions between different cells but also circulating factors and blood supply. A substantial proportion of our work uses cell/organ culture but these can only to a limited extent replace whole animal experiments.

Reduction

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

Reduction

Animal numbers will be minimized by making sure there is no duplication of previous work, optimising breeding programmes, performing pilot studies and designing experiments with the assistance of a 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

Rodents are the lowest species in which suitable models replicate features of human urinary tract disease. Avian species are necessary for chorio-allantoic grafting experiments. Zebrafish will be utilised for examining the roles of new genes and performing drug screens to identify novel drugs to treat chronic kidney disease. The protocols in this licence will keep suffering of the animals to a minimum by proper training of staff, using minimum volumes and lowest number of procedures and completing experiments in the shortest possible time. All animals will be inspected regularly to ensure there are no signs of ill health.

PROJECT 254

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.

Word limit; 1000 words

Project Title	Analysing chromatin modifying enzymes in vivo
Key Words	Genes, knockout, histone, epigenetic
Expected duration of the project	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):

Every human cell contains approximately 20,000 genes, but only around a third of these are active in any given cell type. A liver cell, for instance, will need to make enzymes in order to detoxify alcohol, but genes that are required in the brain have no use in the liver and so are deactivated. Individual cell types must decide which genes to switch on in order to function and then keep all of the remaining genes quiet. This is known as ‘gene regulation’. An effective method of deactivating the unwanted genes is to wrap them up in their own packaging material, a type of protein called ‘histones’. Histones take the 2 metres of DNA in each cell and package it into a nucleus only 10 microns wide, roughly the same thickness as a human hair. Histones, by their nature, hide DNA away and therefore physically regulate how the genes are read. My lab studies a family of enzymes which chemically modify histones, tightening their grip around genes, and thereby switching them off. To understand how each of these enzymes works, we use gene editing methods to create ‘knock-out’ animal models in which the histone modifying enzyme of interest has been inactivated. These knock-out mice allow us to address the physiological requirement for individual enzymes in the context of the entire 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)?

Histone modifying enzymes are potentially excellent drug targets. They are ubiquitously expressed and therefore any type of malfunctioning cell is a potential target. Furthermore, as enzymes, they contain an active site which makes them ‘druggable’ using small molecule inhibitors. Histone deacetylase (HDAC) inhibitors are already used clinically to treat cutaneous T-cell lymphoma. However, their mode

of action in cancer cells and why they exhibit toxicity towards certain types of tumours is still unknown. This work will help define which enzymes and cell types are best suited to ‘epigenetic’ therapy.

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

Our work to study histone modifying enzymes in vivo will use mice. The mouse is a good model for human development and disease. They have similar physiology (heart, brain, nervous system, etc.) and contain 20,000 genes, the same number as humans, whose function is highly conserved. Using standard genetic engineering techniques, we are able to inactivate individual genes and test the effect of their absence to the developing mouse embryo and individual tissue types. We expect to use approximately 5,000 mice in our studies over a five year period.

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

The majority of experiments performed under this license will involve the analysis of cells and tissues isolated from knock-out mice i.e. mice where one, or more genes has been deleted. Deletion of individual genes for histone modifying enzymes can have a variety of effects on the mouse. Some genes are essential and their gene knock-outs lead to lethality as an embryo. The deletion of other genes have no effect at all. We are able to get around embryonic lethality by labelling individual genes with a specific DNA sequence (LoxP site) which marks the gene for 'inactivation' in the presence of an enzyme (Cre). By introducing Cre into only a subset of cells e.g. white blood cells, we can limit gene inactivation to specific tissue types. Sometimes this may cause the development of a harmful phenotype (e.g. tumours, neurological signs, compromised immune system) after a certain age. Tumours may cause a number of physical symptoms in the mouse, such as weight loss, pain, difficulty breathing, a failure to eat or drink, tremors and reduced mobility. In all studies mice will be monitored daily and euthanized before reaching that stage to limit any potential suffering. Our experience with these methods is that the majority of mice will not exceed a mild, or moderate severity.

Application of the 3Rs

Replacement

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

Replacement

The 3-dimensional structures and cell-cell contacts required for organ development cannot be replicated in the petri dish with cells in tissue culture. Similarly, cancer progress is dependent on its microenvironment for growth, while simultaneously

evading the immune system, conditions which cannot be mimicked outside of animal models. However, parallel studies on the same chromatin associated proteins will be performed in cell lines, and results from these experiments will be used to inform and REPLACE the in vivo experiments.

Reduction

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

Reduction

The majority of our experiments are performed on tissue taken from mice following 'euthanasia'. Therefore, we will most effectively reduce animal numbers through careful colony management, accurate genotyping and avoiding the production of excess animals. The number of animals will be further minimised by co-ordinating experiments to make maximal use of tissues from individual animals. We will seek advice from the college bioinformatics service to apply the appropriate statistics to use the minimum number of animals while producing a significant 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 mouse model represents a close match in terms of embryonic development and physiology to humans. The mouse model also has the greatest number of reagents available for generating targeted mutations allowing us study the activity of individual proteins at a genetic level. Working practices will be based upon providing the most refined experience for the animal and causing the minimal possible stress. Examples include: 1) using tunnel/cup handling techniques, which will benefit GA mice developing a moderate phenotype and tumour burdened mice who are also potentially quite sick. 2) Experiments for new drug dosing regimes will be based on in vitro data and pilot studies so the experience of the animal is as refined as possible; and 3) where appropriate, animals may be scruffed for ultrasound imaging rather than putting them through an anaesthetic event.

PROJECT 255

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.

Word limit; 1000 words

Project Title	Immunology of respiratory infection and inflammation
Key Words	Lungs, infection, vaccination, age.
Expected duration of the project	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;
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):

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.

What 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.

PROJECT 256

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.

Word limit; 1000 words

Project Title	Mechanisms of sensory transduction, pain and analgesia
Key Words	Pain, neuropathy, inflammation, analgesia, nerves
Expected duration of the project	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):

The aims of this project licence are to identify the mechanisms and biological processes involved in chronic pain and to identify new targets for its treatment. Chronic pain syndromes occur in 10-20% of the adult population and are notoriously difficult to treat. Currently used drugs are largely ineffective and many have severe side effects. There has recently been progress in understanding the main sensory transport mechanisms in sensory neurons. Knowledge of these underlying mechanisms and the way in which the spinal cord and brain handle sensory information in normal and in injured or disease situations are essential since they can reveal likely points at which to focus novel therapies to treat pain.

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

It is believed that the results from this licence will provide a major contribution to the understanding of sensory disorders, including chronic pain, and will lead to the discovery of new analgesic drugs with greater effectiveness and fewer side effects than those currently available.

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

Rodents will be used as they are the lowest vertebrate group on which these types of experiment can be conducted and there is extensive relevant information on rodents. The total number of any single rodent species used in these models is expected to be less than 500 per year. The use of genetically altered mice will be used for examining the role of particular targets in the pain process.

In the context of what you propose to do to 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 described here are aimed at allowing an investigation of the mechanisms involved in pain processes. Although we will investigate classical 'acute pain' models, where the painful stimulus is of a short duration, of greater use will be models of chronic pain. Many of the proposed models are well established; though we will also look at new models, particularly those closely mimicking human disorders, such as nerve conditions associated with disease; pain caused by inflammation, osteoarthritis, diabetes and bone cancer pain. Most behavioural tests will measure the increased sensitivity of test animals to mild mechanical or thermal stimulation. For most of the protocols the most likely adverse effect will be sparing of an injured limb. The severity of the models will be limited as far as possible (mild to moderate) by limiting the time for which animals are kept following surgery or induction of pain and by the use of painkilling medicine. At the end of each protocol the animals will be 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

It is possible in some instances to use cell systems to analyse, for example, the effect of inflammatory mediators on receptor or ion channel function and such studies will be used where feasible. However, while we will study individual nerves cells in culture, it has not yet proved possible to generate stable cell lines of mammalian sensory nerve cells, our main cell type of interest, to mimic the long term effects of nerve damage or inflammation that occur in the body, or to copy the complex system of nerves that control the pain signals. In these instances the use of animals is essential. We will continually stay aware of current research literature and refine our animal models to make them more suited to the situation in humans. Where we have evidence that a model is not predictive it will no longer be used and, where possible, animal models will be replaced with *in vitro* alternatives.

Reduction

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

Reduction

Where possible each animal will be used as its own control. Where this is not possible groups of animals will be utilised. For all the experiments proposed we will use a group size which is the smallest compatible with achieving statistically

meaningful and robust results using appropriate statistical analysis. We will consult statisticians where necessary.REDACTED

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 in these studies as their extensive use in biological research has provided considerable information already on pain pathways. In addition, genetically-altered mice may be used where appropriate. These are particularly useful for defining the role of a particular target in the inflammatory or pain process, or for providing the means for examining the activity of a particular compound at a human target protein in the body. Rodents are the lowest vertebrate group on which these types of experiment can be conducted and many well characterised and limited severity models have been developed in these animals. Whilst we aim to use the animal models described in this licence to examine specifically the pain associated with chronic disease in order to understand the mechanisms underlying pain pathways and to test the effects of novel painkiller medicines, the severity of the models will be limited as far as possible by limiting the time for which animals are kept following surgery or induction of pain and, where possible, by the use of painkilling medicine.

PROJECT 257

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.

Word limit; 1000 words

Project Title	Early diagnosis and treatment of pancreatic cancer
Key Words	Pancreatic cancer, Early diagnosis, Combination therapies, Primary Cilia, Hippo
Expected duration of the project	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;
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 fundamental objectives of this research are to identify the initiating events that lead to pancreatic cancer so that we can diagnose the disease whilst it is still treatable and to develop personalised treatment regimens.

In the great majority of cases of pancreatic cancer the disease is already well established at the time of diagnosis, and even for the few of those patients for whom surgery is then still an option only some 6% survive more than five years. In stark contrast, in the (currently very rare) cases of early diagnosis the rate of survival beyond five years is 57%. It is quite clear therefore that early detection of the disease is crucial.

The oncogene gene Kras, is abnormally activated in most cells that give rise to pancreatic cancer. Research has shown that this is not sufficient to give rise to disease, additional events are required before cells can become cancerous. We are investigating two of these initiating events, the loss of the primary cilium, which acts as the cells antenna enabling cells to communicate and inactivation of the Hippo signaling pathway. We believe that both of these events can promote initiation of pancreatic cancer. The aim of this project is to understand if this is the case, how these events are regulated and if these can be used to detect early disease in patients.

There have been no improvements in detection for thirty years. It has recently been shown that there are different subtypes of pancreatic cancer and each subtype responds differently to the current treatment strategies. Another aim of this project will be to test new combinations of therapies on pancreatic tumours in mice that

represent each human subtype. This will enable us to develop personalised treatment regimens.

What 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 our understanding of the initiating events that lead to pancreatic cancer. This will have direct benefit to the scientific community striving to understand this disease. Our work will be made publicly available in journal articles. Genetically altered mice generated during the project will be made available to other researchers. We anticipate this project will identify at least 3 potential biomarkers, which following clinical validation, can be used to detect pancreatic cancer earlier. This will increase the number of treatment options available to clinicians to treat pancreatic cancer patients and may even enable us to detect pre-cancerous lesions before a tumour develops. This project will also identify new combinations of treatments to specifically target each pancreatic cancer sub-type. The results from these studies will form the basis for clinical trials leading to the development of personalised treatment regimens. This work is part of a larger consortium called Presicion Panc, comprised of research scientists and clinicians across the UK. Together we will improve both diagnosis and treatment for pancreatic cancer patients.

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

We will use up to 8000 mice in our studies 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?

At least a fifth of the animals used in this study will suffer only mild effects. We will use mice to model human pancreatic cancer. Depending on the mutations the mice have they may develop pancreatic tumours from 6 weeks or not until 9 months old. The tumours may invade other organs. Tumour cells will also be injected into the pancreas of healthy mice involving surgery. This is necessary as this produces a reproducible system to test new therapies and also causes less harmful effects to the mice than genetic pancreatic cancer mouse models. Therapies will include known and new drug combinations as well as radiotherapy. At the first sign of suffering mice will be humanly killed. Tumour growth and metastases will be monitored at regular intervals to animals do not succumb to tumour burden before they are humanely killed. At the end of experiments mice will be humanely killed. At the end of the project any genetically altered mice that could be of use to future projects or other scientists will be maintained under a renewed licence or sperm/embryos will be frozen down for long term preservation.

Application of the 3Rs

Replacement

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

Replacement

We will replace the use of some animals by making pancreatic organoids from the pancreas and pancreatic tumours from mice. These are 3D cultures of cells in a dish that recapitulate most physiological aspects of the pancreas or pancreatic cancer. However, we cannot fully recapitulate the complexity of a living organism or tumour in culture, in particular this does not allow us to study the interactions between the tumour and surrounding cells that can influence tumour growth. Therefore it is necessary to use an animal model. We have chosen mice because unlike lower animals they share 98% of their genome with humans and because mouse models that replicate human pancreatic cancer already exist and are well characterised.

Reduction

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

Reduction

We will use enough mice so that the data we produce is meaningful but not beyond this. We will use established protocols so that minimal pilot experiments are required.

As in humans, there is variability in the development and onset of pancreatic cancer in mice. To control for this variability would require large numbers of mice and so to avoid this we will use a method to deliver standardised tumour cells to the mouse pancreas that generates highly reproducible tumours and therefore reduces the numbers 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

All animals will be closely monitored for early indications of distress. Specific endpoints have been set out and upon reaching these animals will be humanely killed. We shall use ear notching the least severe method for identification, this tissue can also be used for genotyping. Only healthy animals will be used for breeding. Where possible genetic mutations will only be activated once tissue is in culture and therefore the mice will not experience any adverse effects. Pancreatitis will be induced using an injectable inflammatory agent, Caerulein, rather than

surgical methods. Caerulein is milder than other agents, the dose can be modulated and the effects are reversible upon withdrawal. We will implant predefined tumour cells into the mouse pancreas rather than using animals that are genetically predisposed to develop tumours, because this will generate tumours with predictable development and less side effects on the animals. We will inject into the tail of the pancreas as this results in less aggressive tumours and required a smaller incision reducing the impact of surgery. Inhalation anaesthesia will be used when needed as this is less invasive and easier to regulate. Specialised radiation platforms will be used to ensure tumour specific targeting and avoid unwanted side effects. All agents to be tested will be provided via a route causing minimal discomfort and that best recapitulate the delivery route that would be used in patients. We will also trial the use of mini pumps to prevent repeated administration of agents. Pain relief will always be given before and after surgeries or any intervention with the potential to cause pain. Tumour growth will be closely monitored using non-invasive imaging such as ultrasound or MRI. A fluorescence based method will be used for whole body imaging to detect metastasis. Specific endpoint have been set out for tumour size, upon reaching these animals will be humanely killed.

PROJECT 258

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.

Word limit; 1000 words

Project Title	Therapy targeting Chemokine receptor
Key Words	Transplantation, cell migration, chemokine
Expected duration of the project	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;
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):

We aim to identify the biological pathways responsible for cellular movement during inflammation associated with transplantation. Furthermore, we will identify methods for preventing or reversing this process in animal models that are applicable to the human immune system, specifically the conditions of solid organ 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)?

Organ transplantation is the treatment of choice for many patients with end-stage diseases. Waiting lists are lengthening but the supply of donor organs has not expanded to meet this demand. Chronic rejection resulting in late loss of graft function now represents the greatest clinical challenge, with many patients re-joining the waiting list years after an initial transplant operation. Indeed, as many as a third of patients joining the kidney transplant waiting list have already chronically rejected one or more previous transplants. The work that will be carried out under this licence is a continuation of research that has already identified a potential anti-inflammatory reagent. The data produced will help screen compounds at an early stage which have a potential beneficial role in prolonging graft survival. In addition it will also identify compounds with unacceptable side effects or unproductive line of enquiry. This will directly benefit our group and others (academic and industrial groups' actively targeting inflammatory pathways) and stop unnecessary use of animals in future. The benefit is likely to be realised in longer term (outside the time frame of this licence).

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

It is estimated that approximately 500 mice will be used during this project. Previous experience with this model has allowed statistical modelling to determine minimum group sizes of 7.

In the context of what you propose to do to 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 use humanised mouse, air-pouch, skin transplant models to decipher mechanisms governing the migration of immune cells that can be modified by therapy. We intend to employ a murine skin transplant assay. This model has some specific advantages, the surgical procedure is rapid, easy to learn, and does not necessarily need microsurgical equipment. Compared with other transplantation models, skin graft rejection is a limited and very reproducible immunological reaction. Furthermore, the rejection process does not significantly affect the wellbeing of the animal. The air pouch model causes less pain or distress than other models for studying inflammatory processes and enables a range of measures of immune function and modulation to be assessed in a reproducible manner. REDACTED Mice will however be monitored post injection of these molecules and post surgery to ensure that they show no adverse reaction. Animals under general anaesthesia will not be left unattended at any time. Body temperature will be monitored throughout the procedure and animals recovering from surgery will be monitored until fully recovered from the anaesthetic. Post-operative analgesia will be administered as required. Statistical calculations are used to determine the minimum number of animals that are necessary to show a significant anti-rejection effect. At the end of experiment all animals will be humanely killed. The expected severity of the procedures is moderate.

Application of the 3Rs

Replacement

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

Replacement

We have successfully tested the potential of our engineered chemokine molecules to modulate the passage of immune cells across blood vessel walls in tissue culture. Although cell culture assays can indicate the potential anti-inflammatory effects of a new reagent, they cannot reliably predict the effect that reagent has in protecting a transplanted organ in the body This is because immune cells in test tube might exhibit different characteristics compared to those in a natural environment. They are therefore likely to respond to the reagent in a different way. In addition, the effect of the reagent on the whole body (i.e. any toxicity) and the effect of the body on the reagent (i.e. how much reagent reaches the transplant and how fast the drug is

broken down) cannot be accurately modelled by computer simulations or cells in a culture dish

REDACTED

In order to develop this novel reagent for anti-rejection therapy it is vital that it is tested in an animal transplant model. Therefore, after extensive testing in cell culture assays, some testing in animal models of transplantation is necessary. Without it, we could not reliably assess the likely beneficial effects of new potential anti-rejection reagent in man.

Reduction

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

Reduction

Statistical techniques have been used to ensure we use only the minimum number of mice for each stage of the experimental procedure.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 allow sufficient modelling of the transplant rejection process and this would not be possible in "lower" species. In addition, genetically altered mice lacking certain chemokine receptors are available.

Suffering following skin transplantation will be minimised through administration of post-operative analgesia in accordance with veterinary advice. Additionally, many experiments will utilise mice that receive a transplant with human white blood cells in order to maximise the clinical implications learned from these experiments. This will improve upon existing animal models, many of which have limited applicability to human disease and the development of new medications.

PROJECT 259

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Investigations to improve resilience to, and control of, African Swine fever
Key Words	Disease, Resilience
Expected duration of the project	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;
No	(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):

African swine fever (ASF) is a highly infectious disease of domestic and wild pigs, such as wild boar, caused by the African swine fever virus (ASFV) that has a high death rate and causes significant animal welfare and economic impacts. There is currently no vaccine or other treatment for ASF and control of outbreaks relies on killing of infected, and neighbouring, herds and restricting animal movements. Such movement restrictions can have negative effects on animal welfare. ASF is present in many parts of Africa, where it impacts backyard farmers and prevents development of the pork industry. In addition the disease entered the Caucasus region in 2007 and it has since spread to many countries for example, Armenia, Russia, Ukraine, Belarus, Poland, Lithuania, Latvia, Estonia and most recently the Czech Republic.

In contrast to domestic pigs and wild boar, which both get sick and can die from the disease warthogs, which are the natural host of the virus in Africa, become infected without signs of illness. Previous studies have identified differences between warthogs and domestic pigs in a key gene that regulates many immune responses to viruses and may be responsible for the damage caused by infection. The objective of this project is to study if domestic pigs which have been modified so that they have the warthog version of this key gene will respond to infection with ASFV in a similar way to warthogs and, therefore, be able to survive 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 benefits of this project are to advance basic knowledge and assess the potential of genetic modification to enhanced quality of life of production animals. Specifically

the proposed challenge studies will generate data and knowledge on whether introducing genetic changes can enable animals to survive ASFV infection. In the absence of a vaccine against this disease, and together with work by our collaborators to develop genetically altered animals, this will ultimately enhance animal welfare, limit economic losses due to African swine fever disease and contribute to enhance food security worldwide.

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

Approximately 56 domestic pigs, some of which will have been genetically engineered.

In the context of what you propose to do to 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 experience some signs of African swine fever clinical disease, which include fever, reduced appetite, lethargy, depression, conjunctivitis, vomiting, diarrhoea and in-coordination. It is expected that clinical monitoring will limit the severity to moderate for the majority of animals. However, to demonstrate if the genetic alterations are able to change the course of disease such that these animals can survive infection some animals may experience severe clinical signs such as dormancy, reluctance to stand, lameness/joint swelling, respiratory signs (laboured breathing), conjunctivitis, skin blotching and fever for a short time <12h. Animals will be euthanized at the end of the experiment or upon reaching set humane end point. It is not expected that the genetic changes will result in a clinical adverse effect in the animals.

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 replicate the complex factors that influence a host's ability to overcome the ill effects of virus infection in vitro. In particular, the gene we are focussing on in this study is a key regulatory gene that has a multitude of effects in different cell types. In vitro studies cannot accurately replicate the multitude and interaction of effects that changes in this regulatory pathway will have in an animal. There is therefore no alternative method to predict the effect that alteration of the sequences of specific genes will have on the ability of an animal to resist the ill effects of ASFV infection. Swine are the target, and only, host of African swine fever virus and are therefore the only appropriate species to monitor infection.

Reduction

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

Reduction

The experiments to be carried out are peer reviewed to allow a thorough examination of the experimental design, including review by statisticians with expertise in design of animal studies.

Pilot experiment/s will establish the best dose and expected variation in clinical signs induced with the virus strain selected. This data will inform on the final numbers of animals in subsequent experiments comparing pigs with different gene sequences. To control variability, to allow smaller group sizes, animals will be of similar age, weight and genetic background and measurements of clinical signs will be blinded with respect to the treatment group. To avoid the requirement to sample un-inoculated control animals, blood samples will be taken prior to inoculation to provide a value to compare response after infection to.

Refinement

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

Refinement

No animal species besides swine/suidae (domestic and wild pigs such as wild boar and warthogs) are known to be naturally infected with ASF virus. A moderately virulent strain of ASFV will be used as such strains produce less severe signs of the disease than highly virulent strains. Our considerable experience with swine fever infections has lead us to develop and successfully apply swine fever specific monitoring protocols. This includes a 10 parameter clinical monitoring scheme that is assessed twice daily. The infected pigs will be monitored at increased frequency as signs develop to ensure they experience the minimum level of disease signs whilst allowing the scientific objectives to be achieved. Animals will be assessed against this clinical scoring protocol and euthanized upon reaching predefined criteria.

PROJECT 260

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Safety and efficacy testing of veterinary products
Key Words	
Expected duration of the project	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.

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 overall aim of this licence is to conduct safety and efficacy testing in support of marketing authorisation applications for new veterinary products, or for revisions to authorisations for licensed 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)?

The successful licensing of new veterinary products will provide additional tools for veterinarians and animal care workers in the fight against pathogens on commercial livestock facilities and will result in reduced suffering for animals and reduced financial burden for farmers.

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

Over the 5 years of the licence we expect to carry out procedures in cattle, sheep, pigs, poultry, mice and possibly horses. The maximum number of animals over the five years of the licence is expected to be around 16,000 but may be considerably lower than this.

In the context of what you propose to do to 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 products being tested under this licence are in the final stage of licensing and as such any adverse effects following use are likely to be minor (such as transient injection site reactions or pyrexia, a common side effect of vaccination). On occasions more severe adverse reactions may occur but this is extremely rare for products at this stage of testing (less than one animal in a 1000 would be expected

to have a more extreme reaction to any product). For animals included on efficacy studies, untreated / challenged animals are expected to develop clinical disease during the study. The severity of the disease models used is such that only mild to moderate levels of disease are expected. The majority of the animals used on studies will be euthanased at the end (often they are euthanased to provide additional samples for testing as part of the protocol) since animals which are administered unlicensed products or pathogens, cannot be returned to the food chain. On some occasions it is however possible to return the animals to stock.

Application of the 3Rs

Replacement

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

Replacement

At this time the regulations for approval of most veterinary products require the conduct of in-vivo studies to provide safety and efficacy data for inclusion in the regulatory submission. While in-vitro models are available for some procedures required in the licensing documentation (such as minimum inhibitory concentration - MIC, to provide information relating to efficacy of an antibiotic against pathogens), in-vivo studies are required to generate the vast majority of the information required for the portfolio. In some cases, in-vitro product data does not always correlate with in-vivo data, possilbly due to complicating factors that are present in the host animal but not in the in-vitro system. Every effort will however be made to identify in-vitro testing that would replace in-vivo testing where acceptable to the regulatory authorities.

Reduction

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

Reduction

During the design of studies, reduction in the number of animals used is always included in discusions to ensure that the minimum viable number of animals, sufficient to fulfil the objectives of the study, are selected. For safety studies, the number of animals to be used is generally fixed by the guidelines and there is little scope for revision. For efficacy studies, more flexibility is available and the number of animals to be used is generally confirmed by statisticians following review of the data, in order to produce a suitably powered experiment.

Where possible a reduction in the number of control animals used is included in the study design (where statistical comparison with treated animals is not required and therefore matched numbers are not appropriate).

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 in studies are of the age, breed, sex and species required for the product under test (and in line with regulations).

The animal models are continually refined with a review of the outcome of all disease model studies being carried out on completion. This will include a statistical review of the outcome (where relevant) in order to determine whether in the future, it may be feasible for a reduced number of animals to be included in the study.

A review of any issues raised during the study with regard to animal welfare and any changes made during the study to correct the issues are also carried out to ensure that the lessons learned are taken into consideration in future studies. This may require a further validation of the challenge model if clinical parameters do not meet specifications which may mean that further refinement may be necessary.

PROJECT 261

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.

Word limit; 1000 words

Project Title	Inflammation in Alzheimer's disease
Key Words	inflammacion, amyloid, tau, traumatic brain injury, neurodegeneration
Expected duration of the project	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):

Summary of the project

The brain lesions that appear in the brain of Alzheimer's disease patients, including amyloid plaques and neurofibrillary tangles (NFT), can be recapitulated in transgenic mouse and rat models of Alzheimer's disease (AD) . The brains of AD patients and animal models also show increased activation of glial (support) brain cells, generating inflammation. tOur aim is to study the effects and mechanism by which inflammatory and anti-inflammatory molecules affect the the formation these brain lesions and the loss of neurons and memory . Because traumatic brain injury (TBI) is a major risk factor for AD due to an increase in neuroinflammation, we will also investigate this association using animal models of TBI. We plan to investigate the role of anti-inflammatory therapies in these animal models, looking at whether they improve memory and to explore new techniques to detect changes in the brain by imaging in live 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)?

Within the past decade, our understanding of the pathogenic mechanisms in Alzheimer's disease (AD) has dramatically advanced because of the development of transgenic mouse models that recapitulate the key pathological and behavioural symptoms of the disease. These mouse models have allowed investigators to test detailed questions about how pathology develops and to evaluate potential therapies that could slow down the development of this disease. This project aims to understand how inflammation affects the progression of AD in transgenic mice and rats and how certain molecular pathways have an effect on this inflammatory

response. The results of this work will allow identification of new targets for therapeutic intervention and will help to understand the role of inflammation in AD.

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

We plan to use mice (around 2200) and rats (around 1400), in many cases genetically modified as maximum over 5 years. This project covers the work of several groups of research. We will use rats because their bigger brains allow better visualization and resolution during imaging studies, compared with 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?

In general, the level of severity will be mild or moderate. Any animal showing suffering that is greater than minor or transient including paralysis, or showing signs of abnormal behaviour, will be humanely killed immediately. If the animal is of particular interest, advice will be sought promptly from the HO inspector. All animals will be closely monitored after any surgery, and additional analgesia and/or antibiotics provided as necessary. A small group of animals (up to 550) will be used for traumatic brain injury modelling, carried out using two different animal models, the Controlled cortical impact (CCI) model, which has severe classification and a non-invasive controlled pressure wave model the blast injury models (moderate severity). The CCI method uses a rigid impactor to deliver mechanical energy onto intact brain meninges exposed following a craniotomy. Impact is made under precise parameters at a set velocity to achieve a pre-determined deformation depth. The non-invasive controlled pressure wave blast model mimics the type of injury that happens after a blast explosion, which consist of a non-invasive controlled pressure wave injury which is not expected to cause overt injury to the animal.which has severe classification. Post-procedure animals will be very closely monitored and given anti-inflammatory and analgesic drugs to reduce pain. Following TBI the animals may exhibit some locomotor impairment and some loss of senses, but not severe such as paralysis. Any animal experiencing a high level of distress or a reduction in body weight of more than 20% will be humanely killed. In our experience, less than 5% of the animals died after CCI. We will use the CCI model to investigate TBI’s association with AD using neuropathological, genetic and imaging techniques.

Application of the 3Rs

Replacement

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

Replacement

Mice models of AD reproduce in great extend the pathology in humans (amyloid plaques and neurofibrillary tangles) and memory loss and will allow us to understand the pathogenesis of AD, which cannot be detected in vitro. There are some invertebrate models of AD, but the brain anatomy is not similar to humans and the behavioural abnormalities of AD are difficult to address. Cell models do not show the relationship between different cell types in the brain and do not allow to monitor behavioural changes or alterations in brain anatomy

Reduction

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

Reduction

The number of animals will be minimised by doing 1) the experiments in vitro using (neuroblastoma N2a) cells to determine whether we have a positive result 2) using a short number of wild-type animal for treatment in vivo. 3) initially using no more than 3 animals per group of AD transgenic mice for testing novel compounds, typically at two or three dose levels around the expected pharmacological dose. For comparisons using larger groups, group size will be estimated by statistical analysis. We will be conducting the experiments in order to be able to publish according to the ARRIVE guidelines and will use randomisation, blinding, ethical statements, experimental procedures, details of experimental animals (number, strains, sideeffects), sample size, husbandry where appropriate and avoid biases.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 AD are extremely valuable tools in AD research. The two identifiable hallmarks of AD, amyloid plaques and NFT, have been reproduced in several of these models. Previous work has been done on inflammatory aspects, in which we are particularly interested, and the role of key proteins involved in the development of AD. In addition, a correlation of cognitive performance with the protein/plaque pathology has been demonstrated in behavioural studies. The majority of the protocols are minimally invasive or moderate and therefore longitudinal studies in the same subject may be performed.

The CCI model of traumatic brain injury is the least severe model of invasive TBI to reach our objectives, in order to minimize harm. Besides, this model is very reproducible, so the results obtained are more reliable and we need to use fewer animals. We will also use the non-invasive controlled pressure wave model, which is

not expected to cause overt injury to the animal. The classification of the Blast model severity is moderate. We will minimize the suffering by providing anaesthesia and analgesia.

PROJECT 262

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.

Word limit; 1000 words

Project Title	Development of effective pain management protocols for mice
Key Words	Analgesia, Pain, Mouse
Expected duration of the project	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;
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):

This project aims to improve the pain relief administered to laboratory mice that undergo potentially painful procedures in scientific research. We estimate that annually ~13% (~400k UK, >4 million worldwide) of mice used in research will undergo potentially painful procedures where effective pain relief could reduce or prevent the pain experienced. Unfortunately, many of the current pain relieving drugs routinely administered to mice appear may not be sufficiently effective to fully alleviate moderate or severe pain in mice (eg. post-surgery). This represents a major challenge for both animal welfare and the value of the scientific data collected from these mice.

To achieve improved pain relief for mice we need:

- A better means of testing pain-relieving drugs for their effectiveness in treating procedure-related pain;

- To determine whether new pain-relieving drugs are better than those currently being used;

- To determine whether or not the differences in the effectiveness of pain-relieving drugs observed between mouse strains relates to differences in the underlying experience of pain.

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

Unalleviated and therefore uncontrolled pain in laboratory mice remains a considerable welfare concern. Pain also has a range of pathological effects that can reduce the validity of data collected, either by direct effects on the mouse's normal

body functions or by increasing variation in the biological measurements that are being taken. Both these effects can increase the number of mice required in a wide range of different types of research. Effective pain management is therefore integral to the 3Rs that underpin animal-based research. This project will determine which pain relieving drugs are the most effective, for a specific type (strain) of mouse following a routinely used surgical procedure allowing recommendations to be made and implemented to improve the welfare of mice used in scientific research and the quality of the science carried out on these animals.

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

We will use up to 500 mice 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?

Measurement of the effectiveness of pain relieving medicines will first be carried out by training mice to push their noses through metal bars, to reach a sweet fluid reward. The bars are heated so that the mice stop drinking and withdraw when this becomes painful. This will need to be done repeatedly, so it is important to use low temperatures to prevent the mouse burning or injuring itself. To make this painful, the mouse's nose must be made more sensitive, and this is done using bee venom. The dose used will produce a mild reaction, not as great as we would experience if stung by a bee. Because we will need to repeat these treatments, we consider that they will be of a moderate severity. Once we have established which pain relieving medicines are likely to be most effective, we will test this in mice undergoing vasectomy surgery. Some of the mice will get pain relief, but others will not, as we need to be able to show that the medicines used are effective. The degree of pain following surgery is likely to be moderate for about 2-4h, and then mild. At the end of each study, most of the animals will be killed humanely. Mice that have undergone vasectomy surgery may be suitable for use in the breeding programs at our institution, so may be retained and used for this purpose. This would avoid other mice undergoing surgery for use on the breeding programs.

Application of the 3Rs

Replacement

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

Replacement

As we are aiming to develop effective means of pain relief in mice, we cannot study this in isolated cells, or in other species of animal. Pain is a complicated emotion and sensation, that can only be experienced by a conscious animal, with a fully

functioning brain. Although some of the cellular mechanisms can be studied in cells in a dish, or in isolated pieces of the nervous system, this would not enable us to determine effective pain medicines for use after surgery, or other painful procedures in mice.

Reduction

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

Reduction

We have designed our studies carefully, using both factorial design, and cross-over studies when possible, so that animal numbers are the minimum needed to obtain reliable data. The animal numbers have been carefully calculated using the appropriate statistical methods and data from previous related studies. To develop the methods of pain assessment that we will use, we will need to conduct some pilot studies using a limited number of mice. These pilot studies where possible will use animals that have been bred as part of other unrelated breeding projects or are surplus. The use of these animals that were generated during other projects will avoid the use of animals needing to be bred for these pilot 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

We need to carry out our studies in mice, as these are the species that we hope will benefit from the work. We have chosen a method of assessing pain that allows the mouse to avoid the pain (by stopping poking his nose onto the heat source, to get a reward). We have also sensitised the mouse's nose so that the heat used will not cause damage to his skin. For the surgery, we have chosen a procedure (vasectomy) that causes moderate pain, that lasts for a relatively short time, but is sufficient to show that the medicines tested will be effective.

Throughout the study, all mice will be given environmental enrichment, and will be handled using handling tubes because this can reduce stress.

PROJECT 263

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.

Word limit; 1000 words

Project Title	Therapeutic development for sphingolipid diseases
Key Words	Genetic, disease, neurodegeneration, cancer, sphingolipids
Expected duration of the project	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;
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.

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 applicant takes care of patients suffering from a rare family of inherited, relentless and fatal diseases all of which affect the nervous system but may have systemic features (called the sphingolipidoses). These diseases affect all ages but are very severe in babies and children.

The individual diseases called (Gaucher disease, Krabbe disease and GM2 gangliosidosis – Tay-Sachs and Sandhoff diseases) all affect the way the body recycles complex fatty molecules mainly in the brain. Cancers also occur in patients with Gaucher disease – which gives a clue about the cause and possible treatment of such cancers in the general population. We have recently found that identical cancers arise in the mouse model of Gaucher disease.

The overall aim of this application is to generate the data necessary to expand understanding of the fundamental processes which result in sphingolipid disease and use this data in the development of effective 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 will advance clinical treatment of a group of closely related but cruel and relentlessly progressive hereditary defects in the brain and other tissues by directly and safely restoring the defective functions. We will explore corrective gene transfer as well as medication also that has promising actions to improve the outcomes for patients who will otherwise die and endure extreme distress and disability – for there are no effective cures. We will also apply these techniques to test in detail whether in one condition (Gaucher disease) we will be able to prevent associated cancers - we

already have evidence indicating that this may be possible and to justify possible recommendations in patients, need to find out exactly how this therapy might work.

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

We will only use mice and plan to use less than 27000 over five years. We appreciate that this is a large estimate over the period but now it must reflect not only the minority of animals undergoing procedures to test treatments and work out what exactly causes the disease, but also the much greater number of healthy animals which have traits that are required for the complex breeding purposes to obtain those for special study in the particular diseases. These will either not suffer or have only mild effects from the breeding. An additional reason for the much larger number than formerly estimated is the expected requirement to move to improved facilities in 2019: this will require re-derivation of the strains for safety and health management and establishment in the new facility.

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

Partial treatment or belonging to a control group with no active treatment (as required for valid research studies) may lead to mice with genetic diseases living longer with signs of muscle weakness, tremor and weight loss. We will monitor our animals very closely to ensure that their well-being and capacity to fend for themselves is impaired as little as possible and that there is no expectation of pain or sign of distress. A proportion of animals will develop tumours of the blood (lymphoma or myeloma) which is observed in human patients with Gaucher disease. Cancer prone animals (less than 15% of the total number of animals used) will be closely monitored and killed humanely before they develop clinical signs of lymphoma and myeloma (which include enlarged lymph nodes, anaemia [pale paws and ears], loss of body condition, muscle weakness and reduced exercise tolerance). In all cases where signs of pain or where animals start to show behavioural signs, difficulty moving due to muscle weakness or signs of weight loss or body condition will be killed immediately using a humane method. The licence will allow the breeding and maintenance of mice, some of which may develop muscle weakness, tremor and tumours. In addition, most animals (approx. 7650) will be used in experiments where animals will receive treatments, blood sampling and in some cases non-invasive imaging (such as MRI) and exposure to irradiation (in less than 10% of all animals). Some of these procedures may require anaesthesia (for example surgical interventions to inject into the brain) or for restraint (for example imaging and irradiation). Whenever surgery is performed, animals will receive pain relief treatment. In addition, the surgeons who work under this licence have the necessary experience to perform the procedure causing minimal suffering on the animals. The treatments tested under this licence will either improve the health of the animal or have no effect. All animals are killed humanely at the end of the experiment.Most of

the animals used in the licence will be used to obtain those with disease traits but will not show disease themselves; their use in breeding must be noted but the effects of procedures on them will be mild. The work reflects a complex design and it has been challenging to fit this into the new licence format. The revised numbers now include all the experiments envisioned: all are of direct medical significance and/or importance for the severe and life-shortening diseases we study and in patients for whom we provide care. Finally we justify the modified application because these diseases mainly affect young adults and children whose great clinical needs cannot at present be provided for, because the research is closely linked to the discovery and application of specific treatments and because at this stage there is no alternative to the use of animal models.

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 conduct appropriate signalling and in vitro studies using human blood cells. Normally, these are undertaken as confirmatory studies of molecular mechanism but unfortunately only contribute incomplete information because of the ethical constraints of human clinical research.

It is too early to test all the treatments such as gene therapy in patients and we have models in mice that very closely resemble the human condition. Here the diseases can be more safely and comprehensively studied.

There are no alternatives for decisive testing at this stage but we will use samples from patients as soon as possible - and also do experimental medicine in human patients rather than animals as soon as we have the confidence (and permission) to do so.

Reduction

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

Reduction

From previous development we know that our proposed treatments should have big effects when used in the correct way: we will design the research to give clear results which can be examined in great detail to minimize the numbers of animals that would have to be used to test our expectations for human use. Before carrying out regulated procedures, the experimental plan will be analysed using suitable power estimations.

We have long experience of, and access to biostatisticians who will advise on the design of our studies to ensure that suffering is kept to a minimum for the least number of animals according to guidelines; proper controls will be used to add confidence to the findings. For investigations into treatment and cause of disease, mating will be optimised: where we mate young mildly affected mice and carriers to obtain twice the yield of those required that model the disease and reduce production of healthy unneeded animals.

For very rare animals needed to test the potential effects of modifying traits, we will use the new CRISPR-cas9 technology that allows models to be bred efficiently with the disease traits using a few mice and only two or three rounds of breeding. To minimize the chances of passing on unwanted random traits induced by this technology, the first animals obtained with the desired trait will be bred with their parents rather than interbreeding from siblings. CRISPR will minimise the number of breeding cycles required and will reduce the risk of transmitting unintended genetic defects to the offspring.

After collection, the data will be analysed by appropriate statistical methods that meet internationally recognised standards of research practice and experimental design set out in the Animal Research: Reporting of In Vivo Experiments (ARRIVE) NC3Rs guideline documents (National Centre for the Replacement Refinement & reduction of Animals in research -www.nc3rs.org.uk/ARRIVE).

Strong beneficial effects of our treatments or genetic factors in the disease reduce the number of animals required to evaluate the effectiveness (or toxicity) in study designs. Fortunately, small focussed study groups usually prove adequate for us to obtain clear information so that repetition will not be 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

There are several models of the severe diseases we study in animals; however we use mice which have exactly the same genetic problems that cause the human conditions. These mice will be the smallest and least feeling or threatened and distressed animals we can find usefully to test; and we plan to study them under strict legally enforced surveillance and regulations.

To get clear and efficient results, we plan only to do the minimum work on small numbers in each group of mice; so far as possible we make point of using all the materials and cells obtained from these animals.

Every effort has been put into the design and humane conduct of this work and we will be observing the animals to be sure that any suffering is kept mild or at the most moderate and for as short a period as is required to be informative.

There are no alternative experimental models of Gaucher disease and our mice develop the very same cancers that occur in human patients. For the disorders of the brain, previous studies in cats, sheep, dogs and monkeys have been done by American collaborators built on our research. Now these are reported, we will carry out licensed procedures only in mice to discover the basic causes of the diseases as well as the information needed to support the delivery of new medicines (a tablet for the cancer and a gene therapy for the brain disorders in children).

In the cancers, related to Gaucher disease (lymphoma, myeloma and rare liver tumours), we will predict their onset with signs in the blood that anticipate development of full-blown cancer. These will prevent unnecessary suffering to the animal well in advance – as in patients. No animals will be kept until they would die of the disease. Instead they will be carefully monitored using a pre-tested clinical score and killed at an earlier stage to minimise suffering.

Our procedures are well-tolerated: mice make a quick recovery and care is taken to keep them warm with access to food and water during convalescence. With long experience, humane endpoints have been developed; we recognise that high-level monitoring may be needed to avoid distress. When drugs are used: substrate reduction therapy and chaperones, anti-inflammatory agents, and immune-suppression protocols, doses will be based on published data, veterinary, and our experience. We will not develop drugs that require primary testing in mice, since pilot studies will have been already done by our collaborator. Agents with significant side effects (e.g. weight loss) will be used at low doses in the first instance; and substitutions will be actively explored.

PROJECT 264

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.

Word limit; 1000 words

Project Title	Delineating the Pathogenesis of Renal Disease
Key Words	Kidney, Renal, Genetics, Disease
Expected duration of the project	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;
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):

From 1990 to 2013, chronic kidney disease (CKD) was the top non-communicable cause of death and its impact in terms of years of life lost is increasing. In 2011 over 100,000 people underwent treatment for end stage renal disease. Overall, renal disease can affect up to 15% of adults and yet there are very few effective treatments for kidney disease outside of transplantation or dialysis.

We aim to understand the genetic basis and pathways that result in a variety of renal diseases. Our objective is to understand how variations in genes can result in diseases affecting kidney function and how they operate in normal kidneys. The identification of genes that contribute to renal disease will allow us to identify important pathways contributing disease. We will also examine patient populations for variants in the genes we have identified and in related genes In particular, we will focus on the early events that occur during disease as this is very difficult to study in patients (we do not see patients until disease has already progressed to the stage where the kidneys are already damaged).

What 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 gaining a better understanding of how different genes result in different kidney diseases we hope to help understand disease in patients and help identify new ways of treating disease. Firstly, we may be able to identify subsets of patients through genetics that we know have a particular disease; a key problem is that when kidneys are damaged it is difficult to distinguish what has happened as many renal diseases have very similar outcomes. Being able to define specific groups of patients will make drug studies more efficient. Secondly, by studying early events in kidney

disease we may be able to identify markers that indicate how disease will progress. This will aid clinicians in the treatment and management of patients and hopefully improve outcomes if we can predict the type of disease a patient is likely to suffer and how severe it will be. Finally, by understanding how disease progresses and what alterations occur during the disease we aim to identify new targets for therapeutic interventions that would ultimately lead to new or better therapies for specific renal diseases.

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

Approximately 11,800 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 with renal disease will deteriorate over time, but by careful monitoring, we do not expect mice to reach end stage renal failure. The techniques we are employing are generally non-invasive and well tolerated, such as blood sampling and urine collection, and would be no more than a patient would experience in a clinical setting. Time for recovery is left between tests to ensure the mice have time to recover and the overall number of tests is kept to a minimum in order to limit stress. From patient studies we understand the effects of kidney failure on other organs, we will therefore kept to an absolute minimum the number of mice that are allowed to reach end stage renal failure. This will only be allowed to happened when there will be a specific scientific question that we cannot determine form existing data. Through simple tests like dipstick analysis of spot urine collections or blood tests we can monitor disease progression very closely and intervene before mice experience the more aggressive symptoms of renal disease. Renal disease can lead to other complications such as changes in blood pressure or heart disease but as we are focusing on the early stages of renal disease we aim to avoid such co-morbidities through our careful monitoring of disease. All studies are carefully designed and carried out to standard operating procedures. Where appropriate we use general anaesthetics and limit the frequency of these. Some techniques require individual housing during the procedure, which may be stressful. The maximum expected level of severity is moderate. At the end of the 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

The kidney is a complex organ linked into the blood and urinary system in three dimensional multicellular systems involving a complex extracellular matrix. It's function is affected by blood pressure and many diseases involve an inflammatory component, such as fibrosis, and therefore a functioning immune system is required. This precludes using *in vitro* studies. The mouse is a good model for renal disease and shares many similarities and physiological traits with humans. Lower species, such as fish, can be used to study the kidney but they have a much simpler organ that is not useful for modelling all human diseases.

Reduction

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

Reduction

We use statistical methods to analyse our results and to calculate the numbers that we require for an experiment in order to be able to observe a trait and generate robust and accurate data. These calculations are based on experience with different techniques. We have access to a trained statistician who is able to help us achieve this and to develop better approaches. We also make the most use of individual animals and store excess tissue in the form of fixed and frozen kidneys for 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

We will minimise harm to animals by applying the highest possible welfare management and monitoring so that problems are picked up and treated quickly and inform future practice. We will use anaesthetic when pain is likely to occur, for example in a procedure such as blood sampling. We will collect data by the least invasive and most efficient route, with careful aseptic techniques. The frequency and impact of techniques on the mice will be taken into careful consideration to determine the final course of action; for example implanting a minipump may be preferable to carrying out a number of injections. We will anticipate problems by prior planning and putting in place appropriate monitoring and treatment protocols when, for example, generating a new genetically modified animal for the first time or carrying out a new procedure. Through careful monitoring we will minimise the impact of disease on the mice and keep the number of mice reaching the later stages of renal disease to a minimum. All procedures are carried out to the highest of standards with thorough staff training, defined protocols and extensive data collection.

PROJECT 265

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.

Word limit; 1000 words

Project Title	Basic mechanisms of chronic neurodegeneration
Key Words	TSE, Neurodegeneration, Mouse models
Expected duration of the project	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):

This research programme aims to understand the basic mechanisms underlying the development of neurodegenerative diseases, primarily focusing on transmissible spongiform encephalopathies (TSEs) or prion diseases. Neurodegenerative diseases currently have no cure and as a result create an enormous social and economic impact. Current therapies have a modest impact on symptoms and limited or no impact on disease progression. The prevalence of these diseases is predicted to rise dramatically over the next decade.

The earliest events in neurodegeneration are poorly understood although there is evidence to suggest that there are common molecular and cellular pathways leading to the destruction of central nervous system cells called neurons. A common feature of a number of neurodegenerative diseases is the presence of misfolded proteins. Although different misfolded proteins are found in different diseases the pathway to neurodegeneration is thought to be similar between the different diseases. We will use unique rodent models which allow direct experimental control of the amount, position and accumulation of misfolded protein. We will use these models to determine the early cellular events involved in these diseases as well as the potential protective effects factors that may help to slow down disease progression.

Our programme of work will therefore;

- Define the pathways leading to neurodegeneration
- Define host pathogen interactions in TSEs
- Define interactions between neurodegenerative pathways and the immune system which might exacerbate neurodegeneration

- Identify normal function of proteins involved in neurodegenerative diseases

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

The aim of our research is to advance our understanding of poorly understood cell mechanisms that underlie the destruction of the brain and neuron function in neurodegenerative diseases. The potential benefits associated with understanding these basic mechanisms are significant both scientifically and medically. It is anticipated that the information gained will contribute to world wide efforts to understand these life destroying diseases as well as contributing to the ongoing search for options for medical intervention and treatment.

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

We will use a maximum of 10000 mice over 5 years. The size of experimental groups will be based on previous work discussed with a statistician. This will be used to estimate the minimum number of rodents required for establishing significant differences between groups.

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

All procedures detailed here are to be performed in rodents and do not exceed 'moderate' severity. The animal models and protocols to be used here have been developed by us and our colleagues over numerous years in order to study progression of neurodegenerative disease. All experiments are to be performed by appropriately trained staff and are essential for the success of this project. Well defined clinical scoring regimes are in place in order to give a defined humane endpoint and prevent needless suffering. Animals will be humanely sacrificed at the end of experiment.

Application of the 3Rs

Replacement

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

Replacement

As neurons are not present outside the animal kingdom, studies on protection and degeneration of the nervous system require the use of animals and there is no feasible alternative that would entirely replace the living animal and would allow the objectives to be met. To study the interactions between cell types in the progression of TSE disease it is necessary to use animal models. Additionally, the volume of information available relating to the rodent model as well as our extensive

experience with this model makes this the species of choice for our purposes. It is unfortunately not possible to accurately study the involvement of these complex systems in neurodegeneration without the use of animals and the models used in this project are the best available to address the main experimental aims.

At present there is no more reliable method that allows TSE infectivity to be estimated than mouse models. However new studies examining cells studied outside of the normal environment (for example in artificial culture media) are being developed and we will continue to optimise these techniques and aim to introduce their use if they are found to be as reliable as our work on mouse models.

Reduction

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

Reduction

All studies involving animals are subject to completion of a study protocol form which must be approved by both veterinary staff and the unit manager. Where a study involves a novel technique or large numbers of animals it must also be approved by selected members of the AWERB including researchers, statistician and TSE researchers independent from the study prior to commencing studies.

The use of mice where specific genes have been altered may also show shorter disease incubation periods and thus may allow the use of fewer animals in studies.

We also have collaborations with other scientists whereby maximum use is made of animal tissues at the end of 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

Animal suffering will be limited in our studies by our strict monitoring of severity limits which are very well developed and are identified by experienced staff. We also use many procedures that do not produce significant trauma or suffering. Substances and treatments will be administered at non-toxic dosages and if unknown, this will be tested in a carefully graded dose-finding protocol.

PROJECT 266

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.

Word limit; 1000 words

Project Title	Using non-mammalian vertebrates to study development
Key Words	Development, Gene regulation
Expected duration of the project	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):

During embryonic development, a single cell (the fertilised egg) gives rise to many different kinds of cells, each with their own function (for example, red blood cells, which carry oxygen around the body and muscle cells, which contract). One of the major questions in biology is how this process of differentiation occurs. It has become widely accepted amongst developmental biologists that cells in the embryo communicate with each other through cell-cell signalling such that when a cell receives a message from another cell it responds by turning certain genes on or off. This differential gene expression is thought to underpin the establishment of different types of cells and tissues.

The purpose of the project is to increase our understanding of embryonic development. In order to do this, we use frogs (**Xenopus laevis and Xenopus tropicalis)** and fish **(Danio rerio and Alcolapia),** which provide an excellent source of large, accessible embryos. We use embryos to find out what particular genes do in development: What tells a cell to become a muscle cell? How do cells talk to each other? How do cells moderate their responses? We address these questions by either causing an embryo to have too much or too little of a specific gene. Sometimes we need to raise lines of fish or frogs that are genetically altered. We do this to produce fish or frogs that have sets of neurons or muscle cells that are fluorescent. Using special microscopes, the development of these cells can be watched *in vivo* providing insight into developmental processes. Generating a mutation in a specific gene will prevent it from working. Analysing animals that have been targeted such that specific genes do not work (mutants) provide valuable insight into the normal function of that gene.

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

Developmental mechanisms are very similar between frogs, fish and mammals, including humans. This means that what we learn about the genes that regulate frog and fish development is very likely to be applicable to human development. Notably, it is often these same genes important for embryonic development that malfunction in cells that become cancerous. In other words, better understanding of the mechanisms of development gained by the basic research undertaken under this project licence could ultimately underpin the development of new treatments for human disease such as cancer.

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

Over the course of this project: • 30 Xenopus laevis • 320 Xenopus tropicalis • 1100 Zebrafish • 160 Alcolapia

In the context of what you propose to do to 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 obtain Xenopus eggs, a female frog is injected with a hormone to induce her to release eggs. The hormone has no ill-effect on the frog, and only accelerates the natural process of ovulation. The females are injected by licensed researchers, experienced in handling frogs. Each female lays hundreds of eggs, providing a large quantity of experimental material. Injections are carried out once every four months, which allows the frogs a rest period during which they produce more eggs. As long as the female frogs stay healthy they can live for many years in a laboratory colony. Zebrafish will produce embryos in the morning, if a male and female have been placed together the evening before. There is no need to induce zebrafish to lay eggs. Alcolapia are mouth brooders; this mating behaviour means that we need to collect egg and sperm from these fish and carry out in vitro fertilisations. This requires mild sedation and gentle manipulation of the fish. All animals will remain in our aquaria as long as they provide eggs, or for up to 6 years. Once animals are post reproductive they are 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 order to study how developmental biology works we need to have access to embryos. Frogs and fish provide large broods of externally developing embryos. These animals are vertebrates: they have backbones, like humans do. This means

studying embryos from these animals (rather than flies or worms) is best for understanding many aspects of human development. Fish and frog embryos are excellent model organisms to study developmental processes that happen at the earliest stages of development, because these non-mammalian vertebrate embryos are accessible, developing outside the mother. Gene targeting and over expression methods are exceptionally effective in these models, allowing discriminating and informative experiments to generate new knowledge about the molecules being investigated.

Reduction

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

Reduction

As a group, researchers REDACTED coordinate when they do experiments so that when a frog or fish provides a spawning of embryos lots of experiments can be done by different researchers using the same spawning. The embryos are not limiting, so statistically significant results can be obtained from a single spawning. Generating 3 biological repeats requires using eggs from 3 females, so often 3 separate ovulations 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

- Using non-mammalian vertebrates is better for developmental biology because these animals provide more eggs than mice or rats, and these eggs develop outside the mother.
- We have refined our hormone injections into female frogs so that we inject lowest dose to induce ovulation.
- We have reduced the density of frogs and fish in each tank which keeps them healthy and productive.

We provide enrichment to the tanks and monitor animals daily for any sign of ill health.

PROJECT 267

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.

Word limit; 1000 words

Project Title	Regulation of iron metabolism
Key Words	iron, metabolism, anaemia, diets, drugs
Expected duration of the project	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;
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):

Anaemia due to dietary iron deficiency (IDA), chronic disease (ACD) and iron overload caused by haemochromatosis are major health issues affecting millions of people worldwide. For example, one third of the population of pre-school children in the world suffer from IDA. If severe, anaemia can lead to death and even mild IDA leads to impaired cognitive function in children and fatigue resulting in loss of productivity in adults. Type 2 diabetes, anaemia of kidney disease and breast cancer are disorders with links to iron metabolism in the body that affect quality of life and mortality rate in populations globally. The aim of this project is to understand what controls body iron levels and evaluate new drugs and natural products for the treatments of anaemia, iron overload, kidney disease, type 2 diabetes and cancer in humankind.

What 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 interventions which can regulate iron metabolism will benefit the treatment of iron-related diseases. Typical studies will involve using normal mice to investigate iron metabolism genes. Their iron metabolism will be modified by established laboratory methods such as special diet or the administration of substances which influence iron in the body. Blood samples may be taken and at the end of the study, the animals may be terminally anaesthetized and tissues taken for analysis. Potential new treatments may be given so that their effects may be assessed during the study. The research is aimed at evaluating new drugs to treat serious diseases such as anaemia and/or iron overload, use of natural products to treat type 2 diabetes and cancer with the overall aim of alleviating symptoms of these disorders in human patients.

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

Adult mice, a total of 1500 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?

Some studies may cause adverse effects such as diabetes, acute kidney disease or anaemia but suffering will be strictly controlled by timely use of humane end point. Specifically, animals may be fed altered diets and become anaemic, diabetic or develop kidney disease. Animals may develop tumours due to tumour cell administration under the skin and may lose weight. Animals may have injections of substances including glucose, insulin, iron substitutes, potential treatment agents and undergo blood sampling. However, in all cases, close monitoring and the application of early humane endpoints will mean that animal suffering is minimised as far as possible in pursuit of scientific objective.

Application of the 3Rs

Replacement

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

Replacement

Mice have a wide array of well-characterised genetic defects in iron metabolism. Mice are the lowest vertebrate group which adequately model human iron metabolism.

Cell cultures are useful for investigating certain fundamental mechanisms at the cellular level and we are conducting parallel studies with *in vitro* systems, and in cultured cells wherever feasible, to reduce the numbers of animals used. However, the regulation of iron metabolism involves interactions between organs and tissues, and also between different cell types within tissues which have yet to be understood. It is currently not yet possible to investigate such complex interactions without using animals.

Reduction

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

Reduction

Power analysis is used to estimate animal numbers when a large sample size is required. Usually, the designs involve the comparison of different compounds or varying dose regimes and the factorial design will be used as is necessary. Our

previous experience suggests that a group of six mice in a 2x2 factorial design gives confidence and statistical differences among the experimental variables. Control mice are normally given inert drug vehicles and fed the control diets. Stringent efforts to define the hypothesis carefully, choosing a suitable animal model, designing appropriate experimental design will enhance the reduction of animal numbers that are used overall 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

Studies on iron absorption from foods and iron compounds or potential iron tablets require an animal model to understand fully how and the amount that is absorbed. Mice adequately model human iron metabolism, hence the number that will be needed will be calculated and the minimum number will be used strictly when each experiment is planned. Proposed studies will use diets to create the different conditions such as anaemia and type 2 diabetes in the animals. Natural products such as fenugreek and amino acids are food ingredients that are safe and will not cause any discomfort to the animals during the period of the experiments. Appetite to eat will be facilitated by making the food wet and water will be sweetened to encourage drinking. Animals will be properly cared for and handled gently to avoid anxiety and stress. Animals will be housed and provided with bedding that meet specific healthy maintenance requirements. This will ensure that animals are not subjected to factors that might influence their responses and outcomes, thereby preventing repetition of the study.

PROJECT 268

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.

Word limit; 1000 words

Project Title	Epigenetic regulation links genotype to phenotype
Key Words	Development, Gene expression, Epigenetics
Expected duration of the project	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):

Our genetic information as written into chromosomes is about 2 metres long. This is 4 orders of magnitude greater than the size of the nucleus, the cellular compartment that contains our chromosomes. Fitting the genome into cells therefore is a formidable task. This is even more remarkable as the strategies for packing the genome have to allow for the genome to perform its vital functions, in particular the regulated activity of genes which define the identity of different cell types, such as lymphocytes or neurons.

Working out the mechanisms that underlie the identity of distinct cell types will benefit our understanding of development, health and disease. Defects in these mechanisms cause human developmental abnormalities such as Cornelia de Lange Syndrome and cancer. Human diseases such as Angelman or Prader-Willi Syndrome are caused by the deregulated activity of genetic information we inherit specifically from our mother or our father. Environmental factors, such as poor nutrition during pregnancy, can also deregulate these mechanisms and may have life-long 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)?

There are clear potential benefits of studying mechanisms that cause human developmental abnormalities such as Angelman and Prader-Willi Syndrome and cancer. We also hope to progress our knowledge of the impact of environmental factors. If we understand these mechanisms we will be able to better prevent or treat the diseases they cause. There is therefore a reasonable chance that the work under

this licence will identify potential therapeutic targets for human developmental syndromes as well as cancer.

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

We will use approximately 25000 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?

Our protocols will be of a mild to moderate level of severity. We will create and breed mice with relevant altered genes, assess their behavioural features and explore whether dietary changes or drug treatments alter the expression of genes of interest. We use cells and tissues that we isolate from genetically modified mice in order to study the mechanisms that underlie the identity of distinct cell types, with most experiments undertaken using cell culture. Genetic modifications are refined so that that only specific cell types are affected, and the impact on the mice is mild. Some in vivo experiments are required to test and challenge the conclusions from our in vitro work. Mice will be exposed to environmental factors, such as drugs or poor nutrition during pregnancy. A small number of experiments can involve surgery under anaesthetic so that the mice feel no pain. Other in vivo experiments assess the behaviour of mice or subject mice to imaging (under anaesthetic), comparable to scans that are applied to human patients. The great majority of experiments are ex vivo, in which case the animal is humanely killed to obtain cells and tissues for analysis Animals will be killed by a humane method at the end of the project period.

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 cell lines for our study of differentiation and gene regulation has severe limitations. In previous studies we found that the behaviour of cell lines differed markedly from that of primary cells in our experimental systems, and we would have been grossly misled had we studied the regulation of these genes only in cell lines.

We also need to use animals to study the effects of diet, the effects of gene mutation on behaviour, and for imaging in order to monitor the level of gene expression in animals over time.

The success of this project therefore requires mice, mainly as a source of primary cells but also to study whole body systems.

Reduction

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

Reduction

While statistics cannot predict the success of discovery research, we can use statistics to work out probabilities for obtaining desired genotypes from complex genetic crosses and to guide us in experimental design and data analysis, thereby keeping numbers of animals needed to a minimum. We are actively a growing the community of computational biologists, bioinformaticians and statisticians within our own group and the institute overall and we regularly seek their expert advice on statistics and data analysis as well as computational modelling.

Reduction of in vivo experimentation is also achieved by the extensive use of in vitro culture systems, as described under 'refinement' below.

Refinement

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

Refinement

Where possible we will use non-protected species (yeast, C. elegans and D. melanogaster) as model systems.

Vertebrate-specific biological processes will be studied in cell lines where cell lines are appropriate models.

Due to the limitations of non-vertebrate model systems and the inherent blocks to some biochemical processes in available cell lines, the success of this project requires mice. Primary cells are required to investigate the transcriptional and chromatin-based regulation of cell type specific gene expression programmes because transformed cell lines do not faithfully recapitulate the transcriptional and chromatin-based regulation of cell type specific gene expression programmes. Our long-term strategy has been to combine primary cells with advanced in vitro experimentation. To this end we have developed and refined suitable in vitro systems. Primary cells isolated from mice are used because mice are genetically tractable and represent a reasonable model for cell lineage choice and differentiation.

Suffering will be minimised by the use of anaesthesia and analgesia as appropriate. In the unlikely event of distress, animals will be killed using a humane method unless, in the opinion of the Named Veterinary Surgeon, such complications can be

remedied promptly and successfully using no more than minor interventions. Immunocompromised mice will be protected from pathogens where possible.

Use of cells for in vitro experiments to further our understanding of developmental gene regulation depends on the use of cells and tissues from genetically modified mouse strains for two main reasons. Firstly, it enables the isolation of primary cells at defined developmental stages and in sufficient numbers. Secondly, genetically modified mouse strains allow us to test the effect of specific genes. The production, breeding and maintenance of genetically modified mice are therefore vital to our research.

There are no severe protocols in this project.

PROJECT 269

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.

Word limit; 1000 words

Project Title	Study of Innate and Adaptive Immune Responses in Health and Disease
Key Words	Cancer, Autoimmunity, Infection, Vaccination, MHC
Expected duration of the project	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):

The overall purpose of our research programme is to develop strategies for boosting natural immunity against tumours. Our approach is to study the precise way in which experimental tumours initiate immunity, the nature of that immune response and how it becomes suppressed or inactivated by the tumour. When we have achieved sufficient understanding, we will use the information to devise ways of overcoming suppression or inactivation.

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

Immunotherapy offers the benefit of treating a wide range of cancers and autoimmunity with highly specific reagents. This will allow us to treat patients with reagents specific to the disease, either utilising or "educating" the patient's own immune system to either destroy cancer cells while leaving normal tissue mostly untouched or re-establishing the recognition of a patient’s own tissue as something not to respond to. This project will enable new targets for this modulation to be identified and allow us to further develop previously identified targets for human therapy.

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

We will use mice for these experiments and will expect to use 9000 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?

Due to the nature of the experiments there will be some adverse effects from the procedures used. These however, will be managed to minimise the severity. The main adverse effects of the procedures in this project will be from irradiation, injections, removal of blood to assess immune responses and the growth of tumours. To mitigate any adverse effects the mice, procedures have been selected to cause the least amount of adverse effects such that the moderate level of severity indicated for most protocols is, in many instances or performed experiments, reduced to mild. As part of the protocol mice are closely monitored to minimise any distress. At the end of the experiments mice will be culled and their tissues used to investigate/analyse the immune responses induced. In addition, cells will be used to generate cell lines which can be used in the lab and reduce the requirement 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

Because immune responses can only develop in a complete immune system, and cannot be induced or studied at the same level of detail in the lab involving many different cells and pathways, our work can only be undertaken using preclinical mouse models.

Reduction

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

Reduction

We are continually assessing the numbers of mice used in our experiments. Using statistical methods, we have identified the minimum number of mice required to allow valid conclusions to be drawn from the work. In addition, we are developing new methods and techniques that will allow us to perform a more detailed analysis of immune responses from 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

This project is concerned with understanding immune responses to cancer and ways to boost this in individuals. We will use mice for this study since there are a number of very well characterised cancer models that represent a very similar disease to that seen in humans. These models allow us to investigate the fundamental features of inducing an effective anti-cancer immune response. These cancer models and methods are taken from either published, commercial, or research sources. Consequently, any potential adverse effects are usually known or available knowledge will be applied to predict them. Thus, enabling us to minimise these effects to reduce distress.

PROJECT 270

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.

Word limit; 1000 words

Project Title	Cortical and sub-cortical motor control
Key Words	Cortex, Spinal cord, Reticular formation
Expected duration of the project	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;
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):

This project has the overall aim of improving our understanding of the different brain and spinal cord centres involved in the control of movement, and translating the knowledge into improvements in therapy for patients recovering from injury, such as after stroke or spinal cord injury.

Specifically, we aim to understand the relative contributions of different parts of the nervous system to movement control in healthy animals, and how information is processed within each neural centre. We will then map how the surviving centres change after damage. We also aim to understand the processes which can change neural connections within these circuits, and to use this knowledge to devise stimulus protocols which can modify connections.

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

Stroke is currently the leading cause of disability in the UK. There are around 150,000 new strokes annually, one quarter in individuals aged under 65. The UK has 1.2m stroke survivors, around half of whom live with a disability that affects their everyday life. Total care costs for stroke in the UK are estimated at £8.2 billion (all figures taken from The Stroke Association). Therapeutic options for improved rehabilitation are limited, especially for hand function – one reason for this is a poor understanding of the scientific basis for motor control, and the processes underlying its recovery after insult. The information gained by this project will allow us to devise principled new strategies for therapy to improve rehabilitation. If this leads to even small improvements in function, it will translate into major social and economic impact. Motoneuron disease is a rapidly progressing, fatal disease which produces

degeneration of the cells in brain and spinal cord which control movement. Current animal models for this disease use rodent models, but these lack an important connection seen only in primates, which may be critical to the biology of the disease. We will test a new technique to produce a primate model of motoneuron disease. If successful, this could provide important new details about how the disease spreads, as well as provide a way of testing disease-modifying therapies in future. Essential tremor is a common movement disorder, which produces excess shaking of the limbs. Recent results from human patients have suggested that the disease arises from problems in a part of the brain called the cerebellum. In this project, we will test whether inducing specific deficits in the cerebellum in primates can generate a model of essential tremor. Again, this will elucidate important features of the disease, as well as potentially aid the development of novel therapeutics in future.

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

90 macaque monkeys over 5 years 250 rats over 5 years

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

Monkeys will be trained to accept some restraint (a neck collar), and to perform a behavioural task. They are motivated to perform the task by having restricted access to food, and occasionally fluid; food and fluid rewards are then given for correct task performance. After training is complete, they are surgically implanted with a headpiece to allow head stabilisation and electrodes to record muscle activity from the forelimb. Recordings will then be made from the central nervous system in the conscious state, whilst the animal performs the task. The most common adverse effects are associated with wound infections associated with the chronic implants. In a small proportion of animals, focal surgical lesions will be made on one side of the brain. In the days immediately following, these animals may need nursing help with feeding due to impaired movement ability. However, as in human stroke patients with small lesions they often show a rapid recovery. In other animals, viruses will be used to insert genes to produce excess levels of protein thought to be involved in clinical disease, such as motoneuron disease. Rats may be prepared for recording by a surgery to inject neural tracers or novel genetic material, after which they are allowed to survive for a few weeks. Subsequent experiments involve terminal anaesthesia, and then making electrophysiological recordings or removing brain samples for analysis in vitro. Recovery from the initial surgery is unlikely to show adverse effects, and no adverse effects can occur in the final terminal procedure. The macaque experiments will have moderate severity, although the licence limit of ‘severe’ may be reached for short periods in some animals associated with the period immediately after a lesion. Rat experiments will be of moderate severity. At the end of experiments, 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

This project investigates the complex interplay of brain circuits in different regions, and as such must be carried out in intact organisms. The laboratory does run a substantial programme of experiments in healthy human volunteers and patients; however, these can only produce indirect data. Detailed understanding at the level of single neurons and their connections can only be achieved using the invasive approaches possible in animals.

Reduction

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

Reduction

We use sophisticated multi-electrode recording methods, which ensure that the maximum of data is gathered from each animal. Experiments in awake monkeys often yield sufficient data for publication from just two animals. Experiments under terminal anaesthesia use advanced anaesthetic methods to maintain the animals in good condition for extended periods (around 70 hours for macaques); this again enables us to gather extensive datasets from each animal.

Refinement

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

Refinement

Some basic circuit properties can be investigated in rats. However, the neural centres and connections controlling movement differ in key aspects in primates compared with non-primate species. To ensure that our results are directly applicable to human patients, we must use old world primates such as macaques.

Our techniques have been refined over many years, and we continually seek to improve them. All recovery surgeries are carried out under full aseptic conditions, with advanced anaesthetic regimes which produce rapid and uneventful recovery. Full programmes of post-operative pain management are in place.

PROJECT 271

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.

Word limit; 1000 words

Project Title	Pharmacokinetics of novel therapeutic agents and disease modification in oncology
Key Words	Drug discovery, ADME, cancer
Expected duration of the project	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;
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 overall aim of this project is to develop novel therapeutic compounds to combat human diseases, including cancer. This involves identification of proteins that are important in causing the disease, understand its chemical structure and function, then designing novel compounds that can control them.

The first aim of this project is to provide animal tissues and cells from freshly-killed animals to test novel compounds in the laboratory, where relevant cell lines for in vitro testing are not available. Each animal can provide sufficient material to test multiple compounds, and this helps reducing the number of subsequent experiments with live animals.

The second aim is to understand how the novel compounds behave and are processed by the body (ADME profiling), which can lead to better design of drug-like compounds. We aim to find compounds that are able to reach the target disease tissue without significant health risks. The most promising compounds are then tested in disease models at Astex (cancer) or externally (cancer and other diseases).

The third aim is to test the activity of the potential anti-cancer compounds in the animal models. The overall effects we desire is the killing of cancer or slowing of its growth. Our investigation also requires studying of key molecular events that are important in cancer cell survival, then the impact of our novel compounds on these events.

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

Our investigation will lead to identification of novel therapeutic drug candidates which can be tested in human patients. If testing in clinic is successful, these will widen the therapeutic options for patients. Our investigation will lead to further knowledge of the diseases and builds experience of drug-discovery. Early studies, such as the ADME studies, are used to identify the areas of molecules that should be improved. Building data like these improves the efficiency of novel drug designing.

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

Majority of our studies will use mice. We expect to be using up to 39,300 mice in total during the 5-year period of this licence. We will perform smaller number of experiments with rats, with the total of up to 2,600 animals in the same 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?

Majority of the animals will experience mild to moderate adverse effects due to non-surgical intervention (blood sampling, injection, oral dosing), therapeutic drugs, tumour growth or the combination of all. For the early phase of compound development, each animal will be given injection or oral doses of novel compound at low levels that is not expected to have any therapeutic effects, and blood samples taken to determine the compound level in circulation. These procedures should only cause momentary discomfort immediately after. The therapeutic dose levels and regimen are estimated for the promising compounds and their tolerability tested in pilot experiments. Upon repeat dosing of an anti-cancer compound, we expect that the animals experience mild to moderate side effects such as weight loss, transient diarrhoea and changes in normal behaviour. Since the compounds are novel and despite our effort to predict toxicity risks in vitro, it is impossible to avoid rare events where the animals experience severe adverse effects or die due to toxic effects. We monitor animals on this type of pilot studies daily or more frequently in order to stop suffering as soon as possible. The studies are repeated with lower doses until a tolerated dose schedule is found. Efficacy of anti-cancer compounds is tested, initially, in subcutaneous xenograft models in which mice are growing human cancer under the skin. In these, each animal receives tumour implantations, which is minimally invasive and can be monitored externally. Any animal, on or off drug treatment, with its tumour approaching a set maximum burden (14 mm in average length in mice, and 25 mm in average length in rats) or appearing to be breaking up will be killed. Therapeutic compounds are administered at the dose that was found to cause adverse effects of moderate levels or less. Treated animals will be monitored to measure efficacy or killed to investigate the drug effects in tumours ex vivo. With most models, some animals (typically 5-20%, depending on the cell line) fail to develop tumours. These may be killed or re-used to investigate the drug exposure and tolerability of novel therapy. Only the most promising compounds may be tested in leukaemia or mammary tumour models which are more invasive and complex than

subcutaneous models, and requires extra tumour monitoring methods. Hollow fibre assay offers a means to test multiple cell lines in parallel, where animals receive implantation of multiple fibres containing cells under the skin followed by treatment. We also aim to improve in vitro screening by using fresh tissues which this programme provides. Animals are humanely killed without any treatment, or receive procedures under general anaesthesia and killed before recovering from anaesthesia. Some animals may receive prior injection of non-therapeutic compound(s) which may cause mild and transient discomfort. Any animals that suffer adverse effects likely to exceed stated severity will be killed. All animals used in the experiment will be killed on completion 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 have built multiple ways to test novel compounds, including an extensive list of in vitro biology, ADME and toxicity studies which replaces some of the animal work. Drug properties and efficacy are, however, dependent on the complex system involving multiple organs which cannot be mimicked sufficiently in vitro, so the overall effects of the compounds can only be tested reliably in animals.

For some diseases, cell lines that best represent the target organ and diseases are rare. Primary cells and tissues from limited number of freshly-killed animals can provide materials to test compounds prior to proceeding to in vivo studies. This replaces testing using many live animals.

Reduction

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

Reduction

We will follow the in vitro compound selection system to avoid testing compounds that are sub-optimal in studies involving large groups of animals.

Where blood collection is needed, we use multiple micro-sampling from each animal, reducing the overall number of animals.

We will re-use animals that failed to develop tumours (typically 5-20% of mice subcutaneously implanted with tumours) in tolerability and ADME studies.

Ex vivo assays reduce overall usage of animals as organs from each animal provides sufficient material to test several compounds.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 primarily mouse for screening. Established protocols for testing drugs and historical data are widely available in the literature for this species. Ways of testing treatment effects on human cancers in mice are also well-established, allowing investigation of clinically-relevant disease tissues. We will perform the screening using subcutaneous tumour models as it is the least invasive and causes the least discomfort to the animals.

Rats are another well-characterised species for which many study protocols are established. These are also useful in predicting the drug-like properties and activity of compounds in humans.

Whenever possible, pilot experiments will be performed using the same strain, sex and supplier of mice as those intended for later studies involving disease models to ensure consistency and better prediction. Tolerability of drug treatments may be confirmed in tumour-bearing animals so that we are aware of the combined adverse effects of treatment and tumour development, if any.

Appropriate statistical methods will be used to design experiments and to confirm the finding so that the scientific data reported are reliable.

We consult current and emerging guidelines on animal research and implement improvement in regulated procedure when applicable. These include attempts to reduce stress by sugar-dipping oral dosing needle to make it easier for the animals, and using coloured restrainers. Use of temporary tail vein cannulation may also replace surgical cannulation of animals.

PROJECT 272

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.

Word limit; 1000 words

Project Title	Detecting bladder volume and pressure from sacral nerve signals in sheep
Key Words	bladder, sacral nerves, neuroprosthesis, sheep
Expected duration of the project	3 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:
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):

Accidents in people can cause damage to the spinal cord. This causes paralysis and incontinence for which there is no cure. In this project, we aim to develop a new treatment for urinary incontinence. To do this, we plan to design an intelligent implant able to monitor the bladder, for use in man. This has never been done.

Our objectives are:

1. to place in surgery a biocompatible implant on the nerves controlling the bladder;
2. to analyse, during surgery, electrical signals from the nerves controlling the bladder;
3. to continue recording electrical signals with the implant in awake animals during their normal activities.

Altogether, the project will deliver a new implant and surgical protocols for bladder control.

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

This project could benefit people or animals (such as companion dogs) that have sustained damage to the spinal cord. In the United Kingdom, around 50,000 people live with spinal cord injury, with about 1,000 new cases every year. This cost annually approximately £1 billion, which is 1% of the total NHS budget. Worldwide, spinal cord injury affects about 2.5 million people with approximately 130,000 new cases each year. This new treatment will offer affected humans a method to better manage urinary incontinence, instead of using drugs or bladder catheterisation that currently reduce life expectancy. We will also describe a new surgical technique for

this implant with less adverse effects for people. For the scientific community, our results will advance knowledge in: (i) the design and surgical implantation of implants for nerves; (ii) nerve signal processing techniques. It will be applicable to other medical conditions and to radar and sonar systems.

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

We plan to use a maximum of 16 sheep over 3 years.

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

All the surgical procedures are of moderate severity and will be conducted under general anaesthesia. From past experience, pain after the surgeries we propose to do will be mild and transient and can easily be controlled with drugs routinely used for animals. All possible adverse effects we might see are anticipated to be transient and controllable with routine veterinary care and medication. None of the procedures done in awake animals will be invasive, for example, none of these involve breaching the skin.

Application of the 3Rs

Replacement

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

Replacement

We need to use animals to test our implant because there is no other alternative that model the live urinary and nervous system. We need to do some recordings from a live animal, which size is comparable to humans. This is to allow future use in human patients.

Reduction

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

Reduction

We will minimise the number of sheep by using one animal at a time and analysing data before going to the next animal. We will also use animals over long periods of time using non-invasive tests to obtain a maximum of 'real-life' data without having to rely on biopsies or post-mortem evaluation only.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 sheep because their nervous system better mimics that of humans (compared to rats for example). This allows more straightforward application of our treatment to humans or other large animals. We will minimise welfare costs by using totally implantable and biocompatible systems and allow sheep regular access to grazing during the study period. Sheep will be able to express their natural behaviour throughout the project and methods of assessing our implant will remain non-invasive on awake animals.

PROJECT 273

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.

Word limit; 1000 words

Project Title	Repeat Dose& Reproduction Toxicology of Chemicals
Key Words	Toxicology, Reproduction, Regulatory
Expected duration of the project	0 year(s) 6 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:
Yes	(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):

This project licence will authorise the evaluation of chemicals and agrochemicals for potential toxicity. Studies will be conducted based on the rationale set out below at the request of the companies developing the products.

Toxicology evaluation involves the assessment of potential effects of a product upon a number of distinct biological systems and processes. These include systemic and reproductive function. The methodology is based on internationally approved test procedures that have been accepted and enacted by individual government agencies. The procedures are themselves regulated through internationally approved methods for 'Good Laboratory Practice' to ensure a uniform standard to the data generated from such testing methods. The test methods themselves are regulated by government agencies but in General relate to prescribed methodology from OECD (In particular EU), US EPA and Japan MAFF/METI/MHW. It is within this regulatory frame work that safety evaluation will be conducted. A program for testing is established for each new product under consideration. Any available toxicity data will be included in the evaluation 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 results of the safety assessment program provide information on the intrinsic hazard associated with use of a chemical. This hazard information can therefore be used in order to fully assess the risk presented and adopt appropriate risk management strategies.

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

1064 Adult rats (plus 6720 neonatal offspring derived from adults actually receiving treatment)- the offspring do not have procedures carried out on them but have indirect exposure to test items

In the context of what you propose to do to 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 range finding studies the highest severity of effect on adults only is moderate. In general this is only for 25% of animals exposed to the highest dose levels. 25% of animals are vehicle controls and are expected to be at subthreshold severity..A further 25% of animals are likely to be at the subthreshold severity limit as the test design has an expected No Effect dose level All foetal/neonatal animals are expected to be at the subthreshold severity limit as there are no/limited procedures on these animals and no direct test item exposure.

Application of the 3Rs

Replacement

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

Replacement

At present no validated alternative non animal methods have been developed that m eet the criteria established by international regulatory bodies

to fully evaluate the hazards of test items to the systemic and reproductive processe s in order to make informed decisions regarding risk of human/animal exposure

to chemicals

Reduction

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

Reduction

Use of minimum numbers of animals as quoted in the Regulatory test Guidelines suc h as OECD to ensure compliance and is the

expectation of the Regulatory bodies rsponsible for evaluating such test studies to m eet this minimum requirement

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 is the most commonly used species for Regulatory toxicity testing of Chemic als and Agrocheicals and are specified as the species of choice according to

the regualtory Test Guidelines as published by OECD

The test program for systemic toxicity studies will involve the use of small pilot studies with the intention to both establish the nature of any systemic effects and identify the dose-response to the test material. These pilot studies therefore allow for better study design for the subsequent definitive safety test and also identify those products with special hazards in order for a decision to progress is taken

PROJECT 274

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.

Word limit; 1000 words

Project Title	Creation, Breeding and Maintenance of Zebrafish (Danio Rerio)
Key Words	Zebrafish (Danio rerio), Genetic alteration, breeding
Expected duration of the project	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):

The objective of this Licence is breed and maintain zebrafish genetically altered and wild type to support scientific projects using fish embryos in non regulated procedures e.g. to discover new therapies for human diseases in which the vasculature is affected (cancer, diabetes, rheumatoid arthritis, among others) by studying the development of blood vessels in a model like the zebrafish. This will help us to find novel genes involved and to understand how they work

Fish will be bred to produce embryos which may be used in a variety of experimental procedures prior to the free feeding stage (D5).

What 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 we gain from this project will increase considerably the knowledge of the function of genes during development and increase our understanding of their role in development of diseases.

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

We anticipate using a maximum of 30,000 fish during the 5 years of the project.

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

Fish will be bred to obtain embryos for use prior to free feeding stage. Adult fish will be maintained for a maximum of 2 years after which they will be humanely euthanised. Where we wish to collect sperm from male fish they will be sedated and

gently squeezed to obtain sperm that can then be frozen for cryo preservation. Fish will be allowed to recover from this procedure before being returned to the main tanks. Where possible we will use non invasive methods to detect the genotype of embryos e.g swabbing of adult fish, but in a small number of cases it may be necessary to take a small fin biopsy - this will be carried out under anaesthesia and pain relief will be provided following the biopsy.

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 zebrafish larvae prior to free feeding allows the study and understanding of development in whole organisms which are less sentient than mammaliansystems. Zebrafish larvae can also be used in a variety of scientific uses e.g. high throughput screening of drugs that may effect thedeveloping heart or an understanding of fungal infections.

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

Fish will be maintained in small breeding colonies and expanded only as the science requires. We will use cryopreservation of sperm to archive rare genetically altered strains of fish to prevent unnecessary maintenance of live fish.

Novel genetically altered strains will be made available to and shared with other research groups.

Adult fish that fail to breed well will be humanely euthanised.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 breed and maintain healthy fish stocks, and new fish will only be introduced into the facility by the use of bleached embryos to minimise the risk of infection being introduced to the existing stocks.

Fish will only be maintained that are in good health and producing good numbers of eggs.

We will regularly check for advances in the refinement of housing and care for zebrafish and implement thesewhere appropriate.

If it is necessary to take a small biopsy of fin this will be done under anaesthesia and analgesia provided post operatively.

Feeding regimes used will ensure optimal fish health and use of live food will be used as appropriate.

PROJECT 275

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.

Word limit; 1000 words

Project Title	Kainate receptor-dependent plasticity and its role in brain development
Key Words	Autism, Kainate receptors, Synaptic plasticity, Glutamate receptors
Expected duration of the project	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;
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):

Nerve cells communicate and transmit information across structures called synapses. The sending
nerve cell (presynaptic) relays the information by releasing chemical transmitters. The receiving cell
(postsynaptic) detects that signal by specialized receptor proteins present at the synapses and modify
the way neurones are connected. In the adult, synaptic connections can change depending on how
they are used: busy synapses can become stronger or they can become weaker and even completely
disappear when poorly used. These processes, called long-term potentiation (LTP) and long-term
depression (LTD) of synaptic plasticity sound simple but, in fact, they require a highly regulated and
coordinated series of events that are the cellular basis for memory formation and learning processes.

Sometimes, an improperly orchestrated LTP or LTD activity can occur, manifesting as cognitive deficits.
This is the case for many neurological disorders such as dementia, Alzheimer's Disease or intellectual
disability. In addition, this plastic remodelling of the brain influences the correct formation of neuronal
networks during the childhood. In this stage, what we see, hear, touch, taste and

learn, will shape
specific circuits in a manner that they are reflecting the experience incoming form the external world.
The main target of the project is a protein called kainate receptor, which is present at the synapses.

My goal is to explore a new and unusual way in which kainate receptor activity can strengthen or weaken the synaptic connections, thus affecting the power of our brains to learn and memorize new
things. In addition, kainate receptors are present in very high levels at young synapses, when the
external experience is shaping them, and are reduced as the development progresses and the adult
patterns of neuronal circuits and connectivity are established.

How kainate receptors modulate other receptors in the synapse will be studied first, and then the
synapse capacity of being potentiated or depressed and eliminated to form a normal network of
connections will be tested. This is important because when it goes wrong, it is believed to cause
disorders such as autism, schizophrenia and intellectual disability. If so, the way to impede and prevent
such abnormality will be sought, which would indicate a new way in fight against these neurological
pathologies and its devastating consequences.

What 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 autism has advanced, the role of many factors operating normally in the brain development is still unclear. Existing data indicate that kainate receptors play a role in early processes that will determine the fate of the final brain organisation. Understanding how kainate receptors contribute to establishing the healthy interconnections in the brain will help us try to find the way to prevent the consequences of inappropriate connectivity. This will help us devise a new strategy in the fight against autism and its devastating consequences.

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

Mice, 2200.

In the context of what you propose to do to 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 genetically modified, but this is not expected to cause any adverse effect by itself. Their tissues will be used 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 autism models that does not require the use of brain tissue acutely removed from animals.
The project is intended to result in development of the new transgenic mouse strains engineered to
evaluate the role of kainate receptors in development of brain connectivity. 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 nonneuronal
tissue from the animals is used to the fullest extent 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 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

PROJECT 276

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.

Word limit; 1000 words

Project Title	Thrombopoiesis and application to transfusion
Key Words	platelets, megakaryocytes, transfusion
Expected duration of the project	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;
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.

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):

Platelets are small circulatory cells that are essential to prevent bleeding. If platelet numbers are too low, one can transfuse platelets to decrease the risk of bleeding.

The aim of the project is to better understand how platelets are made in the bone marrow and how genetic defect lead to disease of platelets in patients. We will also apply this knowledge to the production of platelets in the laboratory and the testing of these platelets prior to human trials.

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

The sourcing of platelet from blood donors for transfusion to patients has several drawbacks: 1. Sourcing constant supply, 2. donor-derived infections (such as hepatitis, or HIV) and 3. Immune reaction of the patients against donor blood cells. Producing platelets in vitro would eliminate these 3 issues. The project aims at better understanding how platelet are produced by their bone marrow mother cells (the megakaryocyte) to use this knowledge to produce the cells in the laboratory AND understand how certain proteins that control platelet formation can be targeted by drugs to reduce the platelet count in patients at risk of heart attacks and strokes. In this project we will also test the platelet produced in the laboratory to make sure they function properly after transfusion. This will pave the way for applying to regulators for authorisation to carry out clinical trials with patients.

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

This project proposes to use mice. Approximately 3000 mice will be used over 5 years.

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

The animals will be maintained in state of the art facilities, general husbandry will be done by trained staff and experiments carried out by an experienced team. The facilities will also be a “barrier” to infectious agents (to prevent infections particularly in animal with a weak immune system). The majority of the animals maintained under this licence are not expected to show any detectable adverse effects. Most experiments will require the collection of samples after animals are killed either at the end of procedures whilst terminally anaesthetised or otherwise humanely killed. In some instances animal will be administered drugs or cells via an injection in the tail vein or their tummy (or by mouth if appropriate). We may carry out blood test from the tail vein or one of the bigger vein in the leg. These will only cause very limited and short-lived discomfort. In some instances, to be able to carry out the assay described in the project, the animals will need to have their spleen removed under anaesthetic. The effect from this procedure on the animal will be moderate discomfort and will be limited to the post-operative period.

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 minimise the use of animals by using platelets or stem cells from human volunteers from which we can grow the platelet mother cells (megakaryocytes). However, the experimental approaches that we can use with human cells are limited. Platelets cannot be stored for more than 1 week and therefore patients with specific disease/mutations affecting platelets would have to be bled numerous times in order to study their platelets in details. The number of stem cells that can be obtained from blood is also very small which means megakaryocytes can only be grown in limited quantities to study how they function. In contrast genetically modified mice will give us the opportunity to study the influence of specific genes on platelets and their production by megakaryocytes by giving access to fresh samples to carry out comprehensive laboratory studies.

Moreover, megakaryocytes and platelets do not act in isolation from other cells. Megakaryocytes need the bone marrow environment to produce platelets and platelets will interact with blood vessel and other blood cells to form a clot. Our analysis of this complex process combines experiments on platelets in isolation from other cells, with experiments in a whole animal setting. This is vital to allow us to analyse gene function in the setting and context of these other cells and to test the quality of the platelets we produce for blood transfusion.

Reduction

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

Reduction

Animal numbers bred for use on this Project will be minimised as far as possible by matching breeding to experimental requirements. Pilot studies and power calculations will be employed to refine the number of animals used.

The methods chosen will generate the greatest amount of data for the fewest animals used. We routinely expect to derive multiple data sets from a single animal, by extensive use of modern approaches that allow us to analyse very small blood samples. This will be complemented by a team approach allowing the analysis of several different samples 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

Mice are the species of choice for the proposed investigations because they are a good mammalian model with a well-characterised bone marrow and blood vessels that are similar to humans. Studying megakaryocytes and platelets in mice therefore provides valuable information that will further our understanding of human biology and diseases. In particular testing of platelets produced in the laboratory in mice is a well-recognized quality control experiment without which we could not obtain the authorisation to go on to do clinical studies in human patients.

Genetically-altered mouse technologies are becoming increasing sophisticated, where genes can be turned off within specific cells when required, whiles leaving the rest of the animal unaffected. Such genetically altered animals will be used wherever possible in this project and will greatly reduce the risk of adverse effect to the mouse. This is because only the platelets will be genetically altered, whilst all the other cells in the mouse will be normal.

PROJECT 277

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.

Word limit; 1000 words

Project Title	Maternal fasting and offspring health
Key Words	Ramadan, fasting, pregnancy, cognition
Expected duration of the project	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):

Healthy adult Muslims are required to fast during Ramadan. Pregnant women are exempt; however the majority take part in the daily fast. The impacts of intermittent fasting on the health of the developing fetus and adult offspring are not fully known. A poor or imbalanced diet during pregnancy is known to slow the growth of the fetus and lead to both cardiovascular disease and poor mental health. The aim of the proposed study is to determine whether intermittent fasting in pregnant rats, which models aspects of human fasting during Ramadan, has detrimental effects on growth of the fetus and the cardiovascular and mental health of the offspring. We will also study the role that the bacteria in the intestine play in the development of the brain and whether the mother's diet during pregnancy affects the signals that the bacteria send to the offspring's developing 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)?

Worldwide there are 230 million Muslim women of childbearing age. On average, Muslim women have 3 children. Some women will be pregnant outside Ramadan, but up to ¾ of all pregnancies will coincide with Ramadan at some stage. Therefore there are potentially 517 million babies globally that have been exposed to intermittent fasting before they were born. Studies in Muslim countries suggest that the incidence of mental impairment is greater in children whose mothers fasted while pregnant. Unlike other forms of undernutrition during pregnancy, which are usually driven by poverty, fasting during Ramadan is voluntary. Many Muslim women seek assurance from health practitioners that fasting during pregnancy will not be harmful to their baby; however the advice available to pregnant women is contradictory. Clear guidance upon which women can base an informed choice is lacking; therefore

there is a need to understand what happens to the fetus if the mother chooses to fast while pregnant.

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

We will use rats as an experimental model for this project which will last five years. We estimate that we will use 1550 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?

Experiments will start by removing food from pregnant rats overnight, which is when they are active and would normally eat most food. In some cases this will be for a few days of pregnancy; in others it may be for the majority of pregnancy. The animals will feel hungry, but they will have the opportunity to eat as much as they wish when food is returned each morning. In some experiments we will remove water overnight, in others we will remove both food and water in order to mimic the fasting that humans voluntarily undertake during Ramadan. To study the movement of nutrients across the placenta from the mother to the fetus we will use non-recovery anaesthesia so the animals will not suffer any pain. In other experiments we will let the mothers deliver their litters normally. Some of these animals will be used to study memory, learning and behaviour. Sometimes food will be removed overnight prior to the test so that food can be used as a reward to motivate the animals. In the majority of tests the animal's environment will be enriched to stimulate it to explore or interact with others; therefore there will be no adverse effects. Experiments designed to look at the bacterial content of the intestines will use samples of faeces passed normally by the rats or tissue collected from animals that have been killed by an approved method. The final phase of the study will involve feeding rats either a high salt or high fat diet after they have been weaned. Rats eat such diets readily, although the diets will lead to an increase in blood pressure and the risk of diabetes. Blood pressure will be measured using a device applied to the animal's tail which is similar to a human blood pressure cuff. To do so, the animal must be restrained which causes some distress; however this is minimised by training. Urine will be collected by placing the rats in cages with wire grid floors: they find such cages distressing so we will minimise harm by holding them for as short a time as possible to collect enough urine for analysis (usually 2-4 hours). In order to test for diabetes we will use a glucose tolerance test which involves injecting a glucose solution into the abdomen and then collecting small quantities of blood from the tail repeatedly over the following 2 hours. This will cause brief discomfort when a needle is inserted. The final experiments will involve a study of kidney function; however as this is done under non-recovery anaesthesia it will not cause any pain or distress to the animal.

Application of the 3Rs

Replacement

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

Replacement

We will be able to use human placentas for some of our planned studies; however it is not possible for ethical and safety reasons to involve pregnant women or their children in some of the more invasive experiments designed to help us understand underlying biological mechanisms. We must therefore use an animal species that has a placenta for the work outlined in this licence. As only mammals have placentas, we cannot use a lower species; therefore we have chosen to use rats.

Reduction

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

Reduction

We have designed our experiments carefully so that we can get as much information as possible from each animal. For example, an animal that has been used for behavioural experiments can also go onto provide tissues for follow up molecular experiments. We have based our estimates of animal numbers on our own experience with the model, so we have a realistic understanding of how many rats will be required to complete 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

We have chosen to use rats as they have large litters (usually around 15 pups) so we can get as much information as possible from one pregnancy. Their size makes them preferable to mice as they are better suited to the planned surgical procedures; hence the success rate will be greater. In our earlier studies, we fasted pregnant rats daily for the whole of pregnancy. However, in order to more closely mimic the duration of human fasting during Ramadan we have refined our model to fast animals for 3 days, which is equivalent to 1 month of a human pregnancy.

PROJECT 278

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Characterisation & Repair of Immune Defects in Chronic Lymphocytic Leukemia
Key Words	Blood cancer, Immunity, Cancer treatment, White blood cells
Expected duration of the project	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;
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):

In this project, we want to study the role of our immune system in fighting blood cancer. In particular, we will investigate what are the biological mechanisms that normal white blood cells in our body apply to eradicate or to keep cancer cells in check. We also want to investigate the mechanisms that blood cancer cells use to evade this organismal supervision when a full-blown disease develops. With this knowledge in hand, we will be in a position to improve the cancer treatment options in a clinic by helping our body immune system to eradicate blood cancer cells.

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

These results will further improve our knowledge of how the immune system keeps blood cancer in check. The knowledge obtained will be used to design new principles of blood cancer therapy and make it much more successful in clinic. Apart from improving clinical cancer care, we aim to generate additional knowledge on how the immune system works in a healthy organism. The latter will be of great benefit to basic scientists working on the biology of the normal immune system. Thus, our knowledge contribution is likely to be larger than in blood cancer research only.

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

It is our aim to keep the number of animals as low as possible and we will apply a number of strict measures to achieve this. We are aiming to use approximately 12,000 mice over 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?

The expected adverse effects will be similar to the ones observed in early stages of blood cancer in human. These can range from complete absence of symptoms to enlarged lymph nodes and spleen, with or without a high white blood cell count. In more advances disease stages, after the cancerous cells take over the bone marrow, mice can experience tiredness or weakness. Importantly, all novel anti-cancer therapies we will test are likely to result in alleviation of blood cancer symptoms. Furthermore, all the procedures will be either painless or pain will be reduced by applying appropriate methods of anaesthesia. To avoid any suffering associated with with late stage disease, animals will be humanely euthanized at the end of the rigorously controlled protocol.

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 cell lines that could be grown in tissue culture flasks, and that would mimic the type of disease we aim to study. The type of blood cancer cells of our interest, either human or mouse, do not survive outside organism in a long-term laboratory environment. Furthermore, since we aim to investigate the role of immune system in fighting cancer, it is essential that we use organisms that contain adequate immune system. Hence, the animal model is the only available option for our project. However, we are constantly exploring for possibilities to replace animal experimentation with alternative approaches.

Reduction

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

Reduction

We will perform experiments involving mice *only* if prior results obtained using non-animal tools (e.g. cell lines) are promising and are likely to yield significant and reliable knowledge. We will also apply very rigorous statistical tests (power analysis) to keep the number of animals to an absolute minimum necessary to obtain meaningful and reliable 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

Our mouse model is the only one available to examine the relationships between the immune system and chronic forms of blood cancer. Wherever possible, we will apply non-invasive techniques such as observation and non-invasive imaging to minimise any potential harm. Importantly, in our previous work, we haven’t observed any severe adverse effects associated with our animal research. Thus, the animal welfare costs (harms) are likely to be very low.

PROJECT 279

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.

Word limit; 1000 words

Project Title	Production, Breeding & Maintenance of GA zebrafish
Key Words	Zebrafish, Breeding, Genetically altered
Expected duration of the project	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;
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 purpose of this work is the efficient and standardised production, establishment and archiving of high quality genetically altered Zebrafish, and to supply them for research into the study of normal and abnormal physiology, development, disease modelling or biology.

Zebrafish models have been developed for several human diseases, including blood disorders, diabetes, muscular dystrophy and neurodegenerative diseases. The transparency of the developing zebrafish embryo has enhanced its usefulness for *in vivo* imaging to study developmental processes. One area where much progress has been made is in the study of the genetics of the development of the heart and vascular system, and the nervous system. Increased understanding about the genes involved has also contributed to understanding of these processes across vertebrates.

Processes that occur during very early embryonic development and the study of gene regulation at these early stages are not readily tractable in mammals owing to their *in utero* development. Zebrafish embryos are particularly suitable for such studies at early developmental stages including early cell divisions, maternal to zygotic transition, cell movements, and gene regulatory networks that control cell fate specification.

By good husbandry and welfare we will produce high quality research zebrafish. This will lead to fewer fish being required for experimental need and reduce duplication of work, leading to better quality science.

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

Acquisition of new knowledge with respect to basic mechanisms of physiology, development and cell biology. Without this we would be unable to take advantage of these critical genetic methods. In addition, the findings will be published in peer reviewed journals, thereby sharing with the scientific community so that our data and methods can help others working on similar projects. Our GA zebrafish will also be valuable to the wider community, reducing the need for others to expand fish numbers by recreating them. By providing this service centrally we are able to maximise the efficiency of the breeding colonies and thereby keep the number of animals required to a minimum. The animals are then supplied to scientific projects within the institution which have undergone ethical and peer review.

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

The expected use is a maximum of 28,000 zebrafish over the duration 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?

Breeding of gene variants to homozygosity or onto new genetic backgrounds may cause behavioural and physical abnormalities in the offspring. Some of the genetic variants may cause a phenotype during early stages of embryonic development. Larvae that display such defects would be humanely killed before they reach free-feeding stage. In other cases, defects may be observed later or when the genetic variant is crossed onto a different genetic background (frequency variable and dependent on the gene in question). Defects may also occur as a consequence of general anaesthesia (<1%) and fin clipping for genotyping (<1%). Squeezing the abdomen for egg and sperm isolation may cause skin damage (<1%) or inner organ compression (<1%). Fish will be checked at least daily and only fish with normal or mild phenotypes will be maintained.

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 research projects will involve the use of *in vitro* systems such as cell culture, human tissue assays and computer modelling to complement the animal work. Such details will be expected in the justification for the animals' use to be reviewed by the AWERB.

However, *in vitro* assays cannot adequately model the complete array of molecular, cellular or physiological interactions necessary to fully understand how genetic modifications result in normal or abnormal processes.

Fertilization and development of zebrafish embryos occurs ex utero, and the work therefore helps to address the 3Rs by using alternatives to mammalian models. Adult fish for mutant and transgenic lines will be maintained as heterozygous stocks or as homozygous stocks when they show no overt phenotype.

Zebrafish are a well-established model system with the lowest neurophysiological sensitivity possible for work of this kind.

Reduction

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

Reduction

We continuously look at ways to minimise the number of animals used to propagate mutant models. Unnecessary production and import of genetically altered animals will be avoided by searching cryobanks and databases.

Animals will only be bred if a user requirement has been established, and the breeding programme will be subject to regular review to optimally meet anticipated demand. Spare animals may be made available for use on other scientific projects where appropriate.

Breeding will be optimized, wherever possible, to produce only the genotype required e.g. homozygous breeding pairs.

We generally maintain ~50-80 adult heterozygous or homozygous fish (mutant or transgenic), for each line. Some lines will be kept at higher levels to accommodate research that requires the generation of large numbers of eggs. The lines will be of a range of backgrounds, both wild-type and genetically modified. These animals undergo no procedure other than breeding and genotyping.

For founder lines, we will raise approximately one hundred embryos to adulthood to identify a minimum working stock of positive founders. However, techniques are being developed that increase transgene incorporation and mutant rates. Where possible we will use these to reduce the number of embryos that require raising to identify founders.

Efficient colony management ensures that only colonies that are actively required are mated and produce animals. Cryopreservation of gametes and embryos to archive lines will avoid wastage from the need to maintain colonies by continuous breeding. We require 125 animals in order to freeze lines that are not in active use.

Where possible, the use of CRISPR/Cas9a will be investigated to further reduce the number of fish used and create a more bespoke mutation

Refinement

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

Refinement

With current genome engineering and transposon-based methodologies, it is fairly easy to generate targeted mutations and introduce transgenes in zebrafish. Genome engineering is very efficient with targeting nucleases (10-30% of injected embryos, in our experience; Lim et al., Genome Biology, 2013). Therefore, genetic engineering at desired locations in the genome can be achieved using very few animals.

All the experimental manipulations described will be carried out on embryos immediately after fertilization, and since the animals are under 5 days post fertilization, they do not fall under ASPA protection. The standard protocols, methods and reagents have been optimized for this species and there are acknowledged benefits from their use. The methods chosen are all standard for this type of work.

Published guidelines for best practices will be followed for the duration of the licence

Fin clipping of live fish under anesthesia is widely used to collect samples for DNA extraction. An alternative, potentially less invasive, approach involves obtaining samples by swabbing the skin of briefly anesthetized fish. However, this method has yet to be widely adopted for use in laboratory studies in the biological and biomedical sciences. Under this project licence we will aim to establish a reliable protocol for skin swabbing and move towards this as the main method of obtaining DNA samples. If fin clipping is to be used then where possible, analgesia will be administered.

PROJECT 280

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.

Word limit; 1000 words

Project Title	Understanding and Modulating the Immune System to improve Regeneration.
Key Words	
Expected duration of the project	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):

Due to improved modern treatment, most patients today survive an acute heart attack. Unfortunately, the vast amount of damage to the heart muscle can lead to progressive weakening and decline of heart function, eventually leading to heart failure in about 1 in 5 patients.

There is increasing evidence that the immune system plays a crucial role in maintaining heart health and repairing the heart muscle after a heart attack. Immediately after an acute attack, the immune system is essential to quickly repair and stabilize the wound. However, excessive damage can wrongly activate parts of the immune system which can cause destruction of the body’s own tissues, defined as an autoimmune response. This leads to the immune system attacking the heart muscle, which may further exacerbate heart damage and prevent recovery.

In addition, patients known to suffer from autoimmune diseases such as Systemic Lupus erythematosus (SLE) or Rheumatoid Arthritis (RA), have a significantly increased risk of developing heart disease and a much worse prognosis after a heart attack.

We therefore aim to understand the crosstalk between the heart and the immune system with the final aim to find ways of modulating it to prevent the immune system from targeting the heart while boosting its regenerative function.

To achieve these overarching goals, we propose 5 objectives:

Objective 1: What is the role of the immune system in repair and recovery after a heart attack?

Objective 2: How does the immune response after damage differ between more regenerative organs such as skeletal muscle and skin and non-regenerative organs such as the heart?

Objective 3: How is the heart affected by systemic inflammatory/autoimmune conditions?

Objective 4: How is the immune response after a heart attack changed under inflammatory conditions, such as autoimmune disease?

Objective 5: Can we find ways to 'modulate' the crosstalk between the heart and the immune system to boost heart regeneration?

What 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 major need for treatments that improve diagnosis and quality of life after a heart attack, as well as prevent heart disease caused by inflammatory conditions. As an immediate benefit of this project, we expect to generate valuable data for both pre-clinical and clinical scientists. REDACTED The long-term benefits of this project may be new therapeutic options to improve heart regeneration and treat hyper-inflammatory conditions.

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

We will use adult mice and expect to use approximately 5400 animals during the 5 year program of work, the majority of which will undergo mild to moderate 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 majority of animals will undergo mild or moderate procedures and will be covered with appropriate anaesthesia and analgesia throughout. All surgeries will be performed following LASA/Home Office guidelines for aseptic surgery. After treatment/surgery mice will be monitored closely until full recovery and appropriate pain relieve will be provided. Monitoring will be performed using score sheets to assess pain levels and general health. Humane end points are defined clearly so moderate severity limits are not exceeded. Objective 1: What is the role of the immune system in repair and recovery after a heart attack? In the majority of mice, heart injury will be induced by injection of Isoproterenol as a less invasive alternative to surgical induction of damage. Some animals will be selected for surgery. As these procedures induce heart damage, possible adverse effects might be acute heart complications and the development of heart failure after about 18 weeks. All treated mice will be monitored using score sheets and signs of acute complications or later heart failure are defined as humane endpoints and animals experiencing these

symptoms will be humanely killed. Objective 2: How does the immune response after damage differ between more regenerative organs such as skeletal muscle and skin and non-regenerative organs such as the heart? In some mice, tissue injury will be induced in skeletal muscle or skin. These procedures may cause transient local discomfort, and pain relieve will be provided. Objective 3: How is the heart affected by systemic inflammatory/autoimmune conditions? Mice will be treated to induce inflammation or autoimmunity and the effects of autoimmune disease on the heart will be assessed over time. We will use a model of a systemic autoimmune disease called 'Systemic Lupus erythematosus (SLE)'. Clinical signs of the disease may be pale skin, lessened mobility and hunched appearance. However, our current treatment regime has been optimised to avoid strong effects on general health, but still allow for immunological and cardiac readouts to be obtained. Objective 4: How is the immune response after a heart attack changed under inflammatory conditions, such as autoimmune disease? Mice will be treated to induce inflammation or autoimmunity. After treatment, mice will be allowed to recover fully from any acute responses before tissue injury will be induced. Anticipated adverse effects are as described for objective 1 and 3. Objective 5: Can we find ways to 'modulate' the crosstalk between the heart and the immune system to boost heart regeneration? Tissue injury will be induced as described above, and treatments administered to boost the regenerative arm of the immune system. Anticipated adverse effects are as described for objective 1 and 3 and no additional adverse effects are expected from therapeutic interventions. For all procedures, mice will be monitored by non-invasive imaging over a period of 8-12 weeks. Blood samples will be obtained for immunological readouts. At termination, tissues (serum/blood, spleen, lymph nodes, heart, skeletal muscle, skin) will be collected for ex vivo and in vitro analysis.

Application of the 3Rs

Replacement

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

Replacement

There is to-date no non-animal replacement for the investigation of the interaction between organs (heart, muscle, skin) and systems (immune-system) in the body and possible therapeutic procedures influenced by this. Rodent models are an essential and recognised pre-clinical step.

However, wherever possible animal work for this project will be embedded and supported by a variety of well established *in vitro* and *ex vivo* approaches, including *ex vivo* imaging techniques and the use of human cells and tissues.

Reduction

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

Reduction

We aim to design every experiment in a way to maximise data obtained from a single procedure by using longitudinal paired measures and sharing target organs between researchers within the group at termination. We further continue to collaborate with colleagues in REDACTED to obtain surplus lymphoid tissue from animals that undergo surgeries for other readouts.

Thorough experimental and statistical planning ensures robust and reliable outcome of experiments to avoid the necessity of repeats and reduce animal numbers.

Our breeding strategy aims to generate as few as possible surplus animals, and ‘wrong-genotype’ animals are used as tissue donors and controls for separate 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 will be used as they are a suitable model for exploratory pre-clinical investigations. They are universally used for this kind of research work and the standard protocols, methods and reagents have been optimised for this species, thus ensuring most efficient use of animals. To safeguard animal welfare, we will follow general rules of good laboratory practice and published guidelines for the work with research animals. We will also stay up-to-date on potential novel techniques to increase the 3Rs impact during the course of this project.

PROJECT 281

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.

Word limit; 1000 words

Project Title	Embryology and developmental disorders in zebrafish
Key Words	Neuroscience, embryo development, brain disorders, neurodegeneration
Expected duration of the project	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;
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 overall aim of the programme is to understand how the brain develops in order to be able to better design ways to cure or prevent brain defects, either during pregnancy or neurological diseases during adulthood (such as motor and cognitive neurodegeneration: Amyotrophic lateral sclerosis, spastic paraplegia, Alzheimer, fronto-temporal dementia).

Genome projects have now identified most of the genes present in the vertebrate genome but it remains a huge challenge to understand the function of these genes. Studies in animal model systems are currently the only feasible approach for studying complex gene functions.

This proposal describes a programme of research aiming to elucidate the mechanisms that ensure development, function and survival of neural tissues, using the zebrafish. Our research team is recognised as one of the leading groups in the world in this field of research. We have trained PhD students and post-doctoral fellows and have a range of technical skills that cover all aspects of this research project. We have made it a priority to design experiments that will affect the animal as little as possible and the overall level of severity of the protocols required is MILD.

Our research has four broad objectives: i) To identify and characterise the function and regulation of genes involved in regionalisation of the developing brain; ii) to identify and characterise the function and regulation of genes involved in development, proliferation, function and survival of neurons; iii) to identify the behavioural defects associated to development of abnormal circuits, and iv) to elucidate the cell behaviours that underlie the regionalisation and morphogenesis of neural tissues. A key element for these studies is the use of forward and

reverse genetic screens (from colleagues and/or obtained through protocol 2 and fish bred in protocol 1). The project will identify a collection of genes that are important for brain development and disorders.

To investigate the role of these and other already identified genes in detail, we will generate transgenic lines (protocol 1) that allow visualisation of brain cells in vivo or manipulate gene function in an inducible way. Transient ways to manipulate gene functions and trace cells over time in the live embryos will also be used (protocol 1). Complete analysis of gene function will require detailed neuroanatomical (protocol 3) and behavioural (protocol 4) phenotypic studies. A maximum of around 50,000 zebrafish will be used in the five years covered by this PPL.

Reduction-Refinement-Replacement:

Because our key aim is the understanding of developmental events leading to the formation of the vertebrate brain, most of our research plan can't yet be done in tissue culture. We want to discover the cell behaviour involved in building a functional brain and therefore need to observe behaviour *in vivo.* These observations are made on embryos and larvae younger than 5 days of development (too young to be under the Act). The embryos are coming not only from wildtype crosses but also from transgenic and mutant carriers. We chose the zebrafish as sole model organism, because the embryos are spawn in the water (allowing collection without affecting adult mothers) and are optically clear (allowing for observation of brain development with a very minimum of intervention needed). We therefore make most of our observations without any need for surgery or other intervention giving discomfort to the adult animal. We make use of cell culture system to address refined molecular interactions whenever possible. We have refined our experimental designs such that only mild protocols are required, with the exception of protocol 3. For this protocol of moderate severity, we have reduced the number of adult males used to a minimum required to produce the number of embryos needed to establish mutant lines. This minimum is 20 animals. No animal will ever be put in any stress or 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)?

1. In the last years, the identity of all human genes (and all mice and all zebrafish genes) has been uncovered and numerous lists of genes implicated in specific human disorders have been produced. Yet we understand very little about the function of the vast majority of these genes. Our studies will contribute to the understanding of gene function during embryonic brain development. This includes, neuronal fate specification, neuronal connectivity and establishment of neuronal circuits. It will also provide insight into fundamental processes such as cell migration and cell division. These finding will have direct impact in understanding of human diseases such as neurodevelopmental disorders (eg. Autism Spectrum Disorders,

intellectual disabilities, FoxG1 syndrome), neurodegeneration (eg. Alzheimer, ALS, dementia) and cancers. 2. The characterisation of more genes involved in fundamental processes during early vertebrate brain development will give greater understanding of the molecular and genetic interactions that establish structures in the nervous system. It will also give insight in evolution of the forebrain in vertebrates. 3. A long-term outcome will be an increased understanding of the genes contributing to congenital and neurological defects in vertebrate brain, including humans.

What types and approximate numbers of animals do you expect to use and over what period of time?

Over the next 5 years, we predict that around 50,000 fish will be used. Most of the use is in the form of genetic crosses of genetically modified adults to provide embryos and young larvae (3-5 days post fertilisation) we will analyse mostly through live imaging.

In the context of what you propose to do to 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 procedures we will use routinely has any adverse effect. The exception is procedure 2, planned as we may need to conduct a small forward genetic screen using a very limited number of animal (20 adult males). Procedure 2 will only be used once over these 5 years. The treatment will only give mild to moderate discomfort to the fish and advert long term effect of the drug used will be avoided (animals culled by schedule 1 at 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

As the main goal of our research is the understanding of the cellular and molecular mechanisms required for the formation of the brain in the vertebrate embryo, alternatives not using animal at all are not yet available. The originality and strength of our study is the ability to follow cell behaviour in vivo, using imaging of fluorescent transgenic zebrafish. In vivo studies are the only way to identify the cellular behaviour at the source of developmental defects. Some of the molecular candidates involved in formation of neuronal circuits will be assessed in neuron primary culture. We are currently starting to develop 3D cell culture technologies ("organoids"), hoping to get these to be used for some of our scientific questions, instead of the fish. We did search for more alternatives, using FRAME and other sources suggested on the NC3Rs website, without success.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The principle of reduction is applied for all procedures used. Number of embryos and adults used are defined following power calculation as the minimum required to ensure statistically-relevant results. Most of our work will employ zebrafish larvae prior to independent feeding – effectively being a replacement of mouse use. We will employ the optical clarity of fish larvae to allow the use of new microscopic methods to image brain development or brain disorder models. The aim of much of our work is to use transgenes that do not disrupt normal processes to report on those processes as they happen. So, although the animals are transgenic and therefore fall under the Act, they will not suffer in any way compared to non-GA fish. Creating stable transgenic lines is by itself a way to reduce number of animal used. The establishment of healthy viable adults carrying a transgene allows us to reduce the number of embryos studied compared to the quantity we would have to use in a transient system.

As stated in protocol 2, the number of adult males treated by mutagen is reduced to the minimum required to obtain 1000-2000 progeny by outcross (creation of a suitable F1 generation of heterozygous carriers). The conditions of the treatment have been refined to induce little or no discomfort to the males during and just after treatment. Males are culled under schedule 1 before developing any health problem.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 the zebrafish as sole vertebrate animal model because the embryos are spawn in the water (allowing collection without affecting adult mothers) and are optically clear (allowing for observation of brain development with no intervention needed). We therefore make most of our observations without any need for surgery or other intervention giving discomfort to the adult animal. With the exception of protocol 2, our procedures are of mild severity and do not generate pain or distress. In the rare event that the fish need to be kept at the end of a procedure, it is first monitored for sign of pain/distress (lack of activity, appetite or sexual behaviour). Any individual presenting any sign of pain or distress that is not rapidly curable will be killed under schedule 1.

Protocol 2 is of moderate severity. It has been optimised for time of exposure to the mutagen as well as concentration of mutagen used to inflict the least distress and pain possible. The number of adult males used has been reduced to a minimum of 20 required to produce the number of embryos necessary to screen mutations spread throughout the whole genome. The optimal number of adult has been defined by published studies performed by international labs in the last 20 years. The procedure will be done in an isolated calm area and great care will be given to the fish after each bath in the mutagen. Any individual presenting signs of distress or pain persisting in the hours following each treatment will be culled under schedule 1.

PROJECT 282

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.

Word limit; 1000 words

Project Title	Imaging the microcirculation
Key Words	Angiogenesis, Neurodegeneration
Expected duration of the project	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;
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):

Changes in blood vessel function underlies a number of diseases such as blindness, pain and stroke. We aim to further understand how blood vessels adapt in times of stress, for example low oxygen or hyperglycaemia, which result in production of a protein, called vascular endothelial growth factor (VEGF) that drives formation of new, leaky vessels. This will allow us to improve our knowledge of how changes in blood vessel function are brought about, and eventually how they affect disease. In our models we can use the VEGF protein, which has been shown to eventually contribute to blindness and damage to the nervous system underlying conditions such as pain.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Many differing diseases are caused by blood vessels not working properly. Specifically, the cells lining the blood vessels, and the cells and neurons that the blood vessels supply, usually act together to control blood flow and leakiness of the vessel wall to maintain healthy tissue. When the cells lining blood vessels are exposed to unhealthy conditions (e.g. too much sugar, not enough oxygen) they become dysfunctional and this can lead to organ dysfunction and pain, and ultimately, organ failure or in the case of limbs, the need for amputation. Pain, blindness and stroke are leading causes of decreased quality of life and mortality. In many of these instances we are still unaware of how this endothelial dysfunction leads to improper functioning of the blood vessels. These studies aim to identify how the blood vessels maintain blood flow, without becoming leaky, allowing us to design new drugs that will work to prevent these diseases.

What types and approximate numbers of animals do you expect to use and over what period of time?

500 rats 500 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?

In these experiments we will image directly how blood vessels work in the body (mesentery and the spinal cord). We will do this by aneasthetising the animals, then undertaking surgery to expose blood vessels from either the spinal cord or the connective tissue of the gut (laminectomy or a laparotomy respectively,) and injecting a dye into the blood vessels to allow us to record blood and dye flow through and across the vessel and find out how drugs can affect this. All animals will be terminally anaesthetised and killed at the end of the experiment, and we will use the tissue from these animals to do further analysis of how blood vessels work in these tissues.

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 work we check that the drugs to be used work on isolated blood vessel cells (in vitro, i.e. in a culture dish) and also check the scientific literature. However, these experiments can only be done in the animal as blood vessel function is controlled by many interacting cell types and biological processes and therefore can only done in the animal.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Direct imaging of the blood vessels in animals immediately reduces the number of animals required to be used as we can directly compare this to other studies. Using statistical analysis we can calculate the minimal number of animals required to test if our experiments will work - i.e. the outcomes from the experiments will be unambiguous in telling us whether the changes we are proposing and the mechanisms we are testing are true or not.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 have been used widely in the literature therefore we can directly compare our studies with those have been published. These animals have more comparable blood vessel systems to that in humans. All experiments are done under sterile conditions whilst animals are deeply anaesthetised throughout and will not recover from the anaesthetic or feel pain.

PROJECT 283

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.

Word limit; 1000 words

Project Title	Immunogenicity of biologics
Key Words	immunogenicity, IgG, biologics
Expected duration of the project	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;
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):

Protein immunogenicity, is the ability of proteins to be recognised as foreign (and potentially harmful) by the immune system and therefore removed. The problem of immunogenicity is very important for a new type of drug “biologics” that are widely used in inflammatory diseases like arthritis (the top 5 best selling drugs in 2015 were biologics). Biologics are proteins, often antibodies, which are good drugs because they are very specific, so only act on the target tissue, and do not have “off-target” effects or toxicity. However, although these drug proteins are usually engineered so that they should not look foreign to the patient’s immune system, in many patients the drug protein is recognised as foreign and so the immune system responds like it was an infection, producing antibodies to get rid of the drug protein. These anti-drug antibodies mop up the drug so it does not work any more and can no longer be used to treat the patient. The focus is on understanding how changes to the shape of the protein (its folding), especially those that lead to the protein clumping, result in a stronger immune response to the drug protein, in order to help design better drugs that might avoid this problem.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Biologics are increasingly widely used and are very effective in the treatment of many immune and inflammatory diseases such as arthritis and psoriasis which are very unpleasant for the sufferer. The testing under this project will help understand how they sometimes cause immune responses that prevent their use, so will help to develop better drugs which do not have this problem so that more patients can be treated safely

What types and approximate numbers of animals do you expect to use and over what period of time?

The project will last for 5 years. Animal numbers are as follows Mouse : 1575

In the context of what you propose to do to 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 these studies will be conducted using mice. The mice will be dosed by intraperitoneal injection with test proteins, sometimes with adjuvant (materials that enhance immune responses), or with test proteins that have been modified eg by aggregation. The animals will have booster injections of the same materials at weekly intervals for up to 4 weeks by the same route. At the end of the experiment they will be killed humanely and immune tissue (lymph nodes or spleen) and blood taken. The animals will usually show nothing more than minor discomfort from dosing. They are expected to eat, drink and groom normally after these procedures. The animals will be expected to develop an immune response to the injected proteins. In our experience the immune response to these types of proteins does not cause any noticeable effects but it is possible, although very unlikely, when using new materials that an allergic reaction could occur. Allergic reactions would happen immediately (within minutes) after repeat dosing and signs of allergic reaction would be blueing of the skin and respiratory distress. On the day of dosing the experimenter stays with them for 1 hour after dosing (which is done at the beginning of the day) and they are checked several times more on that day. If they have an allergic reaction that did not resolve within a couple of minutes they would be humanely killed. It is also possible, but very unlikely, that the proteins used might have some inherent toxicity or be contaminated in some way, such as with bacterial products like endotoxin. Again this effect was not seen under the previous licence with these types of protein. Doses used are relatively small (usually a maximum of 2.5 mg) and the materials that will be used do not usually cause damage unless the specific target of the antibody crossreacts with mouse tissue and could cause a “cytokine storm”. This effect would be most likely seen on first dosing and occur fairly rapidly. For every new protein, and every type of modification, sighting studies using one mouse will be conducted where animals are always dosed at the beginning of the day and checked four times more on the day of injection for any sign of adverse effects (piloerection, hunched posture etc). If they had signs that persisted for more than an hour they would be humanely killed. All animals are humanely killed at the end of procedures.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The immune systems of mice and humans are very complex with many different parts working at different times and in different ways together and alone. Parts of the immune system can be modelled in test tubes in isolation but it is not yet possible to study the immune system as a whole in this way. So we require animals with an immune system like humans. The immune system of mice is very well studied, and is sufficiently similar to human to be appropriate for this work. The work is conducted in parallel with investigations on human antibody responses to biologic drugs. A substantial part of the investigations are conducted ex vivo with the tissue/serum obtained.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All experiments will be designed in order to achieve the scientific objectives whilst using the minimum number of animals. Cells and serum are archived for future analyses if alternative end points become apparent from subsequent investigations or from the published literature.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 used for these studies. This species are considered to be of the lowest neurophysiological sensitivity available that will allow the study aims to be achieved. For the majority of studies the severity limit is mild (with the exception of sighting studies as the materials will be unknown) and the procedures are not expected to cause anything but mild discomfort during the injection. Sighting studies will be conducted with a single animal for any new material/preparation to ensure there are no unexpected effects caused by the new material. Animals will be routinely group housed with congenic animals from the same stock date and will have environmental enrichment. In an improvement on previous practice, individual mice used for sighting studies will be identified and will remain with cagemates for the duration of the study. Advice on general animal welfare and for specific concerns about the health/well being of the animals will be sought from animal unit staff including a veterinary specialist who is available to advise on any ill health issues and appropriate care for the animals.

PROJECT 284

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.

Word limit; 1000 words

Project Title	Role of Arp2/3 isoforms in mouse development and tissue homeostasis
Key Words	Arp2/3 complex, actin, cytoskeleton, physiology
Expected duration of the project	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):

The actin cytoskeleton, part of the cells skeleton, provides driving force and structural support for the physical integrity of cells and a wide range of essential cellular processes such as membrane trafficking, cell adhesion and cell migration. The correct regulation of the actin cytoskeleton in space and time is essential during development and throughout the lifetime of multi-cellular organisms. Moreover, deregulation or malfunction of the actin cytoskeleton results in a variety of developmental syndromes and diseases as well as tumour cell metastasis. The investigation of the molecular operation, regulation and organization of the actin cytoskeleton is thus essential to understand human development, physiology and disease. The Arp2/3 complex, consisting of seven proteins (Arp2, Arp3, ArpC1-5), induces the formation of branched actin filament networks that perform an essential function in a wide variety of cellular processes. In humans and mice, the Arp3, ARPC1 and ARPC5 protein subunits are encoded by two different isoforms. The overall aim of this project is to investigate the physiological function of these different Arp2/3 complex subunit isoforms during development and tissue homeostasis.

What 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 advances in fundamental knowledge by allowing us to understand which physiological and developmental processes are controlled by the different Arp2/3 isoforms. Given recent observations with patients with mutations in the ARPC1B subunit of the Arp2/3 complex, it is likely that our analyses will also uncover and provide insights into a variety of human conditions and diseases including tumour metastasis, immunodeficiencies and muscle myopathies. The

insights we will obtain will provide new disease markers and opportunities for therapeutic intervention.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use mice. We will use approximately 3000 per year. However, most animals will only be used for breeding of new mouse 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?

Genetically altered mice may develop moderate or more adverse outcomes, which will depend on which Arp2/3 isoform is being altered and in which tissue the manipulation is performed. Many of these adverse effects will present at embryonic and foetal stages, and are not usually compatible with continued life. The maximum expected level of severity for any procedure conducted within this project is mild and follows strict guidelines in accordance with the Home Office. At the end of procedures, animals 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

The study of Arp2/3 dependent actin polymerization in biochemical assays in the test tube and in cell culture models can provide mechanistic insights into different cellular processes. Such analysis may guide, but will not fully uncover the roles of Arp2/3 isoforms during mouse development and tissue homeostasis. This is because these highly complex processes involve the coordinated interaction of multiple proteins and cellular systems, as well as, their higher level organization in a wide variety of different cell types over time. Currently, no biochemical assay or cell based model can fully recapitulate or take into account all of these factors. Therefore, to understand the importance of Arp2/3 isoforms, for example which cell types and physiological processes depend on them most critically, and at which stages of life they are most important, we need to study the consequences of their loss in whole, living animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use state of the art genome editing to generate mice carrying mutations to reduce the need for extensive breeding programs. Where possible, we will perform in vitro experiments in cell culture using tissues or cells derived from knockout mice to reduce the need performing experiments on living animals. In turn, hypotheses generated from in vitro experiments will also allow us to perform more focused animal experiments, reducing animal usage. Experimental designs will use the minimal number of mice required to obtain statistically significant data. We will maximise the amount of data obtained from each mouse by studying multiple tissues, by analysing them using several different methods. We will review our breeding strategies regularly and cryopreserve any strains which are not under current investigation.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 share similar genetics and physiology with humans and so are an appropriate mammal for providing insights into human development and disease. They are not a primate or an endangered species. Mice are the lowest mammalian organism that expresses all Arp2/3 isoforms that is suitable for efficient genetic modifications. Advanced genome editing works very efficiently in mice and will help limit animal numbers. Mice also have well-established laboratory procedures and advanced genetics, which both help to expedite research progress. 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.

PROJECT 285

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.

Word limit; 1000 words

Project Title	ASSESS & IMPROVE WELFARE OF WILDLIFE MANAGEMENT METHODS
Key Words	Humaneness, Spring traps
Expected duration of the project	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.

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);
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):

The overall aim of the research programme is to obtain data that will identify and classify the humaneness of wildlife management methods, used within the UK and the EU, and develop improvements where possible.

The Pest Act 1954, states that it is unlawful to use a spring trap that has not been approved. All approved traps are listed on the relevant Spring Trap Approval Orders (STAO) for England and each of the devolves (Scotland, Wales and Northern Ireland) for named target species.

For any new trap or a new target species for an existing trap to be added to the STAO, they must undergo an assessment to ascertain whether they meet the humanness criteria required.

Trials will be undertaken to assess the humaneness of spring traps used for wildlife management throughout the UK and EU.

REDACTED

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

This work will underpin national and international legislation designed to ensure that acceptable devices/methods will be used for wildlife management throughout the UK and the EU. The specific benefits of this project are to improve the welfare of wild animals that are subject to management techniques. The requirement for wild animal management is increasing due to the increasing number of human/animal conflicts and in many cases this involves removal/ killing of the animals. The results of this

research will feed into the legislative frameworks of the UK and the EU and should prevent the marketing and selling of inhumane traps.

What types and approximate numbers of animals do you expect to use and over what period of time?

For each trap a maximum of 24 animals (more commonly 10-12) will be used of each target species: Norway rat, woodmouse, grey squirrel, stoat, weasel & wild rabbit. The number of traps tested each year fluctuates and can be anywhere between none and 15.

In the context of what you propose to do to 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 tested singly. Killing traps are designed to kill the target species, and this can occur in a variety of ways (e.g. cervical dislocation, internal bleeding, fracture of the skull) and may involve suffering. Once caught by the trap the animal will be under continuous observation and animals not found to be irreversibly unconscious or dead after a strike by the trap within the specified time limit (Woodmice, Norway Rats, Weasel, Grey Squirrel, Wild Rabbits all 300 sec & Stoat 45 sec) will immediately be killed by a Schedule 1 method. Strikes that do not hit the head or body of the target animal are unlikely to cause a humane death and in such circumstances the target animal would be immediately 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

Computer (simulation) modelling has been discussed and evaluated for this project a nd is currently usd in Canada.

However the development of a similar simulation model would require gathering data from a minimum of 35 animals (for some species many more have been required) based upon a range of similar trap designs. In addition the Canadian computer simulation program does not assess the strike position of any trap under assessment, which is a critical component as to whether a trap would meet the required criteria.

The traps that are submitted for testing in the UK are a diverse range of designs and over the past 5 years no two traps have been of a similar design and therefore computer models could not have been

developed. Hence currently only live animal trials will provide the infomation require d but we will continue to investigate other non animal methods.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

These trials will be undertaken in a sequential manner meaning that as soon as ther e is enough data to determine whether a trap will pass or fail we can halt the trial.

This may be as few as 3 trials to fail a trap and 10 trails to pass a trap.

If we can obtain data from any other world wide trap testing authority that gives us su fficient infomation that the trap does or does not meet the criteria, we will use this dat a rather than undertake any animal trials.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 that we use have to be the species that the trap will be used for due to the differences in behaviour between species and strains. Wild caught animals are more
neophobic (therefore would be expected to approach the trap differently thus effectin g the strike location) and have different muscle and fat distribution, and a different bone
density all of which would effect the level of damage that the animal will suffer. Beha vioural and bone density differences between species, prevent results from one species being used to assess traps for most other species. There are similarities between some species such as stoat and weasel, that allow testing on one species to be used for assessment on the other, where possible and there is evidence of similarities this will be utilised to reduce the number of animal trials required.

In order to minimise suffering, if is physically possible
and will produce data that meets the criteria we will use anaesthetised animals that cannot feel pain, as an initial screen before tests are carried out on live free moving animals

PROJECT 286

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.

Word limit; 1000 words

Project Title	Nutrition for Sustainable Sheep Production
Key Words	Sheep, Nutrition, Production, Environment
Expected duration of the project	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;
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.

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);
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):

Currently over 4 million lambs die each year with a majority in this high mortality being associated with hypothermia due to delayed suckling and exhaustion of energy reserves. In addition, the UK is a major consumer of lamb, with per capita consumption of 4.4 kg/year. However, lamb is high in saturated fatty acids, which are associated with obesity and heart disease in humans. In addition, greenhouse gas emissions (GHG) from agriculture make a significant contribution to global warming. In the UK, GHG emissions from sheep farms range from 6.4-19.7 kg CO_2^e/kg live weight, with those with the lowest emissions having the highest levels of ewe and lamb productivity and low reliance on imported protein sources. This project will investigate nutritional factors affecting ewe and lamb performance, product quality, and the environmental impact of sheep production 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)?

The project will advance knowledge and understanding of the sciences underpinning ruminant nutrition and the complex interactions between genotype, physiological state, nutrient supply and animal metabolism. The results will be used to design feeding strategies for sheep to reduce lamb mortality, by increasing lamb birth weight and neonatal lamb vigour. In addition, enhancing the nutritional value of sheep meat, for example by reducing its fat content and increasing its content of desirable polyunsaturated fatty acids, will contribute to reducing the incidence of disease in man. Increasing sheep productivity and greater reliance on home grown protein sources in sheep diets will help to protect the natural environment by significantly reducing greenhouse gas emissions. In the longer term, increasing ewe and lamb productivity will contribute towards increasing the sustainability of sheep production

and the maintenance of rural communities which are essential for protection of the natural environment.

What types and approximate numbers of animals do you expect to use and over what period of time?

The project will be conducted using pregnant or lactating ewes, and growing lambs. It total, it is anticipated that approximately 500 ewes and 500 lambs will be used in experiments over a 5 year period between December 2017-2022.

In the context of what you propose to do to 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 proposed will involve intra-muscular injection, blood sampling by venepuncture, and collection of rumen fluid by stomach tube, faecal grab sampling and possible restraint of animals for short periods in metabolism crates. In all cases sampling will be the minimum necessary with the overall level of severity being mild. The possibility of adverse effects is low and temporary. However, they may include mild hunger or in-appetence, distress associated with handling and restraint, mild pain associated with venepuncture or rumen fluid sampling. These will be minimised by the use of trained competent staff, appropriate equipment and prompt treatment or veterinary intervention if required. Following inspection by the named veterinary surgeon animals will be returned to the university flock.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The work proposed will investigate the effects of nutritional factors on the whole body metabolism of pregnant or lactating ewes, or growing lambs. There are currently no substitutes that adequately replicate the complex interactions associated with digestion and metabolism that take place.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

For all experiments the minimum number of animals required to ensure statistical significance will be used. This will be determined from previous experience, and by using standard statistical techniques. Where possible factorial and Latin square designs will be used. The use of factorial designs will increase the efficiency of the

experiment by increasing the number of replicates per treatment for the main effects. The use of latin square designs will reduce the number of animals required as each animal acts as its own replicate, with the coefficient of variation being reduced accordingly. All experimental protocols will be reviewed for statistical validity by the REDACTED statistician, prior to approval by the AWERB. Variability both between and within treatment groups will be controlled by carefully selection of experimental animals and blocking as appropriate.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 will be housed and fed diets similar to those used in commercial sheep production systems. At most time's animals will have physical, visual and audial contact with each other. Where animals have to be restrained (e.g. for collection of faeces or urine), animals will have visual and audial contact with each other. Animals will be maintained and cared for by trained and competent staff. All animals will be monitored for signs of ill health and treated as necessary. A record will be kept of all cases and treatments applied. In the unlikely event that a regulated procedure or the accumulation of non-regulated procedures exceeds the severity limit, animals will be immediately removed from the study and appropriate remedial or veterinary care provided in consultation with the named veterinary surgeon.

PROJECT 287

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.

Word limit; 1000 words

Project Title	Stem Cell Therapy for retinal degeneration
Key Words	Retina, cell-therapy, RPE, Photoreceptors
Expected duration of the project	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;
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.

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 overall aim of this project is to develop stem cell therapy for inherited or acquired retinal degeneration, including photoreceptor, Leber congenital amaurosis, Stargardts and age related macular degeneration.

Objectives

- To differentiate, *in vitro,* human embryonic stem cells into RPE and photoreceptor.
- To establish a laser model of retina and RPE layer focal damage.
- To transplant human embryonic stem cell derived RPE and/or photoreceptor cells into the retina of pig eyes, an eye that is similar to the human eye.
- To assess the survival and integration into the host retina of the transplanted cells at different time points following 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)?

There are a number of serious ocular disorders, which result in blindness and are either poorly treated or have no effective treatments. These include retinal degeneration, ocular neovascularisation, age related macular degeneration and uveitis. Although many of these diseases are relatively rare, their impact on the individual patient and the community is substantial. The work in previous projects has already led to clinical trials of gene therapy for retinal dystrophy and we will hopefully initiate several more clinical trials on inherited and acquired disease in the coming years based on the work proposed here. The work proposed in this project is still in a relatively early stage and it is unlikely to lead to clinical application in the duration of this project. However, successful completion of the objectives will be an

essential first step in the development of a treatment for these untreatable, blinding diseases..

What types and approximate numbers of animals do you expect to use and over what period of time?

Over the 5 years of this licence, we estimate that we will use approximately 50 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 are animals known to make use of all of their senses, the retinal insult and corrective treatment have little impact on the animals' welfare. It is known that commercially bred pigs can survive normally even when completely blind. They behaviour normally, can find food and water and are not specifically picked on by other members of the herd. They put on weight at the same rate and are sent to slaughter at the same time as other pigs of the same age. We produce the retinal insult and administer the putative therapy into the eye of the animal, while it is under anaesthetic. When the treatment has taken effect, we can determine how well the retina in the animal is functioning and how it looks. At various time points after treatment, the pigs will be humanely killed. The eyes are then closely examined to obtain the maximum information about the effectiveness of the putative therapy. Determining with the greatest precision the retinal insult and the effects that the treatment has had.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Although a number of morphological and functional analysis can be performed in models of disease in culture prior to use in animals, treatment efficacy can only be proven in animals, as the diseases we aim to treat are complex disorders, involving interactions between multiple cell types and release of endogenous survival factors. Assessments of cell transplantation techniques involve immune and wound healing responses, migration into the retina and integration into the existing neural network.

Current knowledge and techniques are insufficient to model all these interactions, either in a culture system or using computers, well enough to reliably predict treatment outcomes.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

A stepwise approach will be used in this study. Assessment of cell survival following transplantation will be performed first, assessing the result 2 days post-surgery to establish cell survival. New transplantation experiments will only be performed after the first-time point is analysed and cell are observed in the subretinal space. A gradual progression of transplantation from 48 hours to 1 week, 2 weeks, 4 weeks, 6 weeks up to 24 weeks post-transplantation will be dependent on cell survival at each time point, thereby reducing the number of animals to be used. New *in vivo* assessments of the health of the retina will allow us to monitor how transplantation is progressing in living animals rather than in post-mortem retinal tissue. Similarly, a new method of disease scoring for retinal inflammation can follow progression in individual animals *in vivo.*

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

Studies undertaken previously, have allowed continuous refined of the procedures undertaken. Recent refinements include the introduced of new methods to model transplantation that do not require the animal to undergo a lengthy transplantation procedures. Furthermore, the development of improved methods of stem cells production have given far better purity and further decrease the (already low) risk of inflammation post-administration.

For each study, a study plan will be produced detailing all aspects of the work. Internal guidelines and SOPs are constantly reviewed to ensure the most humane procedures are used whilst still achieving the scientific objectives.

Pigs are sociable animals and as such will never be kept in total isolation. They will be housed in at least pairs. Research has shown that in a natural environment pigs spend 75% of their daylight hours in activity – rooting, foraging and exploring. Therefore, it is important to facilitate this type of behaviour. Environmental enrichment, for example, footballs will always be included in the animal's pens. Feed will be scattered across the pen to aid foraging and rooting.

PROJECT 288

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.

Word limit; 1000 words

Project Title	Autoimmune epilepsy in the mammalian brain
Key Words	epilepsy, autoimmune
Expected duration of the project	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;
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):

Some people have epilepsy, which is a problem in the brain that causes repeated electrical storms in the brain, known as epileptic seizures. We do not know why this happens, however, sometimes we suspect that the body's immune system is a problem in epilepsy because patients with very severe seizures respond well to treatments that hold the immune system back or block its function. These facts raise important questions:

1. Are some epilepsies caused by the body attacking itself?

2. If so, are these antibody epilepsies more common than we think, and do they explain epilepsy that has no clear cause?

3. How can antibodies, which ought to be helping us, cause epileptic seizures in the brain?

4. Do antibodies get made in response to seizures that are caused by other things e.g. brain damage or infection?

To answer these questions, we are taking antibodies from children and adults who have epilepsy that can be treated with drugs that block the immune system, growing them in the lab to make more of them, and using them to try and make mice and rats have seizures, so that we can work out how they happen. We are also working out how best to treat the seizures in mice and rats, and also in the children that the antibodies came from.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Children and adults with epilepsy caused by their immune systems will get better treatment, and some people who have seizures that have no identified cause may be shown to have a treatable immune epilepsy and so be treated. It is possible that many epilepsies have an immune system aspect to them.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use about 900 mice and perhaps 400 rats over about 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 suffer some discomfort from having seizures and they may lose some weight for a day or two after the first seizure, but they mostly will recover well. Sometimes, we will want to put recording devices into their brains so we can investigate the process of how antibodies cause seizures and this will mean an operation on the skull. This can be painful, and we will make sure that there is treatment for pain before, during and after the surgery, and we will try and do this in as few animals as possible. We have invented a new way to give animals epilepsy that causes the least harm and suffering possible, and we are also trying to use human brains in these studies too (see below).

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The brain and the immune system are two of the most complicated things in the universe, and we need to study both of them, in a way that means they can 'talk' to each other as they would in man. The only way to do this is to use animals that have active brains and immune systems so that we can make the immune system trigger the seizures. Since we don't know which parts of the brain are the most susceptoble to seizures caused by antibodies, we must use an intact brain in a mouse or rat, which both have similar brain cells and brain proteins to man.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have calculated how many animals we need for the project to produce reliable results, and will not use any more than we strictly need to. We have also developed

the use of human brain tissue in our studies, so we will not use as many animals as we may have done AND we will be able to make sure that rat and mouse brains respond in the same way as human brains, ensuring our research is relevant to human disease and not just to rodents. Group sizes for our experiments are calculated based on the variance in our recordings of electrophysiological indicators such as EEG power, seizure number and power. For example, in a typical in vitro electrophysiology experiment aimed at showing changes in receptor function in response to pregnenolone, a power calculation at an alpha value of 0.8 and with $P<0.05$ would require 8 animals in each group to achieve robust results. With chronic recording, variability will be lower and at similar levels of power and P-value, we would expect only 6 animals are required to show robust and valid differences to 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

The epilepsy model we use is the most refined in the world today, and was designed by me specifically with refinement in mind. Instead of many animals dying due to the severity of the epilepsy, the model is much more subtle, and it is rare for an animal to die from seizures. This means we study the most sensitive animals, and not the ones that are so insensitive to epilepsy that they survive even the most severe seizures. The model also closely mimics what happens in man, meaning that the animals which do suffer are doing so for good reason - to improve the treatment of children with epilepsy. In this regard, we are also running a clinical trial that will work alongside this project, so we can try new treatments in children as we as work out how they work using the animal models.

PROJECT 289

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

Project Title	Desert rodents as models of diet-induced diabetes
Key Words	Diabetes, Pancreas, Insulin, Glucose, Rodent
Expected duration of the project	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;
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 project aims to use desert rodents as models for diet-induced (type 2) diabetes. These species are adapted to low calorie diets in their natural environment and when bought into the laboratory and fed standard rodent food they become obese and develop diabetes, and this can be reversed by return to low calorie diet. Our main focus would be on the Pdx1 gene, which is known to be involved in formation of the pancreas during embryonic development and regulates the insulin gene in response to blood glucose levels. Not surprisingly, this gene is involved in several human diseases and disorders, including type 2 diabetes and pancreatic agenesis (failure of the pancreas to form). Desert rodents possess the most divergent form of this gene yet discovered, to the point where it should not be able to function. We hypothesise that the protein produced by these animals is still able to make a pancreas during embryonic development, but that it is no longer able to respond to blood glucose levels and stimulate insulin production, leading to susceptibility to diet-induced diabetes.

Our project will enable us to uncover alternative mechanisms for building a pancreas and specifying insulin-producing cells during embryonic development, and for regulating the production of insulin in the adult pancreas.

What 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 fundamental biological knowledge on the genetic basis of susceptibility to diet-induced diabetes, and especially the function of the developmentally-important Pdx1 gene. Our use of several different species of rodent will enable us to determine how changes in different regions of the PDX1 protein

impact anatomy and physiology, and how such changes underlie adaptation to extreme environments such as deserts. These findings will have relevance to our understanding of human disease (through the linking of DNA and protein-level events with susceptibility to diet-induced diabetes), and for our understanding of dynamic processes underlying onset, progression and reversal of diabetes.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will utilise up to 850 Mongolian gerbils (Meriones unguiculatus); 700 fat sandrats (Psammomys obesus); 700 mice (including genetically altered mice); 200 fat-tailed gerbils (Pachyuromys duprasi) and 200 spiny mice (Acomys dimidiatus) 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?

All procedures will be mild. We'll use oral administration of glucose to test how well the animals can respond to sugar, using a blood glucose assay similar to that used by human patients, with small blood samples collected from a leg vein (the saphenous vein). Based on their response to this glucose challenge, we'll select animals for breeding to develop a diabetes-prone group of animals, and we'll use DNA collected from ear punches and other tissue to identify the regions of the genome underlying diabetes susceptibility. Building on this, we'll next switch groups of animals onto custom high-energy diets for longer periods, to more closely mimic the onset, progression and reversal of obesity and type 2 diabetes in humans. We'll also collected adult and embryonic tissue samples to investigate how gene expression might differ between diabetic and non-diabetic animals. Finally, we'll develop a genetically-modified mouse that has had one of its own genes replaced with that of one of our desert rodent models, as the mouse breeds more often, and produces more offspring than our other species. Blood sampling should cause no more than transient distress, and we will use aseptic technique and monitor for possible adverse affects of infection or inflammation. No animals are expected to develop diabetes from the brief introduction of high energy diets. Animals will be killed at the end of the procedure to provide tissue samples for gene expression studies.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Some of our experiments involving gene expression and function could in theory be carried out using non-animal systems, such as cell and tissue cultues, but these do not currently exist for our study species. The development of some of these resources is a key part of our longer-term research plans.

The chosen species are the most appropriate as they are the only ones known to possess the divergent Pdx1 gene, and to spontaneously develop obesity and type 2 diabetes in response to a high energy diet. Our rodent models also more closely mimic human popualtions in terms of levels of genetic diversity and variation in susceptibility to developing type 2 diabetes, and such variation is typically lacking in cell and tissue cultures.

Since the onset, progression and reversal of obesity and diabetes affects multiple parts of the body in various ways (including levels of circulating blood glucose and insulin, and gene expression in the pancreas and liver), a whole animal approach is the only possible option to recapitulate all of these processes.

Determining the genetic basis of susceptibility to diabetes requires controlled breeding of animals, and so animal use is unavoidable here.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Rigorous experimental design protocols will be used to ensure statistical significance of results for the lowest number of animals, with randomisation and blocking to ensure robustness of the reasulting data, and immature forms used in preference to adult animals wherever possible.

Where possible, and where experiments will not be adversely affected, animals will be re-used between protocols, and no animal will be used in more than one set of experiments. Prior to re-use the health and welfare of each animal will be assessed in collaboration with our named veterinary surgeon.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 chosen for the project are the most refined as they are accepted “model” species for which there is a record of scientific publications, and data on captive husbandry and requirements for environmental enrichment. Furthermore, we

have chosen these species to provide coverage of the breadth of the gerbil subfamily, enabling us to study large-scale evolutionary processes with the fewest species.

Our recent experiences with the study species, and pilot research grants aimed at improving welfare have enabled us to refine husbandry of these species and ensure the highest levels of animal welfare.

Blood sampling will be performed using aseptic technique to minimise the risk of infection and inflammation, and all experimental animals will be closely monitored for adverse reactions, with any affected animals removed from the experiment immediately.

PROJECT 290

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.

Word limit; 1000 words

Project Title	Role of transcription factors in cytoprotection and tumorigenesis
Key Words	Nrf2, liver disease, lung cancer
Expected duration of the project	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):

Several chemicals that been shown to protect mice and rats against cancer and degenerative disease appear to cause the same battery of about 200 genes to switch on. These genes include ones that code for detoxifying or anti-oxidant enzymes as well as proteins that assist the repair or elimination of damaged molecules.
In humans, these genes are less well expressed as we age, suggesting that the decrease in their protective activities may contribute to a greater susceptibility to cancer and degenerative diseases. Interestingly, they are over-expressed in some rapidly growing cancers, where their protective actions might actually be fighting against the intended effects of chemotherapy
This battery of genes appears to be controlled by a master regulator, called Nrf2. Loss of Nrf2 makes mice very sensitive to the development of non-alcoholic steatohepatitis (NASH) and cirrhosis, which can progress to hepatocellular carcinoma. Specific activation of Nrf2, conversely can reverse this process.
We are working on the molecular events that result in Nr2 activation being able to protect the body against degenerative diseases such as NASH and the initiation of cancers, while also being an undesirable state in some cancers once they have arisen.

What 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 need to understand how activation of Nrf2 can be exploited so as to protect the body against degenerative diseases such as NASH, but can be controlled so as to allow the chemotherapy of cancer to have its full effect.

What types and approximate numbers of animals do you expect to use and over what period of time?

We expect to use 7500 mice. Most will be used in the breeding and maintenance of lines in which the level of Nrf2 activity is altered in a consistent mannner. Some animals will be used in experiments to investigate how Nrf2 protects against degenerative disease and cancer formation and others will be used to examine its role in promoting the growth of some cancers, once they arise.

In the context of what you propose to do to 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 continue to use a highly palatable high-fat diet to induce early changes in the liver that are characteristic of NASH and cirrhosis, and to determine how activators of Nrf2 can protect against them. In some cases, it will be necessary to add fructose to the diet or the drinking water, in order to accelerate these effects being observed. We will modulate Nrf2 activity in the mice by interbreeding them with lines in which genes that modulate this activity have themselves being altered, and/or by dosing the animals with molecules that are known or suspected to activate Nrf2. Mice fed on these diets are expected to put on weight but not to develop liver disease to the extent that this causes any effect on their overall welfare. We intend to study the consequences of Nrf2 activation in cancers once they have formed by using the same mouse lines in which lung cancer can be specifically induced in experimental mice (but not in those simply being used in breeding programmes to produce them). We work closely with another research group that has experience with this lung cancer system and which has developed sensitive end-points to allow the studies to be scientifically reliable without causing significant animal welfare problems.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We can and do study the molecular events involved in Nrf2 activation by small molecules and the down-stream activation of the battery of genes and its consequences using cell cultures in vitro. However, in order to model the development of degenerative disease in the liver and, against this background, to test our ideas about the protective effects of Nrf2 activation, we need to use an intact animal. Similarly, when studying the reasons why Nrf2 might become activated in cancer, and how this might be controlled so as to maximise the benefits of existing cancer therapies, we need to work with the whole organism. The mouse Nrf2 gene

appears to be very similar in its regulation and down-stream effects as that in humans, making the mouse a good model system for our studies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Our mouse breeding colonies will be closely managed, to prevent unnecessary surplus and to ensure that animals stay in good health. When testing experimental hypotheses, experiments will be designed to have sufficient statistical power to yield a robust 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 mouse NASH model we have developed using specially formulated diets is a good approximation to the human disease, while not appearing to cause significant welfare issues other than a degree of obesity. In other words, we can detect signs of disease in the liver after the animal has been killed but, during life, these do not appear to be sufficient to precipitate illness.
The mouse lung cancer model is a state-of-the-art experimental strategy that produces disease that reflects human pathology in only those animals that are to be used for an experimental purpose, Based on the experience ofg other research groups. we do not expect to need more than moderate deviations from normal welfare in order to obtain robust scientific data.

PROJECT 291

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.

Word limit; 1000 words

Project Title	Optimising lung therapy using large animal models
Key Words	Lung disease, prevention, cure, sheep
Expected duration of the project	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:
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):

Lung disease is one of the three biggest killer disease areas in the UK, alongside heart disease and non-lung cancers. It kills 115,000 people each year, the equivalent of one person every five minutes and it places a huge burden on our health care services, accounting for over 700,000 hospital admissions and over 6.1 million hospital bed days in the UK each year.

Research is urgently needed to improve the way we prevent, treat and care for people with lung disease, from the earliest to the end stages of life.

Ordinarily new treatments are developed through a process that involves early testing in preclinical models – and in most instances these models involve the use of mice. Unfortunately this system is largely failing to deliver the new drugs necessary to keep pace with the upward trends in lung disease. New models are urgently required that improve on existing systems.

Sheep have been used as models for many years to predict the safety and efficacy of drugs for lung disease. Recently the potential benefits of studies in sheep were realised in translation into a large clinical trial involving people with cystic fibrosis in the UK, which demonstrated that repeated doses of gene therapy had a meaningful effect on the disease, slowing its progression. The use of sheep was prompted by the realisation that mice were not predictive of humans in this context and a larger species with more relevant physiology and anatomy was required to model the delivery of new treatments to the lung and the assessment of benefits due to those treatments. We believe that this model can extend to benefit other lung diseases.

Our overall plan is to identify new treatment targets through studying sheep models of lung disease and to assess the treatment benefits of new drugs or gene therapies directed at those disease targets.

In addition to cystic fibrosis those disease targets include pneumonia, an inflammation of the lung usually caused by an infection, as well as radiation-induced lung injury (RILI). Both are important with pneumonia and lung infections accounting for over 325,000 hospital admissions and over 3 million bed days each year, and RILI affecting 1 in 10 of the patients that receive radiotherapy for lung cancer every year. The risk of developing RILI is a well-recognised dose-limiting consideration in radiotherapy planning meaning that the majority of patients may have potentially less efficacious modifications made to their treatment plans based on the perceived risk to a minority.

Developing new ttreatments or ways of preventing lung disease is an urgent clinical priority. Much of what we learn from our studies will also be directly relevant to lung disease in domestic and farmed animals.Research into lung-directed gene therapy may help in developing vaccines relevant to lung disease in animals, better understanding of normal and abnormal bacteria in the lungs could lead to more effective methods to control respiratory disease in animals, and any radioprotective strategy developed for humans could potentially also be used for domestic 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)?

In the context of patients, this project will potentially furnish strategies and policy guidelines to help the healthcare professionals responsible for their care to make the most appropriate decisions in relation to their management. Ultimately, that benefit will likely be realised in the form of improved quality of life, and increased life-expectancy.

What types and approximate numbers of animals do you expect to use and over what period of time?

We expect to use 150 sheep over a period of 5 years. These animals will be commercially sourced from farms and/or markets in the UK.

In the context of what you propose to do to 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 sheep will be anaesthetised to allow disease to be induced. The sheep will thereafter recover from anaesthesia and the progress of disease, with or without modulation by a proposed treatment, will be assessed by anaesthetizing animals periodically and sampling tissues and/or fluids from the areas under study. In all of our approaches, we are able to limit the proportion of the lung that becomes functionally abnormal. Because the lung has tremendous reserve capacity, it is our experience that the sheep do not experience adverse effect in the form of shortness

of breath, coughing, or difficulty in breathing, hence appearing essentially unaffected when compared to control animals. The fact that we have to anaesthetise sheep to perform bronchoscopy or related procedures, renders most of the procedures outlined in this license moderate severity. However, it is our experience that sheep tolerate anaesthesia very well and recover to standing within 10-15 minutes after cessation of anaesthesia, and experience no apparent adverse effect. The sheep will be killed at the end of these experiments in order to allow us to examine the lungs at post mortem and take samples that would otherwise be unavailable.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The way in which the lung responds to injury and disease is complex and involves the interplay of multiple systems and factors including the circulation, and the nervous and immune systems. Whilst facets of the response may be modelled using alternatives, it is currently not possible to use these systems in isolation to predict how the human lung will respond. However, where possible we do use alternatives to animals in our studies. For instance, in relation to gene therapy we employ human, mouse and sheep cell culture systems to screen for efficacy in gene transfection. Such studies can prove valuable in screening potential gene transfer agents prior to their initial assessment in mouse, and then in sheep. The latter studies are essential, because only in living animals can the influence of an intact immune system be properly assessed – an important aspect of gene therapy using viral vectors.
In relation to identifying systems that could replace animals in modelling the effects of radiation on the lung we are actively involved in using precision cut lung slices for this purpose. These studies will parallel our progress under this license and we will naturally seek to establish the benefits and limitations of this system as a model of in vivo events.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Every experiment is carefully evaluated by a statistician to determine whether its design is capable of answering the question that is being asked. This involves using prior data to estimate the variability of the phenomena we intend to measure, and our estimation of what would be a clinically useful, or relevant, result. From this it is possible to ensure that the minimum number of animals are used in each instance.

This process is part of an ongoing process throughout the project that subjects every experiment to rigorous evaluation by experts in statistics, ethics and animal care.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 the context of gene therapy rodent studies always precede and inform upon their subsequent application in the sheep. However, the sheep is recognised as being a more appropriate translational model to address issues especially relating to delivery.
In the context of studying the effects of infection and radiation in the lung we have carefully considered the option of studies in rodents. However, it is inevitably extremely difficult to limit injury to a small proportion (<10%) of the total lung volume in small animals – and for example in the context of radiation, almost all investigators simply expose half, or indeed all of the lungs to radiation. These approaches are generally not relevant to commonly used radiotherapy regimes in clinical medicine and the impact on the animal is correspondingly higher, leading to obvious respiratory symptoms. By using larger animals it is possible to limit the volume of lung involved to the extent that respiratory symptoms do not occur. It is also possible to return to the same animal at intervals thereafter to assess the response to radiation relative to unaffected parts of the lungs. These considerations shape our contention that studies in large animals for these purposes are more ethically sound and we do not advocate the suitability of piloting studies in rodents for that reason. However, we will keep a watching brief on developments in the field and where appropriate will use such information to refine our own research.The rationale behind the choice of sheep as our animal model includes the fact that they are large animals whose lungs are anatomically and physiologically similar to humans, and that they are amenable to both bronchoscopy and radiotherapy, 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 subjects.

PROJECT 292

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.

Word limit; 1000 words

Project Title	SUMOylation accelerates supply-rate depression
Key Words	Epilepsy, SUMOylation, Synapsins, Levetiracetam
Expected duration of the project	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;
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):

In the brains of healthy people, intense electrical activity doesn’t lead to seizures, indicating the
presence of an inborn protective anti-epileptic mechanism. Therefore, a fundamental, but understudied
question is why more people don’t suffer from epilepsy, or put another way, what keeps most people
epilepsy free? This is important because finding ways to switch on or enhance this innate protective
pathway could provide powerful tools for reducing or preventing seizures in people with epilepsy.
Supply-rate depression (SRD) has recently been identified as one such potential defensive mechanism.
The new data suggest that SRD is regulated by a process called SUMOylation, which alters the
functions of specific proteins at synapses, the points of communication between nerve cells. Our
hypothesis is that manipulating the SUMOylation of these proteins could protect against seizure activity
by reducing the aberrant transmission of electrical signals between neurones, while leaving the
transmission of normal signals unaffected. The project aims toward understanding the molecular
mechanisms underlying these processes and translating these discoveries as a focus for the design of
new targeted therapies for epilepsy.

What 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 people with epilepsy do not respond well to therapy with the currently available antiepileptics This is because current medicines target processes and structures that normally occur in nerve cells. Thus, they inevitably disturb normal function, creating significant adverse effects. Developing new drugs that target only those processes that occur in the brain during the seizures (such as SRD) would keep the normal functions intact, while having a beneficial therapeutic effect. It has been shown that modification of proteins involved in communication between brain cells targets one such epilepsyinduced process - SRD. Understanding how SRD may be modulated to be initiated earlier will help to find the way to prevent the seizures from developing. This will further help with devising a new strategy in fight against epilepsy and its devastating consequences.

What types and approximate numbers of animals do you expect to use and over what period of time?

Mouse, 1600.

In the context of what you propose to do to 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 specifically designed for this project will be genetically modified, but this should cause no adverse effect by itself. The commercially available animals (synapsin I and synapsin II knockouts) generally exhibit spontaneous seizures, but will be used for experiments before the age at which this starts occurring. 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 epilepsy models that does not require the use of brain tissue acutely removed from
animals.
The project is intended to result in development of the new transgenic mouse strains. We will make
extensive use of the transgenic mouse strains engineered to evaluate the role of SUMOylation on

neurotransmitter release. 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, I will share the tissues with other groups to ensure that neuronal and nonneuronal
tissue from the animals is used to the fullest extent 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 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

PROJECT 293

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.

Word limit; 1000 words

Project Title	Generation, rederivation and cryopreservation of transgenic animal models
Key Words	transgenic production, cryopreservation
Expected duration of the project	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):

This is a Service licence that allows the generation of new animal models that can then be used by researchers to answer particular scientific questions and conduct research using specific animal models of disease including cancer, inherited genetic disorders, neurodegenerative disease and respiratory conditions, many of which have a major impact on human health. The PPL also supports researchers by freezing down sperm or embryos (known as cryopreservation) to preserve these important models and to allow collaborations with other scientists worldwide. This PPL also allows researchers to import unique animal models from their collaborators around the world increasing the opportunities for research into a number of human diseases.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

The project aims to breed mice with altered genes as part of a service for the entire University. The benefits of this service are to provide efficient breeding of genetically altered animals, potentially reducing the numbers of animals used, and also in the standardisation of techniques. The production of genetically altered animals contributes to a wide range of scientific research areas, which may have impact on a number of fields of research. In addition, the project also allows for the development of new techniques which may have positive impacts on animal welfare and refine current techniques.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will be using both rats and mice over the 5 year period of the licence. The scientists requesting our service will determine which species is most suitable for their research. Since this is a REDACTED expect to use a large number of animals but we have checks in place to ensure that the animal production is justified under other breeding and maintenance PPL's. The total number of procedures is expected to be 81,000 animals with a further 9000 animals used in breeding an 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?

The overall severity for the licence is expected to be mild. There are two surgical methods included in the licence that are moderate which will be performed by highly skilled and experienced members of staff (vasectomy and embryo transfer). At the end of procedures animals will either be humanely euthanized (if sperm or embryos are required to be collected), or transferred onto the PPL of the researcher who requested the line to test specific scientific hypotheses of that group.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Production of Genetically altered or transgenic rodent models is not possible without the use of animals. The similarity of rodents and human at a genetic level and the availability of transgenic models for a range of human diseases may allow the translation of any findings in rodent biology to human biology, leading to a better understanding of gene function in health and disease.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

This licence provides cryopreservation of lines for all REDACTED which will allow the long-term storage of precious lines that are not required at that moment but may be in the future. This enables there to be a reduction of animals that are maintained in animal facilities.

For our other procedures, we will continually assess the number of animals used on the service licence by collecting data on each session and comparing this over historic data accumulated during the many years the service has been running.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 purpose of the transgenic core is to provide advice and support to researchers so that they can use the best models appropriate to their area of interests. We have successfully adopted novel transgenic techniques, which allows the creation of new models faster and more efficiently. As the Transgenic core service is an integral part of the animal facilities at REDACTED we continually review our procedures with a focus on technical efficiencies and welfare.

PROJECT 294

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Use of novel imaging techniques to improve IVF outcomes
Key Words	Egg, Embryo, Fertilisation, Imaging, IVF
Expected duration of the project	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):

This project aims to advance the field of assisted reproduction by creating new tools to recognise disease and select the best embryos for return to the patient during IVF treatment.

During IVF several eggs are collected from a woman, however after fertilisation and culture in the clinic, only one embryo can be returned to the mother. Often the procedure results in miscarriage because the embryo fails to develop causing distress to the prospective parents and the need to repeat the treatment.

The aims of this project are to develop new methods of microscopy that can predict the probability of an embryo developing fully. This will be used to select the best available embryo for return to the mother, increasing the chances of success.

In additional aim is to reduce the time spent by embryos in culture, returning them to the mother (the best environment for a developing embryo) sooner, potentially leading to healthier embryos

What 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 include improvements to IVF procedures. Improvements will come in the form of 1) increased chances of a successful pregnancy, due to improved selection criteria for embryos, and 2) Improved health of the offspring due to the embryo spending less time outside of the mother. Additional benefits will be an increased understanding of the biology of the egg and embryo, and the development of new tools for scientific research.

What types and approximate numbers of animals do you expect to use and over what period of time?

Up to 1000 mice to be used over a period of 5 years. This is calculated based on expected yield of 20-30 eggs from one super-ovulated mouse. The experiments in this project will require around 20 eggs for the experimental group (this is limited by space on the microscope) and the same number for controls. Thus two mice are required per experiment. It is estimated that two such experiments may be performed in a week. Over 5 years this leads to approximately 1000 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 application includes one procedure, intraperitoneal injection of hormones 48 (oocyte collection) or 60 and 14 (egg collection) hours prior to the humane end-point (Schedule I killing by cervical dislocation). The expected severity is mild. There are no likely adverse effects, but infection in the injection site is possible. This will be monitored for and the animal killed to prevent suffering in the case of infection.

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 live mammalian oocytes/eggs for study.

There are currently no non-animal alternatives, oocytes and eggs cannot be created in culture. They are only created during foetal life; the only source is therefore in the ovaries of animals. Mice are chosen because they are a mammalian model and can be housed easily under controlled and reproducible conditions. They are compatible with our goal of translating findings into human clinical practise. Previous studies show us that mouse eggs are susceptible to the same problems experienced by human eggs during in-vitro culture. For example DNA damage, spontaneous cell-cycle arrest and chromosome segregation errors, are all factors contributing to failure in human IVF and are present, and can be studied, in mice. The culture conditions used in human IVF also work for mice with few adjustments.

My main objective is to improve IVF treatment for humans and mouse eggs are appropriate as they undergo equivalent IVF and culture. The priming of the mice with hormones (see ‘Reduction’) to increase oocyte/egg yield is analogous to the process humans undergo to increase yield during IVF.

Donated human eggs are not available in sufficient numbers or in a timely manner. They are not fresh, are typically of the lowest quality (failed to fertilise during IVF), and come from a population that can't be well controlled for.

Alternatives to mice will be reviewed periodically during the duration of the licence if technological improvements makes them available e.g. oocyte culture.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Mice will be hormonally primed prior to oocyte/egg collection. This greatly reduces the number of mice required (>2 fold increased oocytes/eggs per mouse). The mice will be used before they develop their own hormonal cycle. This prevents competition between the mouse's own hormones and the injected ones leading to increased oocyte/egg numbers. The total number of mice are calculated as the minimum needed to achieve statistically significant results, based on the investigators prior knowledge of the number of oocytes/eggs obtained from appropriately primed 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 provide an excellent model for human IVF as the cell biology is proven to be translatable.

The process of hormonal priming by intra-peritoneal injection is well established and has been refined over many decades. It has an extremely high probability of success and causes short-lived, minimal suffering to the animal because the people administering the injection are appropriately trained and well practised. Restraint and injection of the animal take ~10 seconds. Mice resume normal behaviour with no signs of distress within a few minutes.

Adverse effects are minimised by using sterile needles and solutions for injection.

The mice will be housed in appropriately sized cages with environmental enrichment, in female only groups, and where possible groups of at least 2 mice per cage. They are housed behind a barrier or in a filtered, environmentally controlled environment to prevent infection and maintain good health.

Mice will be used at a young age (typically within 4-5 weeks of birth) reducing the time spent in captivity.

Mice are checked daily to spot any signs of illness or other problems that may occur (for example infighting).

PROJECT 295

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Cellular senescence in aging and tumour suppression
Key Words	
Expected duration of the project	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;
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):

Background: Senescence is a cell based stress response programme that prevents cells from dividing further. Many premalignant tumours display evidence of senescence induction and it has been shown that if you prevent senescence these premalignant lesions progress rapidly to malignant cancer. As such senescence has traditionally been seen as tumour suppressive.

However we now know that senescence can also be activated, and is important in, processes like wound healing and during development. Additionally the number of senescent cells in your body increases with age and depletion of these cells has been shown to improve age-associated deterioration. Importantly senescent cells signal to the other cells in the tissue around them and in some situations they can even promote cancer through this communication. As such it is now accepted that senescence has both good and bad qualities in aging and cancer progression. To complicate the picture senescence in different tissues, with different senescence inducers may be very different to one another. We also know that senescence is dynamic, meaning that a cell that has been senescent for one day will look and act differently a week later (despite still being senescent). Because of this complexity many more studies are needed to understand the role of senescence in the body during specific contexts.

Objectives: As such the aims and objectives of this project are (1) to understand the key factors involved in the initiation and maintenance of senescence in different tissues with different stimuli; (2) to understand how these effect senescence and aging; (3) to modulate these processes and see if we can promote the good (tumour suppressive) qualities of senescence, while reducing the bad.

What 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: These studies will advance our basic understanding of senescence by interrogation of the physiological consequences of altering key mechanisms of senescent cells. This knowledge will be important in the fields of aging and oncology, wherein the pharmaceutical modulation of these mechanisms may support tumour prevention (as opposed to treatment) regimes. Additionally the processes that underlie aging are not well understood and remain a scientific unknown due to the complexity in modeling aging. As such data we generate in this project will invaluable to the field as a whole.

What types and approximate numbers of animals do you expect to use and over what period of time?

Animal Numbers and Severity: We anticipate using less than 15,000 mice in the next 5 years. Our work plan is aimed at a combination of both tumour models (with a focus on early tumour formation) and health/life- span. As such our work will only be associated with a moderate severity. This includes several cancer models (e.g. melanoma, pancreatic, prostate, liver) to look at the role of senescence pathways in tumour progression in these tissues. External tumour mice will be euthanized once a tumour has reached a maximum size (melanoma or xenograft) or an animal is showing clinical signs. For internal tumours we intend to use time-points to determine the average rate of tumour progression in combination with imaging technologies (MRI and ultrasound), and abdominal palpations where appropriate, to determine the rate of tumour growth. Mice believed to harbor tumours will be placed on enhanced surveillance so that they can be euthanized once displaying clinical signs. Additionally we are developing new models that aim to alter the aging process, and attempt to understand how they affect senescence and organism health. For these studies mice are screened for development of clinical signs associated with the end of life, as well as using a frailty index, and a combination of regulated (e.g. glucose tests to look for pancreatic function) and non-regulated procedures (e.g. muscle function tests) to measure health-span and alterations with age and treatments. Both genetic and pharmacological approaches will be used to determine if we can accelerate or decelerate the onset of these metrics and will be combined with the tumour studies to enable us to understand how a particular process in aging may alter tumour development.

In the context of what you propose to do to 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 all cases mice will be euthanized once an experiment is completed and we will maximize the amount of tissues we collect for biobanking for future use to minimize the amount of mice we need to generate for our studies.

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 work in my laboratory is on cells cultured in petri dishes. These experiments generate data faster and also cheaply. However, many of the effects of senescence are not only on the cell itself but on the surrounding cells, or immune cells, or can even act body wide. Therefore we require animal models to probe these interactions and to study how our induced perturbations alter these. Results from cell culture experiments will be used to design our animal work, and our animal work better informs our experiments on cultured cells.

Our work may one day lead to sufficient definition of the biological variables governing senescence that in the future we may be able to generate sufficiently advanced cell culture models to permit the efficacy testing of potential modifying agents.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To reduce the number of mice we have in our colony, we will keep stocks of frozen sperm and embryos. These can then be thawed and IVF performed to effectively bring a mouse line back to life. Thus, if a mouse strain is not continuously required this can enable us to avoid unnecessary breeding, reducing our animal usage. Wherever possible our breeding cohorts will be established to generate sufficient experimental and control mice with minimal excess stock that are unable to be used for experiments. This is of particular importance in more complicated breeding schemes, with several genetic elements.

To maximise the data obtained from each animal we routinely collect samples from multiple body sites whenever possible and, if requested, provide samples to other scientists to prevent duplication of their experiments.

Experiments will be designed in consultation with our in-house statistician in order to keep cohort size and number of replicates to the minimum number of animals required.

We have also chosen to employ the Hydrodynamic transfection technique that drastically reduces the number of mice used as it enables genetic manipulation within the liver without the need for breeding. This thus reduces the wastage of mice of the wrong genotype associated with breeding Genetically Engineered Mouse

Models (GEMMs). Compared to breeding the mice we need for these experiment we estimate that we have made around a 75% saving.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

Scientists have been spent decades developing mouse models. Pieces of DNA have been taken from jellyfish and bacteria and inserted into the mouse DNA in an inheritable manner, i.e. passed down from parents to offspring. From jellyfish came green fluorescent protein, so that we can see which parts of a tissue express our modifications. From bacteria came elements which enable us to induce (turn on/off) our modifications (e.g. gene deletions). Genetically modified mice permit specific genes to be knocked out, or removed, at specific time-points in the animal's life appropriate to the development of cancer. This removal can also be localised to a specific organ, e.g. liver, kidney, or skin. Mice and rats are the mammalian species of choice for gene knock-out experiments. Mice breed quickly allowing inter-crossing in a reasonable period of time. Mice have a short lifespan enabling the study of genetic manipulations from birth through to old age. Crucially, they recapitulate well human cancer behaviour. There is no other model system that is capable of providing the type of data necessary for our studies.

REDACTED We constantly refine our techniques by, for example, testing the best routes of administration for gene inducing agents. We now give the gene inducing agent doxycycline in the mouse's food whereas it had previously been given in their drinking water with a lot of sugar to sweeten its bitter taste, which would not have been good for the health of the mice and would also have effects upon metabolism and hence our experimental results. If doxycycline is given in the drinking water, for instance for a quick uptake for short term studies, we now use artificial sweetener instead of sugar.

All resonable steps will be taken to reduce animal suffering. Where genetically engineered mice have been previously characterised, careful reading of the scientific literature will ensure that any adverse effects are anticipated and avoided. We constantly optimise our procedures to minimise potential pain, suffering or distress, and enhance animal welfare.

Many of the mice in our studies will develop tumours. Animals will be carefully monitored for any signs of illness or distress and any animal showing such tell tale signs culled immediately. Animal suffering will be kept to a minimum by using the

minimum number of animals and the minimal dose of carcinogens or other chemicals.

PROJECT 296

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.

Word limit; 1000 words

Project Title	Neural stem cells and brain cancer
Key Words	brain cancer, stem cell, therapy
Expected duration of the project	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;
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):

Brain cancer is now a leading cause of cancer-related deaths in the under 40's. The most common brain tumour is glioblastoma and this is driven by cells which have similarities stem cells found in the nervous system. This is one of the most lethal human cancers.

Brain tumours are difficult to treat as the cells often invade the local brain tissue, making surgical removal of the tumour very difficult. Any remaining tumour cells after surgery can trigger regrowth of the tumour at a later date. Over the past 10 years we have learned much about the genetic defects in the tumour cells and the properties of the most aggressive cancer cells. Despite this progress, we lack a detailed understanding of the major pathways and genes functioning that drive tumour growth – what are the key molecular targets for which we could develop new drugs? It is critical that we learn more about how neural stem cells are corrupted to trigger brain tumours and which pathways can be targeted with therapies to stop tumour growth and relapse (after antimitotic and radiotherapy). Comparing normal stem cells to their deregulated brain tumour derived counterparts will be highly informative. Fortunately, we can grow in the laboratory both normal neural stem cells and brain tumour derived stem cells and manipulate these genetically to see which pathways are important in tumour growth, and which could be important targets for drug development.

There are two main aims of this project: 1) to define the key drivers and therapeutic vulnerabilities of brain cancer growth and; 2) to identify new small molecules that could be developed further as new treatments. We will gain this knowledge through genetic modification of normal neural stem cells and testing of their ability to make brain tumour cells when transplanted into an immunocompromised mouse brain. In

parallel we will also generate new cell cultures from patient tumour samples and test how aggressive these remain after we disrupt tumour promoting pathways. Towards the second half of the project we will therefore need to test the new drug-like small molecules to see how well they can suppress the growth of the brain tumours. We will also assess tumour relapse after current treatments fail.

What 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 identification of the key pathways that drive brain tumour growth we will be able to develop new targeted therapies. The basic research is therefore likely to provide new knowledge and insights into how brain tumour growth is controlled. Xenograft tumours (human tumours growing in a mouse brain) are a critical tool to test the effectiveness of newly identified drugs, or existing drug/drug combinations, in suppressing tumour growth. They enable us to monitor tumour cell invasiveness and interaction with neurons and blood vessels which can provide environmental signals to keep the cells dormant/quiescence; this is a problem as they then evade anti-proliferation therapies and eventually drive regrowth of the tumour.

What types and approximate numbers of animals do you expect to use and over what period of time?

There are many subtypes of brain cancers, and therefore we need to explore a large set of different genes and pathways. The mouse is the favoured animal species, as we can reliably transplant cells directly into the brain in regions that do not cause significant symptoms until the tumour has grown significantly. This means that we can track tumours in live mice and get a lot of information before the animals develop significant clinical signs relating to the tumour. We will test new candidate drug-like molecules and therapies to see how they affect tumour growth and use the live imaging of tumour growth to monitor responses. Our studies in mice enable us to quickly determine which genes and pathways are involved in this infiltration, tumour growth, and relapse after anti-proliferative therapies. We will model the disease by genetically engineering into cells a set of the most common driver mutations, typically around 5-10 different pathways. These cells with then be injected into the brain to create the tumours. This reduces the numbers of animals that need to be bred. Around 5000 mice will be needed in total (1000/yr) for the 5 years of this project. We transplant cells into mice that have been genetically modified to eliminate their immune system; in this way the human tumours can form without rejection. All mice are kept in individually vented cages (IVCs) to prevent any risk of infection. We have all of the state-of-the-art equipment needed for this project and close contact to trained veterinary staff who provide input and advice on all 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?

We anticipate that the mice will develop brain tumours. For some experiments, we can monitor tumour growth before symptoms develop. In cases where this is not possible the mice are closely monitored and humanely sacrificed when clinical signs beyond mild are detected The transplant procedure itself may causes some discomfort; however, the brain has no pain receptors and mice rapidly recover from the procedure without any deficits. This is a moderate level of severity. Also, mice are given analgesia and the procedure is performed under general anaesthetic. The experimental procedures to inject tumour cells or substances do not cause significant discomfort or distress to the animals and they recovery almost immediately from the surgery. Once tumours have developed animals will be killed humanely and their brain and tumour tissue will be analysed by detailed histological and molecular tests.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Cell culture models are being used by our laboratory, but often do not enable the long term monitoring of cell behaviours and fail to recreate the cellular complexity that exists in the live adult mouse brain. We need to explore tumour cell behaviour in the appropriate tissue microenvironment (e.g. blood vessels, nerve tracks and cerebral spinal fluid contact) as these are known signals that affect tumour cell proliferation, dormancy and specialisation. It is also vital to study tumour cell behaviour in a whole brain, as we can monitor the extent of infiltration and spread. The physiological effects of anticancer drugs require a whole animal study, as drugs can be affected by the liver. Finally, we need to study how well drugs can enter the brain and cross the blood –brain barrier. This is not possible in laboratory culture.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have carefully selected the group sizes and numbers required based on anticipated statistical power required. All experiments performed in the mice will have had significant cell culture experiments performed to generate the initial hypothesis. We will use non-invasive imaging techniques in some instances to enable tracking of tumour growth and reduction in numbers of mice, as more accurate quantitative data is obtained. We are where possible exploiting organotypic

brain slice cultures to assess tumour aggressiveness which can reduce the numbers of animals 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

Mice are suitable for studies of brain tumour experiments as there is considerable knowledge of mouse brain physiology, histology and molecular biology. Mouse is the preeminent model for genetically modifying cells and we can therefore make use of many available transgenic strains. We have taken many steps to refine our procedures over the past few years. For example, using inhalable gas anaesthetics and smaller needles reduces tissue damage and recovery times. We also use analgesia during surgical procedures. We will use humane endpoint for testing new drugs, in line with guidance from local vets, with close monitoring and killing of any animals with signs of large tumours .

PROJECT 297

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.

Word limit; 1000 words

Project Title	Neuroimmune mechanisms of CNS degeneration and regeneration
Key Words	Neurodegeneration, Brain repair, Immune system, Infection, Microglia
Expected duration of the project	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;
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 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 a 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.

PROJECT 298

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.

Word limit; 1000 words

Project Title	Overlapping mechanisms of sleep and anaesthesia
Key Words	Sleep, sedation, anaesthesia, sleep deprivation, insomnia, neurological disorders,
Expected duration of the project	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;
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):

This project seeks to understand how general anaesthetics and sleep-inducing drugs act with the aim of developing safer, more efficient or more selective agents. We also hope to shed light on the fundamental mystery of why we need to sleep and why the lack of sleep is harmful.

Modern surgery would be impossible without general anaesthetics, yet the underlying mechanisms by which they produce unconsciousness and pain relief are only just beginning to be discovered. Understanding the molecular actions and neuronal pathways involved presents a major intellectual challenge for basic neuroscience, and research into anaesthetic mechanisms can be expected to provide fundamental information on neuronal excitability which has broader applicability. It is widely recognised that the anaesthetic drugs presently used in clinical practice are far from satisfactory. Currently used anaesthetics are relatively "non-specific", mostly act at high concentrations, and affect many targets. Consequently, many patients suffer from undesirable side-effects from the anaesthetic and analgesic drugs used for their perioperative care. Serious morbidity (*e.g.* cardiovascular side effects) can be provoked in already compromised patients, which is an issue of increasing concern in an ageing population. Our Programme seeks to understand which molecular targets are responsible for the desirable effects of the anaesthetics and the neuronal pathways that are involved.

Because we have determined that sedatives and anaesthetics act, to some extent at least, on natural pathways of sleep and arousal, our work is also directed towards understanding why the need for sleep is so powerful and why the lack of sleep can be so damaging. For example, it is possible that dementia is exacerbated by the long-term lack of sleep, and our research might provide novel approaches towards

treatment of this and other neurological disorders. Our work may also provide new approaches towards the development of more selective sedatives and anaesthetics.

What 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 clinical relevance and potential befits of our work is four-fold. First, the prevalence of sleep disorders is large and growing rapidly with approximately 15% of the UK population affected, with the elderly being particularly susceptible. This results in a substantial societal and economic burden. The need for novel drugs to treat insomnia, daytime sleepiness, shift-work sleep disorder, and other sleep disorders is growing. Second, in the hospital, sedative drugs may contribute to the development of delirium. When patients are sedated in intensive care for prolonged periods, delirium keeps patients on mechanical ventilation, thereby prolonging their stay and increasing the risk of complications from infections. Application of sedative drugs during intensive care that produce a more “natural” sleep may promote the restorative benefits of sleep, prevent delirium, and minimise the deleterious effects of sleep disruption which may, in itself, significantly increase morbidity and mortality. Third, during many procedures, for example endoscopy, patients must be both sedated and relatively immobile yet remain compliant. Therefore, depending on the patients’ needs and the specific procedure, drugs that highlight different elements of the sedative-hypnotic continuum are needed. Understanding the neuronal networks involved in the production of the sedative/hypnotic response by different classes of agents will permit rational selections tailored to the patient’s needs and could result in major healthcare benefits. Fourth, there is a growing appreciation that certain neurological disorders such as Alzheimer’s disease may be exacerbated, or even caused, by long-term bad sleep. If this link can be established, it may be possible to slow the onset of such diseases by paying specific attention to sleep regulation In summary, by exploiting what is known at the molecular level, our research should provide insights into both how natural sleep is regulated, as well as how and where general anaesthetics act at the level of neuronal networks. This will inform strategies for the development of new drugs, including those to treat sleep disorders, and these strategies may be applicable to currently intractable neurological disorders.

What types and approximate numbers of animals do you expect to use and over what period of time?

Because we are studying sleep and loss of consciousness induced by anaesthetics, the use of animals is unavoidable. We use mainly use mice. We use mice because genetic engineering allows particular mice to be bred which carry putative anaesthetic targets or circuits that have been modified genetically. This allows us to test specific hypotheses regarding the importance of particular molecular targets or the importance of particular brain circuitry. Thus we can investigate the roles of individual anaesthetic and sleep drug targets in the response on the whole animal. We use, typically, 2000 mice over 5 years. Approximately 200 mice are killed humanely and their brain tissue used immediately. The remainder of the animals are

used in procedures that last only a few hours for individual experiments. Occasionally we use tadpoles to assess anaesthetic potencies. Approximately 200 tadpoles will be used for 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?

Animal suffering is minimal in our experiments because the drugs we study are anaesthetics. By their nature, these drugs render animals insensible to pain. In some experiments where we investigate specific neuronal pathways, we introduce electrodes or fine tubes into the brain to deliver chemicals, or temperature-recording devices in the abdomen. Then, anaesthetics or sleep-inducing drugs are applied to specific parts of the nervous system, animals are closely monitored; the experiment is terminated, if suffering is evident. Such events are rare, however.

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 anaesthetic receptors in the brain and neuronal subtypes contribute to brain physiology and generate sleep, or respond to anaesthetics to produce unconsciousness, it is essential to use native brain tissue. Although properties of individual channel types or receptors are best studied by using cell lines that are able to express certain brain receptors, to study how the channels/receptors work *in vivo* and in their native membrane environments requires the intact animal. The complexity of how receptors and ion channels influence brain physiology and how the channels interact with each other can only be appreciated by using mouse lines (or tissue derived from them) with deleted or modified ion channel genes.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

1. Reduced animal numbers and appropriate statistics: we maintain mouse lines at the minimum needed. To reduce the number of individual mice needed we plan the experiments (e.g. injection of substances and agents) by applying appropriate statistics so that the minimum number of animals are used to give statistically meaningful data.

2. Reducing the numbers of mice needed: a genetic Refinement to identify subtypes of neurons in slices of brain taken after death by visually guided recording of genetically fluorescent cells To understand how particular brain circuits function, we need to know the electrophysiological properties of the neuronal types. We use a technique whereby specific types of neuron can be made fluorescent so that they can be easily visualized under a microscope. This means that these neurons can be efficiently identified in living brain slices, **so requiring far fewer animals to get the same results**.

3. Computer-controlled injections reduce the number of animals needed We are using computer-controlled devices ('Angle 2' Stereotaxic frame manufactured by Leica) that allow for highly improved accuracy of stereotaxic injections/electrode placement **and this greatly reduces the number of animals that need to be used to get a useful experimental results**. In addition, these devices allow us to store target coordinates for each animal. **This reduces surgery time, and thus speeds recovery.**

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 tadpoles and mice. **Tadpoles** are useful to study the potencies of anesthetics that are rapidly metabolised in mammals. This allows true potencies to be determined without being affected by the complexities of metabolism. For many anaesthetics, however, potencies in mammals need to be determined because these better reflect potencies in humans. To get close to the human condition, we study how neurotransmitter receptors, ion channels and neuronal subtypes influence brain physiology using mammals. **Mice** are the only mammals that have easily modifiable genetic systems. Hence the mouse is a model organism for our work.

PROJECT 299

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.

Word limit; 1000 words

Project Title	Models of neurodegenerative disease
Key Words	Neurodegeneration, Mechanisms, Gene Therapy, Small molecules
Expected duration of the project	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):

There are no effective treatments for human neurodegenerative disorders. This is partly because current knowledge of the causes of these disorders is limited. In this project we aim to characterise rodent models of human neurodegenerative disease, and to test novel therapeutic approaches in these models. We will specifically investigate amyotrophic lateral sclerosis (ALS often known as motor neurone disease in the UK), hereditary spastic paraplegia (HSP), and Parkinson’s disease (PD).

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

The benefits of our studies will be twofold. Firstly we will identify the underlying mechanisms of disease in ALS, HSP and PD. Secondly we will perform preclinical studies that will either identify or rule out potential therapeutic approaches. Ruling out treatments is an important step. At present the majority of drugs developed for neurological disease fail in man. Greater rigor during preclinical development is necessary to address this.

What types and approximate numbers of animals do you expect to use and over what period of time?

This programme of work will involve studies in mouse. Over 5 years we expect to use approximately 3000 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?

In order to characterise the disease processes, and to investigate the effect of novel therapies, mice will inevitably develop features of neurodegenerative disease, including loss or dysfunction of motor neurons, which will lead to a progressive loss of motor function. In this project we will use early endpoints with moderate severity. At the end of these experiments we will collect tissues to investigate pathological hallmarks of disease such as swellings in nerve cell fibres, loss of motor neurons in the spinal cord, and biochemical analysis to investigate levels of specific proteins.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Neurodegeneration is a complex process that involves the interplay between numerous cell types. Although we model some aspects of the disease process in tissue culture, ultimately we rely on animal studies to confirm the relevance of these findings, and particularly to determine the clinical benefits of novel therapeutic approaches. Some experiments could be performed in non-protected animals such as flies. However there are some limitations with flies, particularly the lack of some of the key genes involved in neurodegeneration.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We consult statisticians to determine the minimum number of animals required to obtain statistically valid results.

If we used fewer animals it would give ambiguous results, which are not scientifically valid.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 our work involves mouse models, which are currently the most refined models of neurodegeneration that allow for testing and development of therapeutic approaches.

We use animals at early disease stages, avoiding any use of mice with severe neurological symptoms.

We develop refinements to the methodologies involved in measuring motor function, particularly in relation to ALS and HSP models.

PROJECT 300

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.

Word limit; 1000 words

Project Title	Combination immunotherapy for the treatment of cancer
Key Words	Cancer, Immunotherapy, immunology, Radiation therapy, Combination therapy
Expected duration of the project	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;
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):

Immunotherapy targets the patient's own immune system to attack and kill cancer cells. Over the last 5 years or so, a number of important breakthroughs have led to the development of immunotherapies which have shown remarkable success in the clinic. The most successful treatments have been those targeting immune checkpoints which are used by cancer cells to turn off immune responses. However, whilst durable responses are seen, they tend to be limited to subsets of patients, and over half of patients fail to respond. This project aims to improve responses by developing combination therapies which will enhance the activity of immunotherapy and generate more effective 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 aims to develop new combination treatments which will enhance the activity of cancer immunotherapy. It will also increase our knowledge of how such therapies work and what factors may limit their effectiveness, which means we may have a better understanding of why some patients respond to immunotherapy whilst others do not. This information can be used to better design therapies in order to improve outcome through the generation of more potent anti-cancer immune responses. Ultimately, our hope is that we can translate our observations in early phase clinical trials with a view to developing more effective and successful clinical therapies leading to greater benefit in cancer patients. REDACTED

What types and approximate numbers of animals do you expect to use and over what period of time?

We anticipate using 10,000 mice 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?

The majority of animals are not expected to show signs of adverse effects that impact on their general well-being. However, given that we aim to develop therapies which activate potent immune responses, on rare occasions the severity of these signs may be such that the humane end points may be reached. The majority of the procedures will result in no more than transient discomfort and no lasting harm. All the mice will be humanely culled 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 anti-cancer therapies is a major goal for modern biomedical science, and the immune system has great potential for contributing to the control and elimination of malignant disease. Therefore, therapeutic strategies to target the immune system against cancer are an attractive proposition. This project aims to develop and characterise therapeutic combinations designed to enhance anti-cancer immune responses. However, such therapies involve complex interaction between tumour cells, constituents of the tumour microenvironment and components of the host immune system. These dynamic interactions occur between numerous cellular populations, in different tissues and organs including the tumour itself and due to the nature of their complexity they cannot yet be fully reproduced in silico or in vitro. Consequently, it is currently impossible to fully recapitulate the phases of a therapeutic anti-cancer immune response, homing to the site of tumour, and associated immune checkpoints/regulation outside of a living organism. Mouse models represent an ideal system for studying these interactions.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Wherever possible, preliminary studies will be performed *in vitro* and *in silico* in order to reduce the number of animals required for in vivo studies.

Variability will be minimised by using inbred strains housed in identical conditions; for transgenic strains efficient breeding will be employed to minimise the number of animals required to obtain the appropriate genotype.

Each experiment will be designed to provide the maximum amount of information from the minimum number of mice (e.g. sharing controls across experiments to minimize the number of control groups needed). Continuing statistical evaluation will be used to guide experimental design and determine the minimum group size required to give significance to results. Where optimal group sizes have not already been determined pilot studies will be used to assess the number of animals required per cohort to achieve statistically relevant outcome.

Where appropriate *in vivo* imaging will be used to facilitate the detection of tumour responses in real-time in individual animals, so helping 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

Adult mice are the principal species of choice because the murine genome is of a similar size to the human genome, with a great deal of functional conservation between the two species, and there is considerable similarity between gene expression profiles in the murine and human immune system. Numerous genetic mutants exist facilitating the exploration of immune function including transgenic knock-in and knock-out models. Proof-of-concept studies in mice have successfully predicted clinical activity of breakthrough immunotherapeutic agents including immune checkpoint inhibitors which have revolutionised human cancer therapy in the last 5 years. Thus, mice remain an important and highly relevant research model.

We constantly work to improve husbandry, and experimental techniques are continually refined to minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. During experimental procedures animals will be closely monitored by experienced technical or scientific research staff who will assess the health and welfare status of the experimental animals. Appropriate anaesthetic and analgesic regimes will be used as well as appropriate humane methods of culling within the animal facility.

PROJECT 301

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.

Word limit; 1000 words

Project Title	Models of neurodegenerative disease
Key Words	Neurodegeneration, Mechanisms, Gene Therapy, Small molecules
Expected duration of the project	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):

There are no effective treatments for human neurodegenerative disorders. This is partly because current knowledge of the causes of these disorders is limited. In this project we aim to characterise rodent models of human neurodegenerative disease, and to test novel therapeutic approaches in these models. We will specifically investigate amyotrophic lateral sclerosis (ALS often known as motor neurone disease in the UK), hereditary spastic paraplegia (HSP), and Parkinson's disease (PD).

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

The benefits of our studies will be twofold. Firstly we will identify the underlying mechanisms of disease in ALS, HSP and PD. Secondly we will perform preclinical studies that will either identify or rule out potential therapeutic approaches. Ruling out treatments is an important step. At present the majority of drugs developed for neurological disease fail in man. Greater rigor during preclinical development is necessary to address this.

What types and approximate numbers of animals do you expect to use and over what period of time?

This programme of work will involve studies in mouse. Over 5 years we expect to use approximately 3000 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?

In order to characterise the disease processes, and to investigate the effect of novel therapies, mice will inevitably develop features of neurodegenerative disease, including loss or dysfunction of motor neurons, which will lead to a progressive loss of motor function. In this project we will use early endpoints with moderate severity. At the end of these experiments we will collect tissues to investigate pathological hallmarks of disease such as swellings in nerve cell fibres, loss of motor neurons in the spinal cord, and biochemical analysis to investigate levels of specific proteins.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Neurodegeneration is a complex process that involves the interplay between numerous cell types. Although we model some aspects of the disease process in tissue culture, ultimately we rely on animal studies to confirm the relevance of these findings, and particularly to determine the clinical benefits of novel therapeutic approaches. Some experiments could be performed in non-protected animals such as flies. However there are some limitations with flies, particularly the lack of some of the key genes involved in neurodegeneration.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We consult statisticians to determine the minimum number of animals required to obtain statistically valid results.

If we used fewer animals it would give ambiguous results, which are not scientifically valid.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 our work involves mouse models, which are currently the most refined models of neurodegeneration that allow for testing and development of therapeutic approaches.

We use animals at early disease stages, avoiding any use of mice with severe neurological symptoms.

We develop refinements to the methodologies involved in measuring motor function, particularly in relation to ALS and HSP models.

PROJECT 302

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.

Word limit; 1000 words

Project Title	Combination immunotherapy for the treatment of cancer
Key Words	Cancer, Immunotherapy, immunology, Radiation therapy, Combination therapy
Expected duration of the project	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;
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):

Immunotherapy targets the patient's own immune system to attack and kill cancer cells. Over the last 5 years or so, a number of important breakthroughs have led to the development of immunotherapies which have shown remarkable success in the clinic. The most successful treatments have been those targeting immune checkpoints which are used by cancer cells to turn off immune responses. However, whilst durable responses are seen, they tend to be limited to subsets of patients, and over half of patients fail to respond. This project aims to improve responses by developing combination therapies which will enhance the activity of immunotherapy and generate more effective 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 aims to develop new combination treatments which will enhance the activity of cancer immunotherapy. It will also increase our knowledge of how such therapies work and what factors may limit their effectiveness, which means we may have a better understanding of why some patients respond to immunotherapy whilst others do not. This information can be used to better design therapies in order to improve outcome through the generation of more potent anti-cancer immune responses. Ultimately, our hope is that we can translate our observations in early phase clinical trials with a view to developing more effective and successful clinical therapies leading to greater benefit in cancer patients. REDACTED

What types and approximate numbers of animals do you expect to use and over what period of time?

We anticipate using 10,000 mice 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?

The majority of animals are not expected to show signs of adverse effects that impact on their general well-being. However, given that we aim to develop therapies which activate potent immune responses, on rare occasions the severity of these signs may be such that the humane end points may be reached. The majority of the procedures will result in no more than transient discomfort and no lasting harm. All the mice will be humanely culled 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 anti-cancer therapies is a major goal for modern biomedical science, and the immune system has great potential for contributing to the control and elimination of malignant disease. Therefore, therapeutic strategies to target the immune system against cancer are an attractive proposition. This project aims to develop and characterise therapeutic combinations designed to enhance anti-cancer immune responses. However, such therapies involve complex interaction between tumour cells, constituents of the tumour microenvironment and components of the host immune system. These dynamic interactions occur between numerous cellular populations, in different tissues and organs including the tumour itself and due to the nature of their complexity they cannot yet be fully reproduced in silico or in vitro. Consequently, it is currently impossible to fully recapitulate the phases of a therapeutic anti-cancer immune response, homing to the site of tumour, and associated immune checkpoints/regulation outside of a living organism. Mouse models represent an ideal system for studying these interactions.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Wherever possible, preliminary studies will be performed *in vitro* and *in silico* in order to reduce the number of animals required for in vivo studies.

Variability will be minimised by using inbred strains housed in identical conditions; for transgenic strains efficient breeding will be employed to minimise the number of animals required to obtain the appropriate genotype.

Each experiment will be designed to provide the maximum amount of information from the minimum number of mice (e.g. sharing controls across experiments to minimize the number of control groups needed). Continuing statistical evaluation will be used to guide experimental design and determine the minimum group size required to give significance to results. Where optimal group sizes have not already been determined pilot studies will be used to assess the number of animals required per cohort to achieve statistically relevant outcome.

Where appropriate *in vivo* imaging will be used to facilitate the detection of tumour responses in real-time in individual animals, so helping 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

Adult mice are the principal species of choice because the murine genome is of a similar size to the human genome, with a great deal of functional conservation between the two species, and there is considerable similarity between gene expression profiles in the murine and human immune system. Numerous genetic mutants exist facilitating the exploration of immune function including transgenic knock-in and knock-out models. Proof-of-concept studies in mice have successfully predicted clinical activity of breakthrough immunotherapeutic agents including immune checkpoint inhibitors which have revolutionised human cancer therapy in the last 5 years. Thus, mice remain an important and highly relevant research model.

We constantly work to improve husbandry, and experimental techniques are continually refined to minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. During experimental procedures animals will be closely monitored by experienced technical or scientific research staff who will assess the health and welfare status of the experimental animals. Appropriate anaesthetic and analgesic regimes will be used as well as appropriate humane methods of culling within the animal facility.

PROJECT 303

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.

Word limit; 1000 words

Project Title	Friedreich ataxia mouse models
Key Words	Friedreich ataxia, Neurodegenerative disease, genetic disease
Expected duration of the project	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;
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):

This project aims to establish and characterize mouse models of the lethal inherited neurological disorder called Friedreich ataxia (FRDA) and to then use these models to gain understanding of the disease mechanism and to identify novel therapies. We have already generated mouse models that resemble a very mild form of the human FRDA disorder. However, we would now like to establish a more representative FRDA mouse model that will further progress the disease understanding and therapy of FRDA. Specifically, the project will investigate potential treatments aimed at reducing, halting or reversing FRDA disease pathology. This form of pre-clinical study will be of great benefit before proceeding to clinical trials in human individuals. Initial experiments on cells grown in the laboratory and using computers have identified novel potential FRDA-therapeutic drugs that have been tested in human FRDA cells grown in the laboratory. However, once this initial data has been obtained, it is necessary to pursue further studies on living animals to understand the more complex interactions that may occur in a human being to achieve the final medical advance.

What 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 data that is obtained from this project will be published in peer-reviewed journals, which will be of great benefit to all scientists in the FRDA research and medical field and related fields of study. The information obtained from this project can be useful for FRDA patients to achieve successful medical advances in the future. This project aims to advance the understanding of a lethal inherited neurological disorder where children usually become wheel-chair bound in their teens and most commonly die in their twenties. There is currently no known effective

treatment for this disorder. However, this project aims to undertake essential investigations that will ultimately lead to effective treatments.

What types and approximate numbers of animals do you expect to use and over what period of time?

This project aims to use approximately 1,500 mice over a period of 5 years. The least number of mice are bred to maintain the colony and are used to give essential data for experimental protocols. In this project approximately 1,500 mice are bred and maintained in total and, of these, approximately 100 mice are used as the parents for matings and approximately 500 mice are required for other experimental protocols. The remaining 900 mice that are produced as part of the essential breeding will not be of a suitable genetic composition to be useful. Young mice produced under this project may be issued to other authorised projects and mice that do not have the required FRDA mutation and are not required to maintain the colony and cannot be used elsewhere will be humanely killed.

In the context of what you propose to do to 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 intend to establish and characterize mouse models for FRDA by natural breeding of existing genetically altered mice. This project has chosen to use the mouse because this is the lowest vertebrate species that can be used to provide a very useful model of FRDA. The experimental behavioural protocols of this project, which assess coordination ability of the mice, are completely non-invasive and are unlikely to cause any pain, distress or harm. For other protocols that determine the disease-like status of the mice, appropriate anaesthesia is given for the animal to feel no pain. Drugs that have already been tested and shown to be safe in humans and mice will be tested by us in our FRDA mice for short periods of 5 days, and then depending on these initial results, in longer periods of 3 months to assess the safety and efficacy of these drugs in this particular disease model. Adverse events due to FRDA-like disease status, drug effects or route of drug administration effects are very unlikely to occur, but if they do occur, the mice will be immediately killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Some information relevant to understanding FRDA and its treatment have been obtained from studies of bacteria, yeast, worms, fruit flies and human cells grown in

a laboratory. We are currently performing studies using cells grown in the laboratory to determine potential novel FRDA treatments. We also aim to establish further FRDA mouse model cells that can be grown in the laboratory as part of this project. In addition, several groups around the world are now attempting to make neuron and heart cells from FRDA patient skin cells, which may prove useful for future investigations of FRDA. However, in order to better understand the disease and to assess treatment strategies, a living mammalian animal model with complex systems, organs and tissues similar to humans is considered necessary. Use of animals is now required to extend the initial information that has already been achieved from studies of lower organisms or cells grown in the laboratory in order to provide a more complete understanding of this human disorder and to undertake testing of novel drugs before progressing to human clinical trials.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Breeding will be kept to the minimum amount necessary to obtain the experimental groups of mice for disease-like characterisation and therapeutic testing. For all of the experiments, each group will contain between 4 and 16 age- and sex-matched mice, which is the number needed to obtain the essential results.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 animals in which a representational model of FRDA has been developed. At the same time, mice are also considered to be a similar enough mammalian organism to humans to provide invaluable information from preclinical testing of potential therapeutic approaches before progressing to human clinical trials. There are several different types of FRDA mouse model and each model may provide useful and complementary information regarding FRDA disease and therapy. Several methods will be employed in this project, starting with mice at 2 months of age and continuing until 5 months of age, to investigate the early stages of this disease. Suffering of mice will be minimised throughout the project by daily inspection of mice for general signs of ill health, and by our decision to kill mice at 5 months of age before the development of later potentially more severe stages of disease. Suffering will also be minimised by the administration of safe doses of potential therapeutic agents by the mildest available route of administration. Any mice showing signs of pain or distress will be killed.

PROJECT 304

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.

Word limit; 1000 words

Project Title	Chemical Ecotoxicological Testing in Fish
Key Words	Fish, Ecotoxicology, Regulatory risk assessment
Expected duration of the project	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.

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):

Aim: Provision of toxicity data to produce dossiers for use by risk assessors in assessing and managing the risk associated with the production, use and release of chemicals which are used in or may be released into the environment.

Toxicology data is generated in accordance with standardised guidelines which many countries follow. Typically, studies will follow guidelines written by the Organisation for Economic Cooperation and Development (OECD) or by the United States Environmental Protection Agency (US EPA). Following these guidelines ensures good quality, standardised, reliable data can be obtained.

Studies typically involve investigations into effects of chemicals on the development, growth, behaviour and reproduction of fish. Baseline toxicity to fish is also often required – this involves investigating the level of exposure to the particular substance under test that will result in 50% death of a group of fish (acute toxicity 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 production of data forms part of the chemical risk dossiers which are used by regulators and authorities to formulate risk assessments for production and use of chemicals as well as their release into the natural environment. Environmental exposure may cause harm to the natural biota. Chemicals that accumulate in fish can pose a risk to end consumers, or cause chronic damage to the natural environment and biodiversity. Providing data which allows authorities to regulate the production and release of these chemicals is vital for environmental protection.

What types and approximate numbers of animals do you expect to use and over what period of time?

Various species of fish will be used but typically they include rainbow and brown trout, zebrafish, fathead minnow, guppy, carp, bluegill, stickleback, bream and roach. Numbers used will vary depending on the type of studies we are contracted to perform but it is possible that the total use will be up to 20,000 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?

For acute toxicity tests (the most severe of the tests) involving exposure of fish to the test substance in water the top dose will likely cause death – this may be over a very short period of time or at most over 96 hours (4 days). Symptoms may include change of behaviour, respiratory distress, loss of equilibrium and loss of coordination. Typically this means a ‘severe’ category. Fish exposed to lower doses of chemical may be less active or exhibit some of the symptoms given above to a lesser extent and over a shorter period of time. In these cases the severity level will be ‘moderate’ or ‘low’ depending on the period of time the symptoms persist. For non-acute toxicity studies the severity levels will be lower as the studies are designed to show sub-lethal effects. As such, effects will typically fall in the ‘low’ category or below (‘sub-threshold’). As the fish are either bred for purpose on site or sourced from farmed stock (when possible), they cannot be released into the wild. These fish will all be humanely euthanised (including control fish) by a Schedule 1 method at the end of a study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The regulatory guidelines require the use of key model species in the generation of the toxicity data. Fish species are one of these key model species. When a sponsor requests a test involving the use of fish we make sure that there is no alternative test which could be done which does not use fish. We also make sure that the test requested is the one most suitable to answer the scientific question being asked.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will endeavour to ensure that the test is required and suggest a lower severity test or test with fewer numbers of fish if at all possible. For example, performing an acute toxicity test to the OECD guideline typically requires only 42 fish, whereas the US EPA guideline requires 120 fish. In such cases we will do everything possible to ensure that the OECD guideline is the one followed. If a regulator does not allow the use of the OECD test then permission will be sought from the Secretary of State to use the alternative test guidelines before any tests are carried 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

We are a small company with the ability to dedicate more time to both planning the tests and monitoring the fish during the tests. As such, time and effort is applied to ensure that the studies are performed to the highest standard on the first time of asking. The preliminary work we perform is done to make sure that when we have to use fish we do so with as much information as possible to minimise errors and maximise quality data output. If necessary a dose-range finder test may be performed on a minimal number of fish initially (a pilot study) to ensure that the dose range for the definitive study is optimised. This reduces the risk of performing a full scale definitive study (many more fish used than a range finder) with the wrong dose range.

We also dedicate more time than is strictly required by the guidelines to ensure that we are checking the fish regularly. This means that if a fish is suffering and we are allowed to euthanise it we will identify the fish quickly and act to reduce that suffering to an absolute minimum.

We are also working towards another refinement by recording key clinical signs data to inform us when a fish is ill beyond recovery. This will enable us to humanely kill fish at a point prior to waiting for mortality.

PROJECT 305

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.

Word limit; 1000 words

Project Title	New Treatments for Respiratory Diseases
Key Words	asthma, copd, cough
Expected duration of the project	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):

There remain significant unmet needs in the treatment of common lung diseases such as asthma and chronic obstructive pulmonary disease (smokers' disease) which requires further research to help identify possible new treatments. In addition, chronic cough is a debilitating symptom in many patients with lung disease and we currently have inadequate treatments with no new drug class having been introduced in the previous 70 years. One of the hallmarks of these conditions is the increased irritability of the airways so that patients respond inappropriately to innocuous stimuli in their environment such as cold air or exercise with bouts of wheezing and coughing. We aim to investigate why this happens and need to use small animals to help us with this research. We will expose guinea-pigs and rabbits to substances that make them cough or that produce airway irritation so that we can investigate novel medicines that may reduce such changes and that could ultimately be used to reduce these distressing symptoms in patients.

In other experiments guinea-pigs and rabbits may undergo surgery under anaesthesia to allow us to measure their lung function. Substances may be injected intravenously or given by inhalation. We will assess the changes in the airways as a result of these substances. Animals may be treated prior to his with various pharmacological agents. This work is necessary to investigate the impact new medicines may have on improving abnormal lung function which is a hallmark of asthma and smokers 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)?

Both animals and people who suffer from respiratory diseases could benefit from our research as we trying to identify improved medicines for treating such patients. Our research will also provide valuable information on the mechanisms causing the symptoms characterising asthma and smokers disease that will help other researchers working to better understand these diseases.

What types and approximate numbers of animals do you expect to use and over what period of time?

Up to 500 guinea-pigs and rabbits over 5 years to study cough and lung function. We also plan to use some rats for other work investigating inflammation in the lung, but we cannot use rats for work on cough as strangely this species does not cough.

In the context of what you propose to do to 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 in this project will generally only experience mild adverse effects or will be under terminal general anaesthesia. However, some animals may experience moderate symptoms of respiratory distress, some discomfort at sites of injection or rarely weight loss from certain types of drugs. All Animals will be carefully monitored and humanely killed if these symptoms become too debilitating. All animals will be humanely killed at the end of experimentation, but tissues and cells will be harvested at this point to allow further work to be carried out in test tubes.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Patients with asthma, smokers' disease and intractable cough have "irritable" airways involving complex nerve reflexes which are difficult to study in test tubes or isolated cells. Furthermore, certain lower organisms such as mice and rats that are often use in other types of medical research do not cough and so we have to use higher species such as guinea-pigs and rabbits to help with this research.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

By appropriate, careful experimental design we will ensure the minimum number of animals are used per experiment. The protocols we plan to use are based on a very

large amount of experience with experimental design and use of the relevant 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

Mice and rats do not cough and so we need to use higher organisms such as guinea pigs and rabbits. All efforts will be utilized to ensure minimal harm to the animals used in these experiments and where appropriate; experiments will be carried out under general anaesthetic for the whole experiment. However, for some types of work involving the investigation of inflammation in the lung we will use rats as their response to certain types of inflammatory insult are known to mimic responses in man.

PROJECT 306

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.

Word limit; 1000 words

Project Title	Quality Control Testing Of Clinical Products
Key Words	Vaccines, Potency, Biologics, Safety
Expected duration of the project	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.

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):

We provide a service to test specialist clinical products made to prevent life threatening human infections such as anthrax, diphtheria and smallpox. This project ensures that pharmaceutical products requiring highly specialised facilities for regulatory and developmental animal testing are available in the United Kingdom. We provide a service to test specialist clinical products made to prevent life threatening human infections to ensure they are safe for people to use and that they work.

The testing proposed in this licence application is to release new batches of product and check that the product works correctly during its shelf life. Animals are only used to test pharmaceutical products where there is no alternative test which meets regulatory requirements.

We also intend to validate an alternative potency assay which uses antibodies raised to the vaccine and the antibodies are then used in a cell culture to neutralise the disease toxins.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Specialist vaccines and specialist biological products are tested for both safety and potency. The public health benefits of these vaccines are substantial, providing protection from infection and outbreaks of lethal pathogens. Validating the alternative potency assay will allow this new test to replace the existing potency assay which is the one authorised by the regulatory authority . The alternative assay uses mice instead of guinea pigs, reduces the severity to mild and reduces the numbers of animals required.

What types and approximate numbers of animals do you expect to use and over what period of time?

We expect to use less than 16000 guinea pigs, 22000 mice and 300 rabbits during the five years of the licence. The choice of species is described within the European Pharmacopoeia and Marketing Authorisation which states precisely how the pharmaceutical products are tested. The numbers of animals used are proportional to the number of batches which require testing, although more testing may be necessary if a product becomes in demand for public health needs. Initially validating the alternative assay will require more animals in the short term, however there will be fewer required in the medium and long term if this new assay is successful.

In the context of what you propose to do to 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 administered with human pharmaceutical products to determine that they are safe and potent. The safety protocols aim to confirm the product is safe with a representative dose. Some of the protocols determining potency use toxin producing bacteria. There is a rare chance that there could be a complication with toxin neutralisation and precautions are taken to ensure this does not occur. Some animals may develop clinical signs which indicate that they will not survive. We will always try to humanely euthanise these animals before natural death occurs and the criterion used is described in this licence. The main harms identified in clinical signs for this infectious disease are ruffled fur, eye watering, fever and painless abscess. These are serious infectious diseases where some animals may not survive, but rigorous procedures are in place to prevent suffering. Accordingly a severe severity limit applies to this type of work. Surviving animals will be euthanised at the end of the test before safe destruction at the facility. These are hazardous infectious agents and are therefore controlled to prevent exposure or release.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animal use is for releasing new batches of product and testing product already released to the market when there is no alternative currently available. The tests are prescribed by the European Pharmacopoeia and Marketing Authorisation for these products.

We have been able to remove all animal tests for one of our products during the previous licence and will continue the 3Rs principles wherever possible as new techniques become available.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used for each test is defined by regulations and, where possible, testing is grouped to minimise the number of control or standard animals used. By using an advanced statistical analysis we have reduced group sizes by 25%. If the alternative toxin neutralisation potency assay is successful fewer animals will be required in the future.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 for animal testing is dictated by the European Pharmacopoeia and Marketing Authorisation. These are robust tests which provide reliable results enabling the manufacture of safe and effective pharmaceutical products.

The disease progression is relatively predictable and enables us to determine clinical signs as an end point to reduce severity, enabling the humane euthanasia of individual animals once a terminal decline has been recognised.

We are in the process of replacing a potency assay which if successful will reduce the severity from severe to mild and fewer animals will be used.

PROJECT 307

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.

Word limit; 1000 words

Project Title	Anti-cancer drug discovery and development
Key Words	Cancer therapy, Experimental tumour models, Pharmacokinetics, Pharmacodynamics, Efficacy assessment
Expected duration of the project	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;
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 overall aim is to develop and progress an anti-cancer therapeutic (either sourced ‘in-house’ or through academic or industrial collaboration) towards clinical trials over the course of the 5 years with approximately 20 compounds a year being evaluated. This will be achieved through the following objectives:

1. To identify and validate novel targets for developing new therapeutics for the treatment and management of cancer, and to develop relevant experimental models for evaluating these targets.

2. To determine the toxicity, pharmacokinetic and pharmacodynamic profiles and efficacy of putative treatments.

This work will also lead to an increase in the scientific knowledge base in the area of oncology

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Around 1 in 2 people in England develop cancer in their life-time, with cancer now causing around 1 in 4 deaths. This picture is similar throughout the ‘developed’ world. There is thus a clear need to develop new therapeutic agents and strategies for cancer, to continue the progress seen in the past 20 or so years due to the movement towards targeted therapies, with improved survival rates seen for most common solid cancers such as lung, bowel and breast cancer. Work done on this project licence will have clear benefits in terms of identifying compounds with good activity in terms of DMPK properties & efficacy in animal models to progress anti-cancer agents to late-stage preclinical & clinical trials. These benefits are worthwhile

as there is still a need for novel agents & regulations stipulate that in vivo preclinical testing has to be done before the progression of an agent to clinic. What we hope to ensure through good practice in this licence is that the numbers of animals and severity of procedures used is kept to a minimum through good experimental design.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use mice (up to 13,000) and rats (up to 800) 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?

Apart from one procedure to determine MTD which potentially has a Severe category for some of the animal used if a level of compound is exhibits toxicity, all other procedures are of Moderate severity. Once a procedure has finished then the animal will be euthanised by a Schedule 1 procedure.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Although the majority of the work carried out during cancer drug discovery is done *in silico* or *in vitro*, the ultimate goal of the project is to develop agents to treat cancer patients in the clinic. Whilst these analyses give much information about specific interactions, they don't take account of how a drug behaves in a complex system, i.e. is it available in a stable form for sufficient amount of time to interact effectively with the cancer cells and not metabolised to a form that is either inactive or toxic to non-cancer cells, or if a drug target is still accessible when it is subject to physiological control from external systemic factors. Thus it is necessary to evaluate the agent in a fully formed living organism. Therefore some *in vivo* work on experimental animals has to be carried out before progress to the clinic, although through adherence to the 3Rs philosophy and good experimental design, the numbers of animals used is kept to the absolute minimum with the minimal amount of suffering to obtain statistically significant results which will aid progress of an agent into the clinic to treat human cancer patients.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Previous consultation on power analyses with statisticians based on our previous data, and our extensive previous experience, will ensure that the minimum number of animals required for our studies in order to produce a statistically relevant result will be used.

In addition, strategies will be applied to ensure that animal use is kept to a minimum including the following:

- Use of the hollow fibre assay as an intermediate efficacy screen between *in vitro* and xenograft evaluation.
- To reduce control animals required, pharmacodynamic analyses will be carried out in satellite groups along with efficacy experiments.
- Where analytical methods are sufficiently sensitive repeat bleed sampling from the same cohort of animals can be used for plasma bioavailability studies.
- Previous extensive data gathered on tumour implantation take-rates will help guide on using the minimum number of animals to give significantly powerful 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

Mice are the most frequently used species for tumorigenicity and cancer therapy studies and least sentient species of mammal in which transgene technology works reproducibly. Although transgenic techniques are well established in lower species, such as the zebrafish, since therapy will be evaluated in mammals, it is essential that these models are established in the same species. Immunodeficient (‘nude’) mice will be used where cells from a different species (e.g. human, hamster) are being transplanted. In the case of models which have been developed in rats and it would not be possible to transfer to mice (e.g. syngeneic tumours, organ size in the case of surgical manipulations), then rats will be used.

REDACTED