Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2018

Volume 2 (granted between 1st July to 31st December 2018)

PROJECT 1. 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 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	
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 aim of the current programme is to identify the role of blood flow in the formation of heart attack and stroke. In order to do so, we will

- Study the reaction of the inner layer of the blood vessels (the endothelium) with modern genomic techniques that focus on how the switching on or off of certain genes in specific tissues leads to arterial disease. These genomic techniques measure the abundance of all genes that are switched on within a sample of cells. By comparing diseased areas against healthy areas, we determine a set of genes associated with atherosclerosis, termed 'atherosclerotic genes'.

- Develop new computing-based analytical strategies to validate these genomic techniques: the amount of data generated by reading the expression levels of all genes in the diseased and healthy endothelial cells requires a large amount of computing power and highly efficient analysis methods to make full sense of the large volume of data. These tools will be developed alongside the in vivo work we carry out in the group to make full use of all data arising from in vivo experiments

- Identify new bio-molecular targets for imaging and treatment strategies that target specific molecules involved in the progression of arterial disease. This line of work feeds directly into the growing trend in clinical medicine towards molecular imaging and/or molecular medicines, where medicines and imaging agents are designed to selectively target specific molecules that have well-defined roles in the identification/diagnosis and/or progression of 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 direct benefits from this project will be: - identification of new gene networks during development of atherosclerosis. In simpler terms, the various genes that regulate cellular behaviour during the transition from a healthy state to a diseased, atherosclerotic, state interact with each other in a highly complex manner, forming

networks of interaction as opposed to simple (linear) cascades. A good analogy would be to imagine these networks as a spider's web, where each gene is a junction between the crossing strands of the spider's silk. The linear cascade would be like a simple rope ladder, where one rung leads directly to the next. The bio-informatics based approach seeks to identify each of these genes and discern how each of them interacts with all others, resulting in a highly detailed understanding of the disease mechanism at a fundamental molecular level - identification of new epigenetic (miRNA) drivers for atherosclerosis: Following on from the point above, the atherosclerotic genes identified can be switched on or off to differing degrees. Further regulation of the activity of the "switched-on" genes is carried out by another class of molecule, miRNA. Continuing with the spider's web analogy above, disrupting one junction of silk threads would cause geometric changes in the entire web; not just at the site of the broken junction. Translating this mental picture to the gene network, if we were to drastically affect the activity of one or several key gene(s), the entire gene network would be affected and, as a result, the state of the cell as well. Doing this in a population of cells, the progression of disease could be halted or reversed. This is the general principle that would underly miRNA-based treatments or prevention strategies in the future and leads to our final potential benefit: - 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. Wild mice naturally go not develop atherosclerosis. These ApoE-/- mice have a genetic manipulation (a mutation of the ApolipoproteinE gene) that causes them to develop extreme hyperlipidaemia, that is, a high blood cholesterol concentration, which is a major pre-cursor to atherosclerosis in humans. This causes them to develop atherosclerotic plaques. 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 works on the premise that complex systems can be simplified into a set of simple processes that can each be described by mathematical (differential) equations and all interact in a mathematically-known manner. Mathematical descriptions of biology at multiple physical scales from the whole animal down to single cells and single molecules can all be tied together using multiscale computational models, which can then predict the behaviour of those biological processes included in the model. This field 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 as a screening tool 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 reduce 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 (eg: CT, MRI, Ultrasound) 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. As these techniques do no harm to the animal other than side effects of anaesthesia, we can repeatedly image the same mouse over a long period of time, saving on the number of mice needed for our studies. 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 2. NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	The Breeding and Maintenance of Genetically Altered Animals
Key Words	breeding, genetically altered, mutant
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Infectious diseases are still a major cause of death and illness in both high and low income countries and further development of new treatments and vaccines are desperately needed. The mice bred under this licence will allow us to determine the role of specific genes and host defences in resistance to these organisms and aid in the development and evaluation of new drugs and vaccines.

A single Project Licence for this purpose allows effective colony management as personnel with experience in breeding and husbandry of Genetically altered (GA) mice are in full control of the breeding. This has the potential effect of reducing the number of animals produced under one licence rather than producing the same line of animals under several different 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)?

GA mice allow specific manipulation of a gene or gene elements and the subsequent examination of gene activity in a complex physiological environment, and provide a valuable method of understanding the function of particular genes in the development of disease. In this establishment the main aims are to study the biology of infectious disease including immunology, pathogenesis, chemotherapy and vaccination.REDACTED

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

The expected use over a 5 year period is - Mice = 15000

In the context of what you propose to do to 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 genetic alteration, there are no adverse effects expected. The overall level of severity for this licence is mild. However should any mouse show

any unexpected behaviour or signs of ill health the Named Veterinary Surgeon will be consulted. These mice will be used for research under other approved Project Licences.

Application of the 3Rs

Replacement

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

Replacement

During the development of new vaccines and treatments of infectious diseases, the study of the effects of genetic alteration often needs to be addressed as the ‘whole’ animal. Wherever possible, other laboratory based techniques will be used, including cell lines but in many cases meaningful results can only be obtained by the using the living GA mouse.

Reduction

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

Reduction

A centralised facility allows for efficient use of the GA mice produced by controlling the breeding of the mice to match the scientific demand and to allow for the sharing of tissues which will minimise the chance of duplication.

Refinement

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

Refinement

GA mice allow for specific manipulation of a gene or gene elements and provide a valuable method of understanding the function of particular genes in the development of disease. This is considerably more efficient and reproducible than previous techniques (such as antibody mediated cell depletion) and allows us to more closely model the pathology of human infection with these pathogens in mice.

All mice will be housed in individually ventilated cages (to ensure the exposure to environmental pathogens is minimised), in appropriate sized groups in solid floored cages and supplied with adequate bedding and nesting material and environmental enrichment.

All new strains of GA mice that are added to this project are quarantined and health checked before being introduced to the main colony, to check that the high health standards are not compromised.

Health screens are carried out every 6 months to ensure that the mice are in optimal health so that breeding success is ensured and without any adverse effects due to any incurrent disease or infection. This high health standard allows the results of the scientific studies to be reportable and correct, reducing the number of times experiments need to be repeated.

PROJECT 3. NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Epizootiology of Wild Bats
Key Words	Bat, Disease, Public Health, Animal 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;
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 diseases of bats may be of concern to government, because they may harm humans (e.g. rabies) or the economy (e.g. affecting livestock and horses). Surprisingly little is known about the movement and behaviour of bats, the pathology of their diseases, and how these combine to affect the dynamics of disease spread and abundance (their epizootiology) or the risks to society. This project will measure diseases in wild bats in the UK, and undertake basic research to fill critical gaps in our understanding of how bat populations and bat diseases work.

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

Helping government make effective decisions in response to the risks presented by bat-borne disease in the UK (e.g. successful, cost-effective and proportionate) is considered the primary benefit of this work. Of principal concern are diseases such as rabies, which can kill humans. One bat rabies virus is already present in the UK, but there are others which may spread here which would pose greater risks. This project will help us predict the potential establishment and spread of such diseases and help suggest effective responses. As well as producing reports to be used by decision-makers, we intend to publish as much as possible in the scientific literature. This is likely to include descriptions of diseases and their prevalence as well as undescribed aspects of bat population behaviour. This is considered a secondary benefit.

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

This licence is designed to permit us to take samples from wild bats for a range of different types of study. We intend to catch, sample and release bats unharmed back into the wild at their point of capture. All studies will ensure that bats are disturbed as little as possible and most will only be held for a short time. For example, we intend to start a long-term study at one important site (i.e. over many years), and may also

undertake additional projects as these are required, some of which may only last a single season; all connected by the fact that they will be about bats and their diseases, and require the types of samples produced by this licence. Our best first guess is that 1000 sets of samples will be collected by this licence, though the uncertainties of working with wild animals and the requirement to respond to future incidents may change 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?

This project is restricted to taking tissue samples for laboratory analysis; mainly saliva as well as skin and blood samples. As our purpose is to study the natural, unaltered behaviour of wild bats and their diseases, it is important that our work does not provoke any changes by unnecessarily stressing individual bats or affecting their populations. Thus our sampling methods and protocols are designed to be as sensitive as possible and no lasting harms are anticipated. We do not intend to kill any bats as part of this project and once studies are finished they will be allowed to continue their lives in the wild. This produces the least severe category of anticipated harm for the bats i.e. mild. Our biggest challenge is to simultaneously address four realities whilst pursuing this research; (1) bats are difficult to catch and those carrying disease are hard to find, limiting the number of animals that can be shared amongst different studies, (2) some studies require us to continually re-sample bats, year after year, to track individual case-histories, (3) day-to-day bats vary considerably in their sensitivity to disturbance, and workers need to be flexible in how each is treated on a case-by-case basis, and (4) that opportunities or incidents may arise within the 5-year life of this licence that suggest or require new, exciting and important scientific studies. Thus the use of animals here differs substantially from the usual laboratory based approach. We use an accounting framework to manage the cumulative burden we might place on bats across the whole project to ensure that individuals are never over-stressed; by recording and limiting the number and type of samples that can be taken at any single visit, in any one year, or across a number of years. By using a flexible approach to study design and fieldwork we believe we can achieve better quality science whilst validating the distress caused by catching the bat.

Application of the 3Rs

Replacement

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

Replacement

Our purpose is the study of disease in wild bats. We can only understand the natural pathology and dynamics of these diseases in wild populations. Previous work has

suggested that laboratory species are very poor models for the study of bat diseases in bats, and British bats adapt very poorly to captivity making them unsuitable for laboratory use.

Reduction

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

Reduction

Wild bats are hard to obtain and none need to be killed for this work. Thus we can undertake different studies at the same site; integrating the results of one study straight into targeted scientific questions for the next and crucially sharing the same individual bats and the samples they produce. This reduces substantially the number of individuals that would be caught and sampled if studies were conceived and executed independently. Our administrative framework ensures that this tight integration of studies does not produce unintended or cumulative harms.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 includes approaches to improve the experience of bats caught for this work as well as the quality of the science produced for the same amount of harm. We achieve the former by introducing an administrative framework to support the use of very flexible protocols. This permits us to avoid ‘all-or-nothing’ sampling of every bat in the hand and spontaneously shorten protocols in the field if workers find bats to be more sensitive than anticipated (they may be unexpectedly pregnant, or juvenile or underweight), all the while, achieving good science. If they are confident that they will re-catch bats, they might also dilute sampling across numerous visits, substantially easing the burden on the bat at any one time. In addition, our administrative framework also permits us to tightly integrate studies, maximising the scientific return on every bat caught.

PROJECT 4. NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Mouse models of virus and bacteria induced airway disease
Key Words	Virus, Bacteria, Asthma, COPD
Expected duration of the project	5 year(s) 0 months

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

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

Asthma and chronic obstructive pulmonary disease (COPD) are chronic diseases of the lungs. 5.4 million people in the UK suffer from asthma whilst COPD is the third leading cause of death worldwide. How and why asthma and COPD develop and persist is still relatively poorly understood.
As well more mild illnesses such as the common cold, Viral and bacterial infections of the airways cause life-threatening attacks of asthma and COPD. Again, understanding of how these pathogens cause asthma and COPD attacks, and the range of other diseases associated with them is somewhat limited and treatments are in many cases not available or considered inadequate.
This project aims to increase our understanding of the mechanisms underlying asthma, COPD and other viral and bacterial respiratory disease. This will help us to identify, as well as test, more effective 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 potential benefits of the project will be in both advancement of science and development of new drugs. The project will enhance our understanding of immune responses to common respiratory virus and bacterial pathogens, improve knowledge of how these viruses and bacteria interact in co-infections of the airways and examine their roles in the development of asthma and in attacks of asthma and COPD. This information will help us to identify processes which can be targeted by new treatments and provide us with animal models of disease in which initial testing of such new treatments can be undertaken.

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

All experiments will be performed on mice. Over a 5 year period we expect to use no more than 24,000 mice (4,800 per year). This number includes breeding of up to

10,000 genetically altered mice and purchase of up to 14,000 non-genetically altered animals.

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

The maximum severity of procedures is considered moderate. Breeding protocols are considered of mild severity. Infection with some bacteria and viruses such as influenza and respiratory syncytial virus could cause some obvious signs of disease such as lethargy and weight loss. The administration of substances such as viruses, bacteria, or drugs to mice will be done by the least invasive method and where appropriate under anaesthesia. Because we are concerned with respiratory disease, some conscious animals will be made to breath substances which cause them to become breathless, in order to measure their lung function. This will however be transient and animals are expected to recover fully and rapidly from this. All experiments will result in mice being killed humanely, most commonly by overdose of anaesthetic. In the case of breeding procedures, mice may be either used for experimentation as discussed, used for further breeding, or transferred to other researchers for their studies.

Application of the 3Rs

Replacement

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

Replacement

In addition to animals, we perform studies on human volunteers and in cells and tissues donated by those volunteers. Wherever possible studies are performed on human samples. Questions such as those relating to clarification of complex immune system mechanisms often cannot be studied in cells from humans however and studies of people with disease often cannot for ethical reasons supply the types of sample or frequency of sampling required to answer a research question. In these cases we will use mice for our experiments, in which we can make genetic and drug interventions, or manipulate the immune system, allowing us to clarify disease mechanisms and demonstrate cause and effect, with the ultimate goal of developing new, more effective therapies for man.

Reduction

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

Reduction

We will always make sure by surveying the scientific literature that studies are not unnecessarily repeated. Statisticians will be consulted to ensure that the appropriate number of animals is used to ensure statistically, as well as biologically meaningful results. Appropriate negative and positive controls for a given treatment are essential to avoid unnecessary repetition of experiments, but where pilot experiments demonstrate that a control is redundant, study designs will be refined accordingly in future work. Finally, breeding strategies will be optimised to help reduce the number of animals used. All studies will be carried out in a way that enable publishing in adherence with 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 will always make sure by surveying the scientific literature that studies are not unnecessarily repeated. Statisticians will be consulted to ensure that the appropriate number of animals is used to ensure statistically, as well as biologically meaningful results. Appropriate negative and positive controls for a given treatment are essential to avoid unnecessary repetition of experiments, but where pilot experiments demonstrate that a control is redundant, study designs will be refined accordingly in future work. Finally, breeding strategies will be optimised to help reduce the number of animals used. All studies will be carried out in a way that enable publishing in adherence with the ARRIVE guidelines.

PROJECT 5. NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Understanding subtype specific tumour biology
Key Words	Tumour subtypes, Personalized therapy, Systems biology, Stratified medicine, Tumour progression, Metastasis
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Attempts to personalise therapy for patients with pancreatic, colorectal and breast cancers have yet to be successful, in part due to the fact that the biological differences between each patient's tumours have been difficult to define. This project aims to define groups of patients who have similar tumours – not only at the genetic (DNA) level, but also in the metabolites that are fundamental to how cells grow, change over time, respond to their environment, and die. This will help us to tailor specific therapies to individuals in each group and improve their treatment outcomes.

In this project, we will confirm the existence of previously defined subgroups and identify extra subgroups using tumour samples from patients, cell lines and mouse models. Using sophisticated biological and computational analyses, we will identify subgroup-specific genetic changes, metabolites, and their response to various therapies. The accurate and full characterisation of the tumours will lead to the treatment of patients with specific types of tumours with 'best-fit' drugs, and the tests that are needed for this to occur in the clinic. Overall, this proposal will help to identify novel personalized diagnostic and therapeutic strategies for patients with this devastating disease.

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

There is a clear clinical need to match specific patients from pancreatic, colorectal or breast cancer with specific therapies. Current treatments using chemotherapies provide only modest benefit at the cost of significant side effects. This is because: a) these therapies are administered to unselected patient populations irrespective of any other clinicopathological or genetic (DNA) characterisation; b) there is a lack of

clinical tests to match patients to therapies; and c) the cause of these cancers at the DNA levels are relatively poorly defined in pancreatic cancer. In this project, we will derive a more complete understanding of the genetic and other clinical differences in these tumour subsets using integrated computational and experimental biology approach to identify therapies. This approach will identify not only the additional tumour subtypes, but also their accompanying underlying biological abnormalities and the drugs that are likely to be effective. These data will be used clinically to: a) develop diagnostic tests for early detection and classification of tumours; b) develop diagnostics tests for matching appropriate therapies that benefit patients; and c) test promising leads in animal models and subsequent clinical trials.

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

We will use the mouse. The mice that we will use genetically altered to predispose them to various cancers. We will also use immune-compromised and normal mice. The approximate numbers of mice we expect to use during the 5-year project will be approximately between 15,000 to 32,500 that will be determined using statistical and computational methods that helps us to match mouse models closer to patient samples.

In the context of what you propose to do to 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 we do everything possible to minimise adverse effects, these can occur. Possible symptoms include loss of condition, weight loss and effects on organs, e.g. skin or liver. Mice will be checked regularly for signs of ill health. The mice are expected to develop tumours and we will test therapies find if the tumours would reduce. We will use pilot studies to determine any adverse effects if the study will be new. Also, we will use the pilot studies and computational methods to determine the number of animals and reduce them as appropriate.

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 suitable ways to properly model these tumours *in vitro* using cancer cell lines. In order to study tumours that form spontaneously, within a native host environment and at the appropriate time during development, genetically engineered mouse models are the only practical tools available to us. Genetically altered mouse models also allow us to model how the presence of the same mutations found in human cancers affect tumour development and response to therapies. To study the

effect of novel treatments on human tumours, we also use immune-compromised mice that allow the growth of tumour cells from patients. Together, these models are complementary and predict quite well the activity of drugs in the clinic.

Reduction

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

Reduction

Most drug development utilises rodents, so we have a considerable amount of information on existing drugs for comparison with novel therapies. Before any new agent is administered we first perform extensive *in vitro* laboratory experiments to establish the concentrations and exposure time required. Then we test that any novel agents are tolerated in animals at the doses required to give for a predicted efficacious exposure. In addition, we will use our computational methods to predetermine which mouse models match patient profiles, and thereby, minimise the number of animals. Mouse numbers will also be calculated to minimise usage while allowing robust 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

We operate within a very tightly regulated, clean and well administered facility that has an excellent track record for animal care and safety. In all cases we use appropriate anaesthetics, analgesics and procedures to avoid pain, suffering or lasting harm to the animals. When we need to cull them we do so by approved procedures. We have also implemented a real-time, networked database to monitor colony and determine the endpoints earlier to avoid the suffering of the mice. This way we get informed through the databses and get aware of issues with our animals and can respond to them quickly. Additionally, we are refining and replacing our current models with more sophisticated ones that incorporate non-invasive tumour imaging to enable early tumour detection. This will reduce animal numbers, shorten our experiments, and prevent large and potentially debilitating tumours. In this way we hope that efficient and compassionate use of our animals will help us to make a lasting impact on the treatment of these deadly cancers.

PROJECT 6. 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-mesenchymal interaction in tissue regeneration and carcinogenesis
Key Words	colorectal tumours, cancer microenvironment, inflammation, 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):

Adult stem cells are contained within every tissue in the body and are critical for tissue development, maintenance and repair. It is vital that stem cell activity is carefully regulated in health and this is achieved through the action of chemical messages secreted by cells that surround the stem cell (the tumour microenvironment). Cancers arise from loss of control of stem cell division. It has long been considered that this loss of control occurred purely because of the accumulation of faulty genes in the stem cell itself, however recently it has become apparent that derangement in the regulating chemical messages can also result in loss of control of stem cell division resulting in tumour growth. Variation in the importance and influence of these chemical messages in different tissues may influence tumour site predilection

This project will explore the mechanisms involved in stem cell control in health, repair after injury and at the initiation and progression of tumours. We will generate mouse models that mimic the scenario of human cancer patients, including tumours that develop in different areas of the body (e.g in different regions of the intestine). We will also investigate combinations of therapies to see if we can enhance wound healing in the gut which can predispose to cancer formation and/or slow the growth of tumours. The work will focus on cancers of the gastrointestinal tract, which commonly affect human patients.

Specific objectives include

1. To examine the consequences of disrupting cell chemical messages on tissue development and daily maintenance

2. To investigate the supporting cell types and chemical messages important in regulating tissue repair following inflammation and injury, and assessing drug treatments that affect this

3. To investigate the role of chemical messages and the supporting microenvironment in driving cancer initiation and progression

4. To see whether we can use treatments that address the disrupted chemical messages from both the cancer cells themselves and the surrounding supportive cells

Mouse models are necessary as cell culture models cannot replicate the numerous interacting chemical messages that this project aims to study

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

Historically, research looking at the cause of cancer, particularly colorectal cancer, has focused on the accumulation of faulty genes in the cancer cells themselves. This has provided a wealth of information on some of the genes that can lead to cancer and has successfully guided the development of treatments. Recent work, (including our own), has shown that the tumour microenvironment can also have an important role in influencing the behaviour of cancer cells at the earliest stage of tumour formation and that this influence may continue in established tumours, contributing to some of the variable clinical behaviour seen in different tumours. We think this work will further our understanding of the interaction of the tumour cell, the stromal supporting cells and immune cells and establish how these elements interact in tumour initiation, progression and the development of metastasis. We believe this work will help to identify novel drug targets including targeting some signaling pathways that originate from supporting cells, rather than from the cancer cells themselves. By redressing disrupted chemical messages from the supporting cells we believe that this will help standard chemotherapy attack the cancer cells themselves more effectively

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

We expect to use approximately 17500 mice over the 5 years of the project. The majority of these animals will be used for breeding purposes. The numbers required are because the most realistic models of human cancers are those with accumulations of multiple faulty genes. To reproduce these combinations in mouse models requires several generations of breeding. Accurate models of human cancers are required to replicate the tumour biology seen in human patients and to understand the response of tumours to drugs or radiotherapy.

In the context of what you propose to do to 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 mice will develop benign tumours or cancers and these will usually cause predictable symptoms such as weight loss and general ill health. However, in the great majority of cases we can identify those cancers before they cause the

animal any distress and, after humanely killing the animal, we can gain enough information to answer the questions we are asking. For some mice in which the timing of cancer development is uncertain, we will undertake clinical monitoring for evidence of tumours and intervene early to prevent distress. Other mice will undergo induced inflammation or cell DNA damage and/or localised wounding to examine the mechanisms that induce wound regeneration. Daily monitoring of these animals for signs of anaemia and lethargy will prevent excessive morbidity from these interventions. Some interventions and monitoring will involve colonoscopic examination, which will be performed whilst the mice are asleep (anaesthetised) A key aim is to examine the effect of potential wound healing or cancer therapies to develop and test agents that might be used for treatment or prevention of human inflammatory bowel disease or cancer. Administration of these agents may require some mice to have injections, but that will only cause temporary discomfort. Overall, our breeding mice will experience mild severity with animals on experimental protocols experiencing disease of moderate severity. At the end of the work, all mice will be humanely killed.

Application of the 3Rs

Replacement

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

Replacement

Our work is all derived from studies of human cancer patients. We can do some experiments in cancer cell lines derived from human tumours, but these lack certain very important features. We know that the interactions between cancer cells and the supporting normal cells in a tumour are vital determinants of patient prognosis and how well the cancer responds to treatment, and these interactions are very hard to model realistically in cell lines as they lack the complex supporting microenvironmental tissues. Only animal models can faithfully recapitulate these aspects of human disease and mimic a human cancer and, even if not every aspect of tumour growth is the same in humans and mice, there are many similarities that we don’t find in lower animals. For example mice carrying mutations in genes that predispose to bowel cancer in humans also develop multiple bowel tumours that have provided many important insights into the human disease

Reduction

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

Reduction

Our studies are based on the minimum number of mice required to show differences between groups, such as response to a new drug or the effect of a specific gene

fault. These numbers have been generated based on statistics and optimised experimental design. Where possible, we perform initial studies in cell lines or other cell culture systems before turning to mouse models, and will aim to reduce animal numbers used by refining gene induction techniques

Refinement

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

Refinement

Compared with other animals, mice currently provide the only model of human disease that allows routine genetic manipulation, that has sufficient physiological similarity to humans, that comprises a large body of existing resources, and that can be bred with sufficient rapidity. The existing data on likely phenotypes and dosing of mice are far more comprehensive than those for other animals, preventing unnecessary animal use. Even when we cannot be certain of what tumours, if any, a mouse will develop, there are precedents that will allow us to anticipate when phenotypes will develop and whether they could cause the animal any distress. If necessary, pilot experiments will be perform on small numbers of mice in order to determine windows where research can be performed successfully before distress develops. All animals will be monitored daily and any mouse with evidence of distress will be humanely killed. Wherever possible, we shall humanely kill mice before they show any signs of distress.

We shall further refine our experiments using specific techniques, including

- use of anaesthesia for any procedure expected to cause more than momentary distress

- the use of aseptic technique for all procedures

- using targeted and/or inducible mutations so as to minimise ill effects outside the window of study

- use of the most relevant tumour induction methods to answer the experimental question including localised recombination methods

- minimising the delivery of agents to promote or retard cancer growth and using non-invasive methods wherever possible

PROJECT 7. 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	Biological mechanisms of cardiovascular disease
Key Words	Cardiovascular disease, atherosclerosis, heart failure, GWAS
Expected duration of the project	5 year(s) 0 months

Purpose of 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 goal of the project is to investigate the mechanisms of cardiovascular disease such as heart failure, and the causes of heart attacks and blockages of blood vessels by fatty deposits. Cardiovascular disease is the largest cause of death in Western societies, and is also emerging as a major health problem in developing countries. New strategies to prevent or reverse cardiovascular disease are needed to help decrease death from this condition. In the last 20 years, much has been learnt about the mechanisms that regulate the health of blood vessels and how to decrease levels of bad fats in the body. Although fat lowering therapies have been very successful, there are still a significant number of people for whom these treatments are not effective. Hence, there remains a pressing need to identify novel key factors in cardiovascular disease. It is only with the identification of novel pathways which alter the progression of cardiovascular disease that we will be able to develop new treatments for this condition.

It has recently been established that multiple cardiovascular diseases have similar cellular mechanisms which drive the progression of these diseases. For example certain types of white blood cells are responsible for the progression of atherosclerosis, aortic aneurysm formation and poor recovery after a heart attack. In this licence we will investigate novel pathways in multiple cardiovascular models, and by using this methods we will greatly improve our understanding of common cellular pathways across multiple conditions. This is important as patients frequently present with more than one cardiovascular condition however, at the moment we have little information about how these conditions interact with each other. Increasing our understanding of these interactions will help pave the way for better treatment strategies.

It is now recognised that certain conditions such as diabetes worsen cardiovascular disease. In addition, other conditions such as the development high blood pressure during pregnancy (pre-eclampsia) have now been recognised to significantly increase the likelihood of both the mother and child developing cardiovascular

disease later in life. The mechanisms by which these co-morbidities alter the development of cardiovascular disease is not well understood. This lack of understanding of the mechanisms underlying these conditions makes it very difficult to develop treatments for them. In this program of work, we will also investigate how our novel pathways alter cardiovascular disease development in the presence of these additional risk factors.

The major aim of this project is to identify new and better targets for the treatment of cardiovascular diseases. Specifically, the objectives of the licence are:

1. To test the role of novel genes in the development of cardiovascular disease.
2. To characterise the cellular mechanisms by which these genes work
3. To investigate how these genes interact with factors which are known to increase the change of development heart disease such as diabetes
4. To investigate how these genes alter the risk of development complications during pregnancy such as pre-eclampsia and how this affects the cardiovascular health of both mum and offspring.

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

This project will advance our knowledge of factors which contribute to the initiation, progression and regression of cardiovascular diseases, and has the potential to identify new therapeutic targets for future prevention and treatment. Specifically this Project will address: (1) In the short/medium term, it will provide the information on how changes in the vasculature, inflammation and cardiac function are related to disease progression in cardiovascular disease. (2) In the longer term, it will pave the way for novel pharmacologic, genetic or molecular approaches to prevent or reduce cardiovascular disease (3) Help us to identify and manage people at risk from vascular disease complications such as preeclampsia A continued clinical need for prevention and treatment of cardiovascular disease may be addressed in future by identifying new therapeutic targets through better understanding of known biological pathways, and by identifying entirely novel pathways that were not hitherto recognised to contribute to cardiovascular disease pathogeneses.

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

All work will be carried out in mice. In order to ensure that the minimum number of animals are used in each experiment power calculations will be carried out prior to the start of the experiment to establish appropriate sample sizes to be set. For the majority of experiments a significance level of 5% with 80% power will be used to establish statistical significance. When possible experiments will have a factorial design to allow maximum information to be obtained for minimum input and good laboratory practice will be introduced to avoid bias such as randomisation of

treatment and blinded assessment of outcomes. It is estimated that approximately 30000 animals will be used in the life time 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?

Most protocols on the licence are of a moderate severity. There are five severe protocols on this licence. However, it is anticipated that the vast majority of the animals on this licence will experience mild procedures, with a small number undergoing more invasive procedures. We expect adverse effects to be minimal based on our extensive experience and our continuing commitment to animal welfare and application of the 3Rs. In order to answer the aims set out in this licence we will need to use genetic technologies to generate transgenic models of cardiovascular disease. A large proportion of the animals will be used to generate models of cardiovascular disease such as atherosclerosis and hypertension. As is typical with humans it is expected that this will be asymptomatic. Some mice will be generated to model more severe forms of heart disease such as heart failure. As with humans these models can cause adverse effects such as lethargy and discomfort. As we are interested in the processes that initiate cardiovascular disease we will aim to collect data from the majority of mice before these symptoms become apparent. Disease progression may be monitored using non-invasive imaging and measurements of cardiac function which may be conducted under anaesthesia. Some mice may be generated that are prone to sudden death due to rupture of a major blood vessel. As with humans this will result in a very fast death with the mouse losing consciousness within minutes of the rupture. As we are interested in the mechanisms which precede this event we will aim to collect data before this happens. If we observe this adverse effect in certain strains of mice we will use non-invasive imaging such as ultrasound to help us identify mice at risk of sudden death. At the end of these experiments the animals will be humanely killed and tissues collect for biochemical and histological analysis. During our studies, we will use blood samples collected from blood vessels close to the skin to look at factors circulating this blood. This will help us to monitor factors, which we know affect cardiovascular disease such as levels of fat or sugar in the blood. We will also look at the number of white blood cells in the blood, which we know have an impact on cardiovascular disease progression. In addition, we may look for other molecules, which we think, might be able to predict the progression of cardiovascular disease. We will be very careful to ensure that we only take very small blood samples for mice and when we need to take more than one blood sample for example in long term studies that leave enough time between samples for the mouse to fully recover. In addition, we will use non-invasive imaging methods such as ultrasound and MRI to monitor disease progression. Some animals will receive multiple imaging sessions in order for us to monitor disease progression over a long period. For example, mice may have an echocardiography to monitor heart function every month from 3-9 months of age. We will always ensure that all mice are fully recovered and back to normal for their first imaging session before they

have another imaging session. In some studies we may administer substances to alter certain functions which are known to effect the progression of cardiovascular disease such as drugs to alter the response to inflammatory cells. Where possible these drugs will be administered in the food, however, for some drugs we may use injections. When we need to give repeated doses of a drug for longer periods we will implant a small drug delivery device under the skin. As observed with humans consumption of a high fat and or high sugar diet leads to high levels of fat in the blood and can make the body less sensitive to certain hormones such as insulin. We will feed animals high fat and or sugar diets to mimic these changes. These diets are commercially prepared and are formulated to ensure that all other nutrients are balanced and at the correct proportions. Feeding these diets as with humans can result in obesity and can cause the development of type II diabetes. Mice are closely monitored to make sure these circulating sugar levels do not get to high and that obesity does not affect the normal activities of the animal. We will image the progression of cardiovascular disease in some mice using non-invasive imaging methods such as MRI and echo as is commonly done in humans. In almost all cases the animals will be carefully anesthetised to allow these recordings without distress or suffering, in order for us to monitor changes in the circulating factors such as levels of glucose or fats in the blood. This typically involves making a small puncture wound in a blood vessel close to the surface of the skin and the collection of a few drops of blood. Although a small wound is made the animal returns to normal function straightaway. A small number of animals will undergo surgical procedures to allow us to investigate the common causes of cardiovascular disease. One of these models is thoracic aortic banding, a well-established procedure which narrows the aorta and is similar to the problems associated with aortic valve stenosis in humans. To generate this model an incision is made on the chest and a small suture is tied around the aorta to decrease the diameter which results in a gradual thickening of the heart wall. In another model we will tie off a coronary artery on the surface of the heart which will result in a heart attack. As before this is carried out by making an incision in the chest of the mouse. After surgery all animals are given analgesia, are placed in a warm environment and are monitored closely. In most cases animals recover from the surgical procedure within 48h. After surgery the major adverse effect that could occur is heart failure which results in weight loss, reduced mobility and breathlessness. The combination of close monitoring and sensitive imaging techniques means we can identify mice which are entering heart failure before any symptoms become apparent, enabling us to keep distress to a minimum. We will also use surgery to model cardiovascular surgical intervention used in humans such as bypass grafting and angioplasty to look at how genetic interventions alter the outcomes of current surgical treatments for cardiovascular disease. In these studies a vein or artery is grafted into another blood vessel or the vessel has an angioplasty wire inserted to mimic the damage cause by this intervention in humans. Some discomfort is associated with the surgical interventions. Animals will be monitored regularly and surgical discomfort will be alleviated by analgesia. At the end of these

experiments all of the animals will be humanely killed and tissue collected for biochemical and histological analysis. In a very small number of studies, mice may have a combination of surgical models. In some mice, we may implant a device, which allows us to monitor the blood pressure and heart function of the mouse remotely 24h a day. Once this is implanted, the mouse is returned to its own cage This enables us to carry out very detailed analysis of cardiovascular function in a mouse, which is not restrained and critically allows us to look at cardiovascular function when the mouse is asleep and awake. Once the mouse is fully recovered it may under go a second surgery. For example implanted with a device which delivers a certain amount of drug throughout the day. This method means that we do not need to handle the mouse daily to administer the drug. We will use drugs which we think will be beneficial at lowing blood pressure or we may administer drugs which we know when produced by the body in large amounts cause cardiovascular disease such as angiotensin II. Some discomfort is associated with the surgical interventions. Animals will be monitored regularly and surgical discomfort will be alleviated by analgesia. At the end of these experiments all of the animals will be humanely killed and tissue collected for biochemical and histological analysis.

Application of the 3Rs

Replacement

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

Replacement

Cardiovascular disease is a complex interplay between metabolic and inflammatory mechanisms acting in numerous systems such as the vasculature, nervous system and the heart. Despite advancements in computer modelling, *In vitro* cell based systems and the use of clinical studies in patients with cardiovascular disease, these methods are still unable to fully model the complex biological processes in cardiovascular disease. Hence the use of animals is unavoidable if important biological questions about this condition are to be addressed.

We have strong collaborative links with clinicians and now have routine access to blood and tissue samples to isolate inflammatory cells, vascular tissue (e.g. sample of atherosclerotic plaques and aneurysms), and cardiac myocytes from patients with cardiovascular disease. This enables us to investigate the relevance of cellular pathways and individual genes in cardiovascular disease tissue. However, these samples cannot tell us how important these mechanisms are for the initiation and progression of the disease.

Where possible we have established cell based assays to test the role of genes implicated in cardiovascular disease and potential therapeutic strategies in place of in vivo models. We have created cell based models that have been altered so we

can change the expression of our genes of interest and we routinely utilize siRNA as a method to investigate consequence of loss of function of our genes of interest. Cell lines have been useful in establishing mechanism of action e.g. assays to establish interactions between inflammatory cells and endothelial cells. However, cell-based studies cannot address the impact of our manipulations on In vivo disease initiation, progression or regression.

Alternative animals such as Zebra fish have been considered and although these species have proven valuable in certain aspects of cardiovascular research such as angiogenesis and heart repair, they still have significant limitations as models of cardiovascular disease biology. For example, although Zebra fish can develop hyperlipidaemia at this time there are no Zebra fish models of atherosclerosis. Although Zebra fish are an excellent model system for heart regeneration post myocardial infarction this does not make them a suitable model for investigation the role of candidate genes in myocardial infarction pathology. In addition, although Zebra fish can model isolate pathologies associated with cardiovascular disease they cannot recapitulate the multi-factorial nature of cardiovascular disease

Reduction

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

Reduction

The majority of animals are used for breeding or in mild/moderate procedures; approximately 85% of animals. We will manage animal breeding carefully to reduce animal numbers to the minimum required for our phenotyping experiments. We hold weekly lab meetings where we critically review animal usage including the estimated need for animals in the coming months. This enables us to ensure that animal over breeding is kept to a minimum. We work as a team to ensure that maximum use of all available tissue is made from each animal. Power calculations will be carried out prior to the start of the experiment to establish appropriate sample sizes to be set.

We invest in new technologies which require smaller sample sizes and enable us to derive more data from one sample. We have established new technologies which enable us to reduce the use of animals. For example we have recently purchased a mitochondria analysis machine (Seahorse) which require much smaller sample sizes and allows the analysis of 96 samples in parallel. This assay was previously carried out using a Clark electrode where samples are analysed individually with large volumes of mitochondria per assay required. The increased number of replicates has decreased data variability and reduced sample size. It also enables us to investigate multiple conditions at once.

Recruitment and retention of inflammatory cells is a key step in multiple cardiovascular disease pathologies. We have previously used immunohistochemistry

to assess inflammatory cell infiltration in cardiovascular disease. Although immunohistochemistry can give valuable data about location and subtype of inflammatory cells present in that aorta it provides only a snap shot of the aorta and as such has large statistical variability. We have developed a method of aortic and heart tissue digestion, which allows quantification of inflammatory cell content in the whole aorta and heart using flow cytometry thus reducing the number of animals needed in each group.

We have optimised protocols to allow multiple tissues from one mouse to be utilized for multiple assays for example for the isolation of primary vascular smooth muscle cells, macrophages and endothelial cells which means cells, can now be collected from multiple tissue sites allowing multiple researchers to utilize the tissue from one 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

The disease models in this licence are already well established in our laboratory and we have worked hard to optimise animal welfare per- and post-operatively. Detailed protocols have been written in collaboration with other groups who use these techniques in order to ensure best practice. We constantly look for refinements and replacements that we can adopt in our studies. This includes literature searches to check for refinements and possible replacements. This also includes frequent communications with collaborators and other scientists to establish if they have any refinements that would be applicable in our models.

We continue to develop new imaging techniques in rodents to increase sensitivity and decrease variability in our models. We are currently optimising a new imaging method (high resolution μCT) to image atherosclerosis. Traditional methods only give a 2D analysis of atherosclerosis, giving only a snap short of atherosclerosis at a few anatomical locations. μCT enables us to image the volume of atherosclerosis in the whole of the aorta, decreasing the high variability associated with current quantification techniques. It also allows us to investigate location specific changes in atherosclerosis which allows us to look at how changes in blood flow patterns alters the development of atherosclerosis.

We are interested in the biology which causes heart disease as such we are most interested in what happens before animals develop symptoms of heart disease. We use non-invasive imaging such as ultrasound imaging of the heart to monitor the

progression of heart disease. This means the vast majority of our animals will never develop symptoms of heart disease.

As with humans certain strains of mice when fed a high fat diet will develop atherosclerosis. We frequently fed our mice a high fat diet to cause the development of atherosclerosis. Our extensive experience with the model enables us to feed mice for the minimal period of time to cause the required atherosclerotic plaque features. Unlike the diet the mice are normally given high fat diet is softer, hence mice are given wooden sticks and additional chewing material to keep their teeth in a good condition. In addition due to the grease nature of the diet cages are changed more frequently and different types of bedding used to make sure the mice do not develop grease coats.

We have many systems in place to minimise any suffering animal's experience during procedures. They are housed in a controlled environment with optimal lighting, heating, food and with appropriate companions. When we need to administer drugs or agents to mice we always pick the least invasive route, for instance in their food and drinking water or injections just under the skin. We will also use the lowest effective dose when this is known. If we are using substances for the first time we will carry out pilot tests on typically 5-6 animals, always starting with the lowest dose and only increasing when necessary. Anaesthesia and analgesia will be used for any procedures where the pain or discomfort could last longer than a few seconds. All surgery is carried out in a dedicated surgical room under sterile conditions. Animals are always given analgesia before and after surgery and are monitored closely after surgery.

PROJECT 8. 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	Gut/Commensal Bacterial Interactions in Health and Disease
Key Words	Microbiota, Autoimmunity, Infammation, 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.
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 bacteria which inhabit the gastrointestinal (GI) tract, collectively referred to as the microbiota, control many aspects of human health and physiology. They assist in regulating many host metabolic and immunological processes that can either be beneficial or negative to their host. In this way altered bacterial communities in the GI tract have been associated with many human diseases especially those caused by activation of the immune system. In contrast, defined bacteria with beneficial characteristics have been proposed as therapeutics and have been employed successfully in animal models of disease.

A variety of *in vitro* (cell based) and *in silico* (computer modelling) techniques can also be employed to identify new bacterial species and strains with potential to prevent and treat human disease. Although this platform of techniques is useful and important, none can fully recapitulate the complex interplay of pathways responding to bacteria *in vivo* and therefore mouse models are critical for efficacy studies and further understanding of the mechanistic basis of bacterial therapeutic function.

The objectives of this project are as follows:

1. To treat mouse models with bacterial strains or the novel bioactive products they produce to develop new ways of treating human disease.

2. To understand the mechanisms by which these bacterial strains affect the host 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)?

The short term benefits of this licence are that our results will increase the understanding of how the immune system responds to particular strains of gut resident bacteria and enhance the understanding of how the presence of specific bacteria may influence the development and severity of autoimmune disease. Overall, our long term aim for this project is to identify new live biotherapeutic

products isolated from the human microbiome with the ability to treat a range of autoimmune and inflammatory diseases, either as a monotherapy or in combination with existing treatments. This work will also be beneficial to the work of other scientists in a number of disciplines and will hopefully be disseminated in peer reviewed journals and at conferences.

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

We will use mice for this project licence and have estimated that we will use approximately 1000 mice over the period of 5 years. This will allow us to assess how specific immune cells and bacteria influence and regulate a variety of disease states in these models.

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

To treat mouse models with bacterial strains or the novel bioactive products they produce to develop new ways of treating human disease. The majority of experiments will involve the treatment of rodents with bacteria or bacterial products by oral gavage. Oral gavage is the administration of a substance via the oral cavity, using a feeding needle or tube, into the lower oesophagus or stomach. Once introduced, the substance to be dosed is slowly expelled, and the tube is withdrawn slowly. Compared with other methods of oral administration, it is more invasive, difficult and stressful (handling, restraint and introduction of equipment into the animal is required). This is associated with a higher risk of complications, especially if repeated or chronic dosing is neces-sary. However, it is also the most accurate and reliable method for administering substances into the gastrointestinal tract. The mice used under these protocols will at most experience a mild to moderate level of severity and multiple steps are in place to ensure minimum discomfort is experienced by the an-imals. Acclimatisation of the animals to handling in advance, combined with animal facility staff training in competency for manual handling and restraint of animals in the procedure, itself minimises any stress experienced by the animal. Further experiments may involve treatment in a challenge setting e.g. administration of a stimulant to recreate an inflammatory setting. Administration of such substances can take place though a wide variety of routes including, subcutaneous, intravenous, in-tramuscular or intraperitoneal needle injection. Complications associated with these methods can include local irritation, pain, infection and damage to the surrounding tissue. Adverse effects can be minimised by choosing doses based on knowledge of the properties and toxicity of each compound, such that the minimum effective dose required to achieve the aim of the study is used. Generally administrating smaller volumes over multiple injection sites will also minimise adverse reactions. The number of animals used will always be the minimum compatible with sufficient statistical power to generate meaningful results. At the end of the procedures mice will be killed by a home office approved method.

Application of the 3Rs

Replacement

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

Replacement

We are committed to identifying and developing the latest *in vitro* and *in silico* systems for the identification and characterisation of human gut resident bacteria. However, at present these systems have significant limitations which prevent them from fully replacing animal models. In particular, they lack faithful recapitulation of the complex ecological niches represented by the microbiota *in vivo* or the interaction between bacteria and the host at the gastrointestinal interface. This project will provide mode of action insight into how bacteria exert beneficial or negative effects on mammalian systems, which will allow us to further refine our in vitro assays. We will continue to develop *in vitro* assays and maintain up to date knowledge of available technologies in an attempt to replace animal use wherever possible.

Reduction

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

Reduction

The numbers of mice used in experiments will be kept to a minimum by optimal experimental design and the animals used will be bought in from reputable breeding establishments. The size of experimental groups will be decided after consultation with a statistician and we will continue to consult with statisticians for optimisation of the experiments throughout the life of the licence.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 a model system in this project because they have an immune system which is sufficiently similar to humans. Mechanisms related to the bacterial interaction with the host have primarily been investigated already in rodents and they are the industry standard for route to clinical trial.A significant component of the work proposed in this project involves the identification of biomarkers indicative of both health and disease states. For example, we aim to identify markers with prognostic value, diagnostic value or indicative of protective/therapeutic responses. In this way,

we hope to offer refinements to current -methodology whereby endpoints can be reached prior to onset of clinical symptoms.

PROJECT 9. 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 ecology of seabird migration and foraging
Key Words	stable isotope, migration, seabird, foraging, 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:
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):

Seabird populations are currently declining faster than any other comparable group of birds. Understanding factors influencing these declines requires a detailed knowledge of their foraging and migratory behaviour, which is at the core of my research interests. I use logging devices to track the movements and behaviour of seabirds, and use small samples of feathers and blood to provide information on their diet and sex.

The ratio of stable isotopes (chemical elements with different weight and therefore different properties) can be measured in a small sample of bird feathers or blood to provide important information about movement and foraging behaviour. For example, they can be used to quantify the amount of fish that some birds get by scavenging from fishing boats compared with fish caught naturally, which is important for understanding the impact of the Discard Ban, under reform of the EU Common Fisheries Policy.

Environmental change may not impact all members of the population equally. Sex-specific differences in foraging and migratory behaviour are well known but for species where males and females cannot be identified based on external characteristics, they can be sexed using genetic markers amplified from non-destructively sampled tissues.

The key scientific questions I will address are about seabird foraging and movement ecology, with the emphasis on implementing these in terms of conservation.

What are 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 current research is to inform conservation for seabird populations currently under severe threat – globally, seabirds have declined by ~70% over the period 1950 to 2010. This is of concern since these iconic animals provide an essential function in marine ecosystems – regulating plankton, cycling nutrients and

event helping to offset the impacts of climate change. They also have considerable intrinsic value for people. In the UK alone, we have approximately 8 million breeding seabirds of 25 species and for 8 of these species we have >30% of the global population, providing an important link with the marine realm. Moreover, at one small seabird colony in Scotland the local economy is boosted by ~£750,000 per annum because of visitors to this site. Without effective research-informed conservation, the future of seabird populations is bleak.

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

Northern gannet - ~500 individuals 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?

Birds will be taken from the wild, before being returned promptly following blood/feather sampling, measurements and (in some instances) attachment of a small logging device (s). This may cause short-term stress. To the potential for any deleterious effects, these mild protocols will only be performed on those animals considered suitable (based upon reproductive state, condition and qualitatively assessed stress levels).

Application of the 3Rs

Replacement

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

Replacement

Field-based applied research requires sampling of free-living animals – there are currently no viable alternative model systems available to address the questions at the core of my research.

Reduction

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

Reduction

Previous work reveals a degree of variation in strategies among individuals requiring that we sampling ~100 individuals per annum.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 research in largely confined to species that feed on a combination of anthropogenic and natural foods to enable us to understand better how changes in the availability of these foods might impact upon wild bird populations. We will use stable isotopes of non-destructively sampled tissues (i.e. feathers and blood) to avoid destructive sampling or the use of invasive dietary assessment methods such as stomach flushing.

PROJECT 10.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	Do fish have necks: measuring 3D motion of the vertebrae and axial muscle dynamics in suction-feeding fishes.
Key Words	Muscles, Bones, Spinal column, Fish, Biomechanics
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):

This project will study how muscles and bones work together to give humans and animals a flexible neck, by studying the hidden “neck” of fish. The neck’s importance in humans is starkly illustrated by the pain and discomfort caused by disorders of the neck. A neck allows the head to move three-dimensionally and independently of the limbs and body, and was a major turning point in the evolution of land animals. But currently we know relatively little about how the neck evolved, or how its bones and muscles interact to produce motion. Although fish lack a true neck, their backbone could function as a neck by bending to lift the head upwards as fish open their mouths during feeding. If fish do have a hidden “neck”, it is powered by their body muscles, which extend from head to tail. All muscles have a trade-off between how fast they can shorten and how much force they can produce. But by changing their shape as they shorten, muscles may be able to automatically adjust their speed to produce as much (or as little) force as necessary. I will use new imaging tools to study how these bones and muscles of fishes “necks” work by:

1. Measuring 3D motions of the backbone, head, and shoulder girdle

2. Comparing the shape of the vertebrae along the backbone

3. Measuring how the body muscles shorten and change shape

4. Creating a computer model to predict how muscle shortening controls neck motion.

What 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 change our perspective on the origin of the neck and provide insights into how muscles move our bodies. By linking the anatomy and motion of the backbone in living fish, this study will help us understand how the neck may have evolved in fish-like animals that are now extinct. It may also help fish farmers improve the growth and health of their fish. The information on the shape and

shortening of fish muscles can also be applied to understanding human muscles—and how changes in muscle shape during ageing may impact our health. The digital models, computer animations, and videos from this research will be used in outreach programs at a local museum and science clubs to teach and inspire young scientists.

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

I will use three fish species (temperate, tropical freshwater, and tropical marine) that each have differently shaped “neck” bones. Over the one-year project, I expect to use no more than 30 adult fish.

In the context of what you propose to do to 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 severity level of this study is moderate. Some fish will have a minor surgery, where tiny (1mm or smaller) metal spheres will be implanted under the skin in their muscles and bones. This short surgery will be done with general anaesthesia and pain-relief, and fish will be carefully monitored to ensure they recover quickly and go back to swimming and feeding as normal. These fish may experience brief stress when their tank is moved to a different laboratory to record their bone and muscle motion. To minimize stress, the transport will be short, we will keep all the tank conditions (e.g., water temperature, water cleanliness) the same, and fish will have a chance to rest after the move and get used to the new laboratory. At the end of the experiments, all fish 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 goal of this study is to measure how the spinal bones and muscles of a fish move as it lifts its head during feeding. Measurements of these backbone motions and muscle shortening have never been recorded before in any fish species, so this study could with information from published papers. And there are currently no non-animal models that can predict the complex, three-dimensional motions of the bones and muscles of a fish’s backbone. Larval fish do not have a fully developed skeleton, so I need to use adult animals to understand how the bones and muscles of the neck work to produce motion.

Reduction

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

Reduction

This project will observe bone and muscle motion directly in fish, and will not require statistical tests to answer the study questions. Because of this, I only need to use enough fish to judge how movements vary among fish of the same species (compared to how they vary among fish from different species). Based on previous experience, I will need a minimum of 3 fish from each species to do this. However, not all fish can be trained to feed reliably, which is essential for recording bone and muscle motion during feeding. Therefore, I need to get up to 10 fish of each species, and then select the 3 that feed the most often and can be well-trained. The remaining fish will not experience any suffering or ill 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

I have chosen the three species that represent a wide sample of the different “neck” shapes and motions found in fish: ranging from relatively simple backbones that are used to rotate the head upwards a little bit, to specially shaped backbones that are used for extremely large rotations of the head. For these three species, there is enough known about their feeding and anatomy to plan the study and ensure good measurements, and about their habitat and behavior to care for them in an aquarium environment. We have an excellent facility where these fish will be housed according to their specific needs (for example, temperature, water flow, hiding spots), and their health will be carefully monitored by trained aquarium and veterinary staff.

PROJECT 11.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	Identifying and characterising novel anti-schistosomals
Key Words	Schistosoma mansoni, helminth, vaccine, drug
Expected duration of the project	5 year(s) 0 months

Purpose of 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 goal of our research programme is to identify novel vaccine, chemotherapeutic and immunomodulatory agents useful in combating the neglected tropical disease schistosomiasis. By applying *in vitro*, *in vivo* and *ex vivo* models, we will characterise how selected schistosome biomolecules affect parasite development, mammalian cell phenotypes and host/parasite interactions.

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

There is currently no suitable anti-schistosome vaccine and existing control strategies rely on the effectiveness of a single drug, which has a currently unknown mechanism of action and is incapable of preventing reinfection in endemic areas. The identification and characterisation of immunomodulatory biomolecules provides information relative to the mechanisms schistosomes use to orchestrate long-term survival in infected hosts. This information could be used to direct more effective immune responses during our search for urgently-needed, novel anti-schistosomal drugs or vaccines.

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

The mouse model is the most easily adaptable and appropriate vertebrate definitive host for studying schistosomiasis in the laboratory as it is a fully permissive host, supports the full sexual development of the parasite and produces fully viable, infective larval stages. In addition, the mouse model can easily be genetically manipulated with a wide range of knockout and transgenic lines available. This allows for specific dissection of particular host factors that may or may not be involved in the proposed experimental procedures discussed herein. Finally, the mouse is the most highly tested animal model system (lowest vertebrate group) for determining levels of vaccine-induced protection or effects of chemotherapeutic

treatment in our field. We anticipate using 2450 mice in total throughout the 5-year 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?

Protocol (1) involves the percutaneous or intraperitoneal administration of S. mansoni larvae (obtained from snails) to mice (~1600 mice). In order to generate schistosome parasites of different lifecycle stages for our studies, these organisms must develop and mature in a suitable definitive mammalian host. The expected adverse effect of this protocol will be stress due to handling, irritation due to parasite infection and deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit. Protocol (2) will allow us to assess the immunoprophylactic potential of characterised schistosome biomolecules (~350 animals). Here, we will administer protein or DNA vaccines (intraperitoneal, subcutaneous/intradermal or intramuscular injections) to mice before percutaneously infecting them with schistosomes. At seven weeks after infection, we will assess the efficacy of vaccination when compared to control animals. The expected adverse effect of this protocol will involve stress due to handling, mild discomfort of vaccination/muscle electroporation, irritation due to parasite infection and deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit. Protocol (3) allows us to examine the function of schistosome biomolecules (~500 animals). By using RNA interference (RNAi) or targeted drug treatment, we will be able to generate information critical to our understanding of proteins necessary for intra-mammalian parasite development. Here, in vitro manipulated (using RNAi) parasites will be injected intraperitoneally into mice or infected mice will be treated (intraperitoneally, transdermally or orally) with an anti-schistosomal chemotherapeutic agent. Both of these in vivo manipulations can synergistically be used to assess the importance of key schistosome biomolecules. The expected adverse effects of this protocol include stress due to handling, irritation due to chemotherapy administration or parasite infection and deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit. In all protocols, mice will be subjected to a Schedule one method upon termination of experimental procedures.

Application of the 3Rs

Replacement

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

Replacement

Schistosomes are mammalian parasites and, thus, necessitate a suitable mammalian host for completing studies outlined in our research programme. While we can employ other non-mouse alternatives for some study objectives, this is not possible for *in vivo* determination of novel chemotherapies, vaccines or immunomodulatory agents.

Non-mouse alternatives include *in vitro* parasite culturing manipulations, appropriate *in vitro* and *ex vivo* cell model surrogates to assess how a schistosome biomolecule can affect a mammalian cell, molecular biology manipulation of schistosome genes, comparative genomics/bioinformatics to computationally interrogate schistosome biomolecules, functional genomics to suppress schistosome gene expression, epigenetics to assess schistosome development, biochemistry to assess enzyme activity and proteomics/lipidomics/glycomics to understand the biological nature of key schistosome molecules. All of these non-mouse alternatives will be synergistically used to prioritise schistosome biomolecules prior to performing *in vivo* experiments in the mouse model of schistosomiasis.

Reduction

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

Reduction

To minimise the number of mice necessary to obtain statistically relevant results, power calculations for determining optimal group sizes will be derived, inbred mice will be predominantly used for efficacy experiments and careful planning of all experiments will be performed in consultation with IBERS 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

All animal protocols employed in this license have been carefully developed over the last several decades in laboratories around the world and have been the subject of peer review. Our specific experiences utilizing animal models for the study of schistosome/host relationships have come about from ~25 years of practical experimentation. All procedures use animals obtained from a licensed supplier and all animals are housed in conditions that comply with the Animals Directive Code of Practice (2010/63/EU).

PROJECT 12.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 micro-environment of brain tumours.
Key Words	Brain tumour, immune cells, tumour microenvironment, 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:
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 project aims to study the role of various immune cells during tumour growth in the brain. The immune cells of interest include microglia, which are resident immune cells in the brain. Normally, these cells serve as guardians of the brain and respond immediately to infections and injuries for example. This reaction is essential to remove any abnormalities and to allow brain recovery afterwards. However, during tumour growth in the brain these defensive cells change their behaviour and begin to support the tumour causing it to grow and to spread. The reasons for this are not understood. Furthermore, other immune cells like peripheral macrophages, neutrophils and T cells infiltrate these tumours in addition, generating a very complex microenvironment which further supports tumour growth. Thus, understanding the reasons for this pro-tumoural behaviour of the different immune cells is the essential first step to develop therapeutic strategies to interfere with these cells to finally inhibit tumour growth.

We will study the interactions of immune cells and tumour cells using the zebrafish as a model. Due to the optic transparency of the zebrafish larvae we will be able to observe these interactions in the living animal as they occur in real-time. Work under the current project licence has already shown an immediate response of the brain resident immune cells (microglia) to tumour cells and a variety of direct cellular contacts between these immune cells and the cancer cells. Moreover, we were able to show that these immune cell responses have direct tumour promoting consequences. Using this model, we aim to understand the detailed interplay of different immune cells and the tumour now. Furthermore, due to the good accessibility of the zebrafish larvae for chemicals we can test which drugs influence the interactions of immune cells and tumour cells. Using this strategy, we aim to identify drugs that inhibit the pro-tumoural activity of the immune cells or even induce anti-tumoural activity.

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

Our studies will provide a comprehensive understanding of the different immune cells in the microenvironment of a brain tumour. To understand the regulation of these cells within a tumour provides the basis for the development of future therapeutic interference. Here the obvious aim is to find treatments that interfere with immune cell functions specifically within the tumour environment, instead of applying suppressive treatments for the entire immune systems. Such treatments could become an alternative treatment for brain tumours like glioblastoma, the most common malignant brain tumour, since these tumours still resist standard therapies. Furthermore, our studies will advance the scientific knowledge about the interplay of different immune cells in vivo (in the animal). This is important for the scientific community as the immune cells of investigation here are involved in a variety of other diseases including chronic infections/inflammations and multiple sclerosis for example. Our findings will be made available to other scientists in peer reviewed publications and presentations at scientific conferences.

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

We will use the zebrafish (Danio rerio) for our studies. Adult fish will mainly be kept for breeding whilst most experiments will be performed in larval fish that are between 3 days and 30 days old. A limited number adult fish will be used for experiments. For the duration of the project (5 years) we estimate to keep approximately 22500 adult fish and to use approximately 15000 larvae and 5000 adult fish for experiments.

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

Most adult fish kept for breeding will have a genetic alteration. Generally, this has no deleterious effect on fish health and well-being. Thus, keeping genetically modified zebrafish is does not induce any harm to them. In case a genetic alteration is observed to have a deleterious effect on fish health, these fish will be euthanised immediately and will not be used for our research. Tumour growth will be induced in larval zebrafish by two different methods. The first method is based on transplantation of a low number of human tumour cells into the brain of larval zebrafish and monitoring the responses of the immune cells over the next few days. The transplantation has been optimised over the past years under the current project licence. Nevertheless, additional injuries or infections might occur. Fish will be closely monitored and if abnormalities are observed fish will be euthanised immediately. Injected larvae will be kept alive for one week upon transplantation. During this time the tumour cells do not cause any apparent harm and larval fish behave normally. At the end of the week fish will be humanely euthanized. The second method is based on the activation of specific genes (onco-genes) that lead to tumour growth. These genes are activated in larval fish and tumours can be detected from 1 month of age. These fish might be followed for up to 12 months. The induced tumours appear to be slowly growing and no impact on the welfare of the fish has

been detected. Under the current project licence, fish were monitored until the age of 6 months and did not show any adverse effects. At the end of the experiment fish will be humanely euthanized. To observe the response of immune cells to the tumour and their interplay larval zebrafish might be “live imaged”. For this procedure larval fish will be anesthetised and imaged using a laser scanning microscope. This procedure does not cause any harm to the larval fish and upon completion fish develop normally. To understand the role of a specific immune cell type (T cell) during tumour growth some fish need to be injected into their brain with a substance that causes a mild inflammation. These injections can cause additional injuries of blood vessels for example. Fish will be closely observed upon injections and in case these problems occur fish will be humanly euthanized immediately.

Application of the 3Rs

Replacement

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

Replacement

We aim to study the interactions of cells of the immune system and brain tumours. Since immune cells are very sensitive to any alteration in their environment these studies can not be done in cell culture. The zebrafish larva offers the unique opportunity to observe interactions of the immune system and tumours in real time as they occur in the living animal. This is essential to understand these processes and to develop future therapies.

Importantly, these interactions can be observed in zebrafish larvae before day 5 of development. During these early time points of development, the larvae are not considered as sentient animals. A large number of our experiments will be performed in larval zebrafish below the age of 5 days and these replace the use of adults.

Furthermore, before doing experiments in zebrafish we will test the suitability in cell culture. Only treatments that show clear results in cell culture will be tested in the zebrafish larva.

Reduction

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

Reduction

To strongly reduce the number of animals we will do a large number of experiments between day 3 and day 5 of development before the larva is considered a protected animal. Nevertheless, a certain number of larvae will have to be studied post day 5 of development. Based on studies under the previous project licence on immune

cells in tumours, we have clear expectations of the variability within our experiments. Based on this, we will follow statistical guidelines for experimental design, which ensures that the minimal number of animals is being used to reach significant results. This will ensure that the scientific knowledge on immune cell function within a tumour is increased, without using excessive numbers of animals. In case of necessary drug treatments pilot studies will be performed *in vitro* (not in an animal) beforehand to further reduce the number of animals.

Refinement

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

Refinement

The optically transparent zebrafish larva is an excellent model for studying the interactions of immune cells and brain tumours in the living animal. In particular the opportunity to image cellular interactions as they occur in real time in the living animal, combined with the possibility of intervening genetically and pharmacologically, make the zebrafish the model of choice for our studies. Furthermore, a lot of tumour research is done on mice. Zebrafish represent an obvious refinement here as they are the vertebrate model of the lowest perceived sentience.

We will minimise animal suffering by performing the majority of experiments in larval zebrafish and only a limited number of adult fish will be used.

To minimise animal suffering zebrafish will be anaesthetised during experimental procedures and will be allowed to recover afterwards. One of our methods to induce tumour growth is the transplantation of human tumour cells into the larval zebrafish brain. Upon transplantation of human cells zebrafish larvae will be kept at 33-35 C to ensure survival and growth of the human cells. These temperatures do not affect welfare of the larvae and have also been found in natural habitats of zebrafish. Furthermore, our work under the previous project licence has confirmed that zebrafish tolerate these temperatures well. Transplantation protocols have been optimised under the previous project licence in a way that only the minimal number of tumour cells required for the experiments is transplanted. Thus, adverse effects due to the injection could be mitigated. In case the injections cause adverse effects (e.g. damage to membranes or vessels), these will become obvious within the first hours post transplantation and larvae will be humanely euthanized to prevent them from experiencing further harm.

To understand the role of a specific subset of immune cells (T cells) during tumour growth in the brain we need to use adult zebrafish as larval zebrafish don’t have

these cells. For these studies we need to inject specific substances into the brain to attract T cells. These injections might damage membranes or vessels in the brain and up to 16% of injected fish might not survive the procedure. In case these problems occur, it happens within the first hours post injection and no additional adverse effects have been detected in the following days upon injection. In order to minimise the harm to the animals, great care will be taken that only optimally fed animals go into experiments, e.g. fish will get extra food during the pre-experimental days. Fish will be checked by the experimenter at least three time a day during the first

three to five days after injection. After that, the wellbeing of fish will be checked at regular intervals during feeding (usually twice, but at least once per day). Fish will be closely monitored for changes in movement, feeding patterns, and other signs of distress.

All animals will be closely monitored during the course of experiments and in case unexpected signs of stress are observed fish will be presented to a Vet immediately.

PROJECT 13.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 normal and malignant haematopoietic cells
Key Words	Leukaemia, stem cells, haematopoiesis
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Acute leukaemia is a type of cancer of the blood forming cells. There are around 5000 cases each year in the United Kingdom and despite modern treatments the great majority of patients still die from their disease. There is therefore a large unmet need for new treatments for patients as well as a need for greater understanding of disease processes which enable the design of such treatments. In particular, acute leukaemias are driven by disease-causing stem cells which must be completely eliminated in order to cure patients. The purpose of this project is to develop greater understanding of these leukaemia stem cells and to determine how they differ from normal blood forming stem cells. Through understanding these differences we will be able to identify and develop new treatments to bring into the clinic for the benefit 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)?

Expected benefits include new knowledge about which genes and cellular pathways are most important in leukaemia and discovery of new treatment targets to develop into the clinic for patient benefit in the longer term.

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

2000 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, such as X-ray treatment of mice, blood or bone marrow sampling, or injection of cells and compounds are not associated with significant side effects and are of mild-to-moderate severity. Mice injected with blood cancer cells will, when the disease develops, exhibit signs of disease, such as hunched posture, poor levels of socialising and interaction. Under these circumstances, and whenever else a

mouse displays features of ill health, or at the end of each experiment, mice will be humanely killed using a Home Office sanctioned method.

Application of the 3Rs

Replacement

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

Replacement

Blood-forming stem cells are defined by their ability to make normal or leukaemic bone marrow cells following their transfer in to a second animal. Cell culture experimental systems are insufficient for this purpose because they do not provide the required environment to grow a new tumour or normal bone marrow system This is because blood forming tissue is very complex and involves lots of interactions between many different cell types which cannot be reproduced in cell culture. Without the use of mice, the biology of blood forming cells cannot be studied properly.

Use of mouse models of leukaemia is important for a second reason. One of the problems of experiments using human leukaemia cells is that the cells vary from person to person making it difficult to study of the effects of specific mutations. By contrast, mouse models of human leukaemia enable investigation of the biological effects of specific mutation in a controlled and informative way.

Reduction

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

Reduction

Use of mice will be minimised by (i) making use of cell culture model systems wherever possible, (ii) using imaging systems or bone marrow sampling to follow disease development in real time (rather than culling groups of mice at certain time points), (iii) careful experimental design with the help of a statistician, (iv) using pilot experiments before a full scale experiment, (v) using protocols for each experiment which include the objective, interventions, numbers of animals and analysis method (reviewed in every case by the licence holder REDACTED for experimental rigour and the 3Rs) and (vi) freezing down in multiple aliquots of leukaemia samples thus eliminating a requirement for continued production of cohorts 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

Mice have been chosen for the study because they represent the least sentient species from which meaningful experimental data can be generated, while exhibiting considerable genetic and biological similarities to humans with regard to their blood forming system. Only a mammalian blood cell generation model system has the potential to accurately mimic both the anatomy and complex cell biology, including microenvironmental interactions, of human normal and leukaemic blood cell generation. Furthermore, there is considerable experience in the wider scientific community regarding the use of mice as a model system for human diseases of the blood and many reagents exist for the phenotypic characterisation of mouse cells.

The techniques used have been carefully evaluated to minimise distress to the animals. Mice used in surgical procedures will be treated with anaesthesia, analgesia and post-operative rehydration by subcutaneous injection, followed by careful observation. In other areas, irradiation doses will be administered at a level sufficient to induce bone marrow suppression but no other long term impact; bone marrow injections and aspirates will be not be performed routinely, only where the scientific justification is high; and in studies that result in the initiation of leukaemia, mice will be closely monitored for health status and killed by a Home Office approved method when signs of ill health are displayed.

PROJECT 14.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 microenvironment in organ homeostasis and disease
Key Words	Liver regeneration, tissue repair, 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:
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):

Liver disease in the UK is increasing in prevalence. Currently the only treatment for end stage liver disease is liver transplantation. As a result, the number of livers available for transplantation is much lower than required. The aim of this work is to find out 1. How liver disease occurs, 2. How the liver repairs itself following injury and 3. What happens when regeneration goes wrong: does it lead to liver cancer formation? By understanding these processes we can design drugs to specifically target these processes with the ultimate goal of preventing disease and enhancing repair in the liver; thus eliminating the requirement for 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 benefits of this work primarily focus on liver disease and the treatment of such. These includes: 1) the understanding of the adaptive immune system during liver disease and regeneration. 2) Interventions that could promote regeneration. 3) Potential drug targets for chronic liver disease However, many of the processes which occur during liver disease also occur during disease in other tissues so our work in the liver will contribute to disease progression in a range of organs.

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

We will use mouse models of liver injury as our main models. These include genetic modified animals which enables the labelling of specific cell population or the specific ablation of T regulatory cells. Dietary and chemical models of liver injury will be used to simulate different aspects of human liver disease. The planned work will be conducted over five years and we will use a maximum of 8000 animals

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

As we are modelling liver disease, our animals will progressively demonstrate symptoms of the liver disease under the models that simulate chronic liver injury (with dietary or water). For example, weight loss, lack of mobility, become very thin, jaundice. These resembles the symptoms of human chronic liver disease such as liver fibrosis and fatty liver disease. The disease related symptoms will persist during the injury model regime, which range between 2- 6 weeks. In some situations, animals will experience interventions such as injections through intraperitoneal or intravenous, blood sampling from peripheral vein. Animals might experience temporary pain due to the injections but will return to normal behaviour rapidly. The expected level of severity in these liver injury model are categorised as moderate severity. However, we will closely manage these symptoms such as monitoring animal body weight, behaviours, and condition of fur coating to ensure that the animals do not undergo any undue suffering. For animals that undergo intraperitoneal or intravenous injections, extra caution will be taken when performing injections to prevent injury to other organs of the occurrence of haemorrhage. The animals that received liver injury models or ablation of immune cells may experience loss of weight, lack of mobility, and abnormal condition of coat such as piloerection. Animals weight and condition will be monitored frequently, such as: if weight loss exceed 20%, prolonged piloerection, hunched postures, animals will be humanely killed if exceeded the stated severity limit.

Application of the 3Rs

Replacement

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

Replacement

Regeneration in the liver is a complex, multi-staged process in which there are many different cell types interacting with one and other. It is impossible to model such complexity without using animal models. However, most experiments will be carried out in the laboratory at cell level before experimenting on live animals. Throughout the project, we will constantly, seek, review and incorporate alternatives to replace the need for animal studies. For example, in- co-culture experiments to confirm preliminary data, targets, and effect of small molecules/ recombinant protein before for the use *in vivo*.

Reduction

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

Reduction

It is possible to calculate the numbers of animals required for experimentation based on data from previous experiments and results. In all cases we ensure that we have

calculated the minimum number of animals required for the experiment to give us useful data. This approach reduces the animal numbers required, and also reduces the likelihood that the animal experiment would have to be repeated. We also try to develop new models (genetic altered and non-genetic altered) to reduce the number of animal used. For example, we have developed Cas9 expressing cell lines to enable CRISPR-Cas9 genetic screening in vitro to reduce the number of animals used for the programme of work.

For example, we have developed Cas9 expressing cell lines to enable CRISPR-Cas9 genetic screening in vitro to reduce the number of animals used for 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 objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

Our project aims to aim to investigate the effect of dysregulated immune system during liver disease using mouse models. The disruption of the immune system tends to occur during human chronic liver disease, and it is inevitable to use mouse models (both transgenic and non-transgenic) to simulate human chronic liver disease which takes several years to develop in human. We typically use mice for experiments as they model accurately human diseases and organ injuries. Genetic altered mouse model are widely available. We regularly refine the disease models we use to reduce animal symptoms and to improve the effectiveness of our models. For example we are proposing pilot studies with a small cohort to identify the optimal dose for Diphtheria Toxin (DT) administration for our studies to reduce the suffering of animals whilst providing useful information simulating the disease phenotype.

The animal models of chronic liver injury we used in this study (both dietary and transgenic) are well established and published across the field. However, we will perform small scale pilot studies for experiments such as altering the immune system with the FoxP3-GFP-DTR mice. The optimal regime to alter the immune system without triggering systemic autoimmunity will be determined before using this protocol in conjunction with other liver injury model, as we do not intend to trigger systemic autoimmune response in our study The behaviour of animals and signs of discomfort will be monitored throughout the injury regime, we will constant seek improvements on our current protocols through searching published protocols or exchanging knowledge with researchers within the field to reduce discomfort and establish models that are more relevant to human chronic liver disease.

PROJECT 15.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 antimicrobial agents for bacterial pathogens of livestock: light activated CO-releasing molecules
Key Words	Antimicrobial, E. coli
Expected duration of the project	5 year(s) 0 months

Purpose of 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 test the effectiveness of new antimicrobials (CORMs) against pathogens of importance to veterinary and human medicine.

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

The work conducted in this project will increase the knowledge of the mechanisms and effectiveness of these new antimicrobials. If effective, there is potential for a new class of antimicrobials to be taken forward for clinical trials. Due to antimicrobial resistance the list useful antimicrobials is reducing. These new antimicrobials, could increase our arsenal.

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

A pilot study will be undertaken using 2 groups (n = 18) of SPF chicks. Birds will be orally dosed within the first week of hatch with a defined E. coli strain. At 21 days old 1 group will be orally dosed with the antibacterial CORM. At 3 days, 2 weeks and 3 weeks post-treatment 6 birds/group will be killed by a Schedule 1 method and necropsies of the birds performed. Quantitative bacteriology for the dosed strain will be done on spleen, liver and caecal samples obtained post mortem. From previous projects, it is estimated that if pilot studies show promise, up to 300 birds may be used over the course of the project (5 yrs).

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

There is a risk of infection from the colonisation with avian pathogenic E. coli. For initial studies a strain will be used that has been used on numerous occasions in the past with no clinical disease observed. If the treatments appear effective, further studies may use strains with more potential to cause disease. However, birds will be regularly monitored and a score-sheet used to record any symptoms. In the event

where animal welfare appears to be compromised, animals will be euthanized early. At the end of the experiments, all chicks will be euthanized humanely.

Application of the 3Rs

Replacement

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

Replacement

Previous lab work using an insect model (*Galleria mellonella*) has led to a reduction in total protected animal numbers, to be used in this study e.g. toxicity studies were performed in the insect model and cell culture assays.

Reduction

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

Reduction

All experiments will be thoroughly planned in consultation with a qualified statistician to accurately determine the minimum number of animals required to give statistical significance.

Refinement

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

Refinement

Chickens have been selected as they are the prime host for the pathogen under investigation, avian pathogenic *E. coli*. For the initial study a bacterial strain and dose level will be used that has been used on numerous occasions in the past which colonises but is essentially commensal and non-pathogenic in birds. Animals will be monitored closely. In the event where animal welfare appears to be compromised, animals will be euthanized early.

PROJECT 16.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	Exploiting Immunological Tolerance for the Future of Regenerative Medicine
Key Words	Stem cells, Degenerative disease, Regenerative medicine, Allograft rejection, Dendritic 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):

Over the past 80 years, we have witnessed an unprecedented increase in life expectancy. While this may be considered a significant medical success story, extended longevity has brought with it a substantial increase in incidence of chronic and degenerative diseases, such as heart disease, diabetes and Parkinson's disease, which rob those affected of quality of life and productivity. Against this backdrop, stem cells offer the attractive possibility of providing a plentiful source of cell types and tissues to replace those that have become diseased or defective through the natural process of ageing. In particular, recent advances have created so-called 'pluripotent' stem cells that display the unique capacity to differentiate into any one of the ~200 cell types that make up the human body: a single line of induced pluripotent stem cells (iPSC) could, therefore, be used to treat numerous, unrelated diseases. The single greatest barrier to implementing this vision of regenerative medicine is, however, the immune system of the recipient which is poised to reject the replacement tissues. The aim of this project is, therefore, to investigate the viability of a new approach to intervening in rejection which exploits the properties of a cell type, called dendritic cells (DC), which have been shown to guide the immune system to tolerate foreign tissues, thereby establishing a state of 'immunological tolerance'. 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)?

If successful, our approach to tolerance induction may help facilitate the future use of iPSC in regenerative medicine as well as curtailing unwanted immune responses to therapeutic proteins. The lysosomal storage diseases, for example, constitute a significant unmet medical need caused by the absence of specific enzymes that normally clear waste products from cells. Those affected by such conditions rarely survive beyond childhood. Although administration of the missing enzyme can be curative, the immune system perceives the enzyme as foreign and mounts an immune response against it. We therefore propose to exploit our capacity to produce

abundant tolerogenic DCs to intervene in the induction of immune responses to the missing enzyme, thereby establishing a profound state of tolerance. REDACTED

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

Over the course of the next five years, we anticipate breeding a maximum of 17,000 mice in order to obtain all the necessary data to enable us to apply for regulatory approval to begin first-in-man trials. Of these mice, we estimate using approximately 1,000 per annum (5,000 mice in total) to demonstrate robust proof-of-concept.

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

The majority of mice used in this project will be either wild type or genetically altered in ways that will have no discernible effect on either their health or welfare. Future experiments may benefit from the importation of strains of mice in which the genetic modification impacts in predictable ways on their health status, however we currently have no plans to do so. The first experimental model we propose to use involves the grafting of a small portion of tail skin from a suitable donor to the flank of a recipient mouse. Skin grafting does not require an incision through the body wall or sutures and rarely results in any adverse effects beyond the pain and discomfort of the initial procedure which is carefully managed using standard pain relief until the wound has completely healed. The second model involves the implantation of tissues differentiated from iPSC under the fibrous capsule that surrounds the kidney. This site is highly vascularised and supports rapid engraftment whilst also providing a useful readout of rejection. Implantation under the kidney capsule is more invasive, requiring an incision through the body wall to expose the kidney which is subsequently repaired with sutures. Adverse events are likewise rare: occasionally the implanted tissue may grow more rapidly than expected and cause distension of the abdominal wall. The third model will only be used on rare occasions and will involve the immunization of mice with a defined protein antigen using an appropriate adjuvant. Adverse effects are typically transient, such as a localised inflammatory response. Any mice showing signs of pain, suffering or distress in response to any of the models used that cannot be resolved with the help of the vet, will be humanely killed, as will all animals, once the necessary data have been collected.

Application of the 3Rs

Replacement

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

Replacement

The rejection of foreign tissues is a complex physiological phenomenon that cannot be recreated *in vitro* due to the dynamic architecture of the lymphoid organs, where the immune response is first initiated. Although certain features of immune cells *in vitro* may be predictive of their involvement in rejection, *in vivo* models are ultimately required to provide unequivocal proof of our ability to intervene in this process by setting in place a state of immunological tolerance. Nevertheless, we shall continue to replace the use of mice where possible: we have, for instance, introduced *in vitro* techniques to demonstrate the pluripotency of any new iPSC lines we produce, so as to avoid the need for formation of teratomas in recipient mice.

Reduction

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

Reduction

We have identified the greatest source of variability between experiments to be variation between the batches of DC we produce. We shall, therefore, subject each batch to stringent characterisation before embarking on *in vivo* experiments in order to exclude any that do not comply with the expected profile, thereby reducing the need for repetition of experiments. Furthermore, we shall use appropriate power calculations and statistical blocking techniques to maximise the information we are able to gain from each experiment and ensure that the minimum number of mice is used to reach statistical significance.

Refinement

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

Refinement

Our choice of mice is guided by two principles: firstly they represent the lowest vertebrate species whose immune system is sufficiently similar to that of humans to enable us to derive predictive data, and secondly, genetically modified strains and molecular tools are available that permit us to answer fundamental questions about graft acceptance or rejection. We plan to refine our techniques during the course of this project to incorporate any amendments that are likely to be beneficial to the experimental mice, such as the introduction of inhalation anaesthesia, from which they recover
more rapidly, the replacement of Complete Freund's Adjuvant with next generation a djuvants such as TitreMax, known to reduce non-specific inflammation, and the use of appropriate pain relief following surgery.

PROJECT 17.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	Discovery DMPK Studies for New Chemical Entities
Key Words	DMPK, New Chemical Entities, Pharmacokinetics, ADMET, Drug discovery
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):

To provide a complete package of high quality, robust and incisive pre-clinical pharmacokinetic data (what the body does to the drug) using ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicology) studies that will facilitate the rapid identification and selection of the best candidate drugs for further development and avoid any unnecessary and wasteful *in-vivo* tests being applied to inappropriate compounds.

These approaches have been used in the past 20 years by most organisations involved in life-science R&D within UK, Europe and USA.

What are 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 is greater success in clinical trials from more carefully selected compounds and a long-term reduction in the lead-time for a new therapy to reach patients. This will deliver a greater potential to save lives, alleviate suffering and reduce the incidence of adverse effects experienced with existing therapies.

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

For the duration of this licence we will be using rodents only, specifically mice, rats and guinea pigs. For the duration of this licence it is anticipated that we will use 23450 mice, 10350 rats and 160 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?

From non-surgical context, the animals will be dosed with NCE’s and sampled for DMPK data output to meet study objectives. From a surgical context, the animals will be surgical prepared to facilitate sampling (e.g. with Jugular Vein Cannula for blood sampling or Microdialysis probes for localised brain sampling) for DMPK data output

post dosing with NCE's to meet specific study objectives. The expected adverse effects from the non-surgical and surgical models to be used under this licence are a) mild effects associated with the administration of novel compounds, such as hyperventilation and sedation that will not require further intervention or treatment, and b) moderate severity adverse effects due to the animals undergoing surgical procedures, such as pain and discomfort requiring pain relief and fluid replacement. If any adverse effect is observed, which is more than transient, the animals will firstly be treated to alleviate the unwanted symptoms. In cases where the symptoms persist, after consultation with NACWO (Named Animal Care and Welfare Officer) and Vet appropriate measures will be implemented that including termination by schedule 1 method. At the end of all protocols in this licence the animals will be killed humanely 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

Currently, there is still a prerequisite by governmental authorities for new chemical entities (NCE's) to be tested *in-vivo* ADMET studies before first-in-man studies can be started. Also, current *in-vitro* systems lack the complexity of *in-vivo* models and cannot be used as a predictive tool to fully replace *in-vivo* studies. Therefore, animals are still required to assess pre-clinical pharmacokinetics and are an essential part of drug discovery and development research.

Reduction

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

Reduction

In our aim to reduce the overall number of animals used for the duration of this licence, and as a good scientific principle, we always insist that there is a well established scientific rationale for undertaking any *in-vivo* study and that prior *in-vitro* data supports such studies. This careful selection of test compounds from *in-vitro* screening studies ensures that only those compounds with a positive profile for efficacy/potency, physic-chemical properties, metabolic profile and toxicity evaluation will be taken forward for use in regulated procedures in the species of the lowest acceptable order (i.e. rodents).

As part of our commitment to minimise animal numbers, we will design our studies in such a manner that the maximum information can be obtained and where possible

serial samples are taken from the same animal (i.e. by vascular cannulation) so as to negate the need for extra animals or future additional or repeat studies.

The number of animals used for *in-vitro* assays in each experiment will be the minimum needed to provide sufficient cells or tissues. Additionally, we will use the minimum number of animals for the determination of pharmacokinetic parameters so as to achieve statistically relevant data.

In some cases, it may be possible to limit animal numbers by reducing the size of control groups, sharing control groups or using control groups for analysing multiple 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

Non-surgical and surgical rodent models are the research species of choice in drug discovery and development due to their size and substantial amount of literature data already available. These models have been used and validated extensively, and have provided much of our knowledge to date in ADMET studies that are requested for use on this licence to generate the package of high quality, robust and incisive pre-clinical pharmacokinetic data that we aim to provide. They can provide a correlation to humans, such as forming similar metabolites or exhibiting responses to a treatment are much more reflective of humans and in such cases we will ensure the most relevant species are always used.

The choice of animals are also dictated by the governmental bodies as they demand that prior to first-in-man studies, pharmacokinetic data is provided in two species, rodent and a non-rodent, to ensure that the drug is suitable for human use.

We will continuously monitor the literature to implement the latest animal husbandry and environmental enrichment legislation and practices. Furthermore, we will minimise animal suffering by using the most advanced technologies where possible, like non-invasive imaging, and by using appropriate anaesthetics, pain relief and infection controls.

PROJECT 18.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 Production of Antibodies
Key Words	Antibody, Polyclonal, Monoclonal, Immunogen, Antigen
Expected duration of the project	2 year(s) 3 months

Purpose of 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 provide a service for the production of antibodies for both the medical research community and diagnostics manufacturing industry within the UK and Europe. 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 disease and their use as critical tools in the areas of research and medicine, including basic research of cells and their function in disease, diagnostic technology and therapeutic medicine 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)?

Antibodies routinely help scientists to research the function of both healthy and abnormal cells in disease, by the detection of proteins within the cell 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 a clinical environment. This can allow for the rapid diagnosis of life threatening disease and assist in providing clinicians (clinical Scientists and Doctors) with specific information in terms of the most appropriate course of treatment to follow, thus preventing death. The use of antibodies in therapeutics is a fast developing area of medicine, with the use of antibodies as constituents of direct medicine in order to treat various diseases including cancer, auto-immune disorders and infection.

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

Rat 3,500 (5 Years) Mouse 6,500 (5 Years) Guinea Pig 2,500 (5 Years) Rabbit 12,500 (5 Years) Chicken 750 (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. When raising antibodies against DNA, special technology has been developed to do so. This technology involves the use of DNA coated gold particles which are introduced to the animal via bombardment of the skin with pressurised gas. This procedure is carried out under general anaesthesia and has minimal associated effects, which can include slight redness of the skin at the site of inoculation. The production of antibodies against bacteria requires the inoculation of a bacterial liquid(without adjuvant) direct into the blood stream. This methodology can result in the loss of animal body weight and (rarely) the onset of symptoms that appear similar to that of an allergic reaction e.g. laboured breathing, reduced mobility and redness of the eyes with associated light sensitivity. 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. All animals used for the production of antibodies 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 (monoclonal) antibody secreting cells.

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 and Chicken will be made available for the production of antibodies. Selection of a specific species , from one of the above 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 the production of antibodies. 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 the field of antibody production.

PROJECT 19.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 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	
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 aim of the current programme is to identify the role of blood flow in the formation of heart attack and stroke. In order to do so, we will

- Study the reaction of the inner layer of the blood vessels (the endothelium) with modern genomic techniques that focus on how the switching on or off of certain genes in specific tissues leads to arterial disease. These genomic techniques measure the abundance of all genes that are switched on within a sample of cells. By comparing diseased areas against healthy areas, we determine a set of genes associated with atherosclerosis, termed 'atherosclerotic genes'.

- Develop new computing-based analytical strategies to validate these genomic techniques: the amount of data generated by reading the expression levels of all genes in the diseased and healthy endothelial cells requires a large amount of computing power and highly efficient analysis methods to make full sense of the large volume of data. These tools will be developed alongside the in vivo work we carry out in the group to make full use of all data arising from in vivo experiments

- Identify new bio-molecular targets for imaging and treatment strategies that target specific molecules involved in the progression of arterial disease. This line of work feeds directly into the growing trend in clinical medicine towards molecular imaging and/or molecular medicines, where medicines and imaging agents are designed to selectively target specific molecules that have well-defined roles in the identification/diagnosis and/or progression of 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 direct benefits from this project will be: - identification of new gene networks during development of atherosclerosis. In simpler terms, the various genes that regulate cellular behaviour during the transition from a healthy state to a diseased, atherosclerotic, state interact with each other in a highly complex manner, forming

networks of interaction as opposed to simple (linear) cascades. A good analogy would be to imagine these networks as a spider's web, where each gene is a junction between the crossing strands of the spider's silk. The linear cascade would be like a simple rope ladder, where one rung leads directly to the next. The bio-informatics based approach seeks to identify each of these genes and discern how each of them interacts with all others, resulting in a highly detailed understanding of the disease mechanism at a fundamental molecular level - identification of new epigenetic (miRNA) drivers for atherosclerosis: Following on from the point above, the atherosclerotic genes identified can be switched on or off to differing degrees. Further regulation of the activity of the "switched-on" genes is carried out by another class of molecule, miRNA. Continuing with the spider's web analogy above, disrupting one junction of silk threads would cause geometric changes in the entire web; not just at the site of the broken junction. Translating this mental picture to the gene network, if we were to drastically affect the activity of one or several key gene(s), the entire gene network would be affected and, as a result, the state of the cell as well. Doing this in a population of cells, the progression of disease could be halted or reversed. This is the general principle that would underly miRNA-based treatments or prevention strategies in the future and leads to our final potential benefit: - 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. Wild mice naturally go not develop atherosclerosis. These ApoE-/- mice have a genetic manipulation (a mutation of the ApolipoproteinE gene) that causes them to develop extreme hyperlipidaemia, that is, a high blood cholesterol concentration, which is a major pre-cursor to atherosclerosis in humans. This causes them to develop atherosclerotic plaques. 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 works on the premise that complex systems can be simplified into a set of simple processes that can each be described by mathematical (differential) equations and all interact in a mathematically-known manner. Mathematical descriptions of biology at multiple physical scales from the whole animal down to single cells and single molecules can all be tied together using multiscale computational models, which can then predict the behaviour of those biological processes included in the model. This field 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 as a screening tool 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 reduce 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 (eg: CT, MRI, Ultrasound) 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. As these techniques do no harm to the animal other than side effects of anaesthesia, we can repeatedly image the same mouse over a long period of time, saving on the number of mice needed for our studies. 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 20.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 and Maintenance of Genetically Altered Animals
Key Words	breeding, genetically altered, mutant
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Infectious diseases are still a major cause of death and illness in both high and low income countries and further development of new treatments and vaccines are desperately needed. The mice bred under this licence will allow us to determine the role of specific genes and host defences in resistance to these organisms and aid in the development and evaluation of new drugs and vaccines.

A single Project Licence for this purpose allows effective colony management as personnel with experience in breeding and husbandry of Genetically altered (GA) mice are in full control of the breeding. This has the potential effect of reducing the number of animals produced under one licence rather than producing the same line of animals under several different 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)?

GA mice allow specific manipulation of a gene or gene elements and the subsequent examination of gene activity in a complex physiological environment, and provide a valuable method of understanding the function of particular genes in the development of disease. In this establishment the main aims are to study the biology of infectious disease including immunology, pathogenesis, chemotherapy and vaccination.REDACTED

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

The expected use over a 5 year period is - Mice = 15000

In the context of what you propose to do to 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 genetic alteration, there are no adverse effects expected. The overall level of severity for this licence is mild. However should any mouse show

any unexpected behaviour or signs of ill health the Named Veterinary Surgeon will be consulted. These mice will be used for research under other approved Project Licences.

Application of the 3Rs

Replacement

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

Replacement

During the development of new vaccines and treatments of infectious diseases, the study of the effects of genetic alteration often needs to be addressed as the ‘whole’ animal. Wherever possible, other laboratory based techniques will be used, including cell lines but in many cases meaningful results can only be obtained by the using the living GA mouse.

Reduction

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

Reduction

A centralised facility allows for efficient use of the GA mice produced by controlling the breeding of the mice to match the scientific demand and to allow for the sharing of tissues which will minimise the chance of duplication.

Refinement

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

Refinement

GA mice allow for specific manipulation of a gene or gene elements and provide a valuable method of understanding the function of particular genes in the development of disease. This is considerably more efficient and reproducible than previous techniques (such as antibody mediated cell depletion) and allows us to more closely model the pathology of human infection with these pathogens in mice.

All mice will be housed in individually ventilated cages (to ensure the exposure to environmental pathogens is minimised), in appropriate sized groups in solid floored cages and supplied with adequate bedding and nesting material and environmental enrichment.

All new strains of GA mice that are added to this project are quarantined and health checked before being introduced to the main colony, to check that the high health standards are not compromised.

Health screens are carried out every 6 months to ensure that the mice are in optimal health so that breeding success is ensured and without any adverse effects due to any incurrent disease or infection. This high health standard allows the results of the scientific studies to be reportable and correct, reducing the number of times experiments need to be repeated.

PROJECT 21.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	Epizootiology of Wild Bats
Key Words	Bat, Disease, Public Health, Animal 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;
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 diseases of bats may be of concern to government, because they may harm humans (e.g. rabies) or the economy (e.g. affecting livestock and horses). Surprisingly little is known about the movement and behaviour of bats, the pathology of their diseases, and how these combine to affect the dynamics of disease spread and abundance (their epizootiology) or the risks to society. This project will measure diseases in wild bats in the UK, and undertake basic research to fill critical gaps in our understanding of how bat populations and bat diseases work.

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

Helping government make effective decisions in response to the risks presented by bat-borne disease in the UK (e.g. successful, cost-effective and proportionate) is considered the primary benefit of this work. Of principal concern are diseases such as rabies, which can kill humans. One bat rabies virus is already present in the UK, but there are others which may spread here which would pose greater risks. This project will help us predict the potential establishment and spread of such diseases and help suggest effective responses. As well as producing reports to be used by decision-makers, we intend to publish as much as possible in the scientific literature. This is likely to include descriptions of diseases and their prevalence as well as undescribed aspects of bat population behaviour. This is considered a secondary benefit.

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

This licence is designed to permit us to take samples from wild bats for a range of different types of study. We intend to catch, sample and release bats unharmed back into the wild at their point of capture. All studies will ensure that bats are disturbed as little as possible and most will only be held for a short time. For example, we intend to start a long-term study at one important site (i.e. over many years), and may also

undertake additional projects as these are required, some of which may only last a single season; all connected by the fact that they will be about bats and their diseases, and require the types of samples produced by this licence. Our best first guess is that 1000 sets of samples will be collected by this licence, though the uncertainties of working with wild animals and the requirement to respond to future incidents may change 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?

This project is restricted to taking tissue samples for laboratory analysis; mainly saliva as well as skin and blood samples. As our purpose is to study the natural, unaltered behaviour of wild bats and their diseases, it is important that our work does not provoke any changes by unnecessarily stressing individual bats or affecting their populations. Thus our sampling methods and protocols are designed to be as sensitive as possible and no lasting harms are anticipated. We do not intend to kill any bats as part of this project and once studies are finished they will be allowed to continue their lives in the wild. This produces the least severe category of anticipated harm for the bats i.e. mild. Our biggest challenge is to simultaneously address four realities whilst pursuing this research; (1) bats are difficult to catch and those carrying disease are hard to find, limiting the number of animals that can be shared amongst different studies, (2) some studies require us to continually re-sample bats, year after year, to track individual case-histories, (3) day-to-day bats vary considerably in their sensitivity to disturbance, and workers need to be flexible in how each is treated on a case-by-case basis, and (4) that opportunities or incidents may arise within the 5-year life of this licence that suggest or require new, exciting and important scientific studies. Thus the use of animals here differs substantially from the usual laboratory based approach. We use an accounting framework to manage the cumulative burden we might place on bats across the whole project to ensure that individuals are never over-stressed; by recording and limiting the number and type of samples that can be taken at any single visit, in any one year, or across a number of years. By using a flexible approach to study design and fieldwork we believe we can achieve better quality science whilst validating the distress caused by catching the bat.

Application of the 3Rs

Replacement

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

Replacement

Our purpose is the study of disease in wild bats. We can only understand the natural pathology and dynamics of these diseases in wild populations. Previous work has

suggested that laboratory species are very poor models for the study of bat diseases in bats, and British bats adapt very poorly to captivity making them unsuitable for laboratory use.

Reduction

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

Reduction

Wild bats are hard to obtain and none need to be killed for this work. Thus we can undertake different studies at the same site; integrating the results of one study straight into targeted scientific questions for the next and crucially sharing the same individual bats and the samples they produce. This reduces substantially the number of individuals that would be caught and sampled if studies were conceived and executed independently. Our administrative framework ensures that this tight integration of studies does not produce unintended or cumulative harms.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 includes approaches to improve the experience of bats caught for this work as well as the quality of the science produced for the same amount of harm. We achieve the former by introducing an administrative framework to support the use of very flexible protocols. This permits us to avoid ‘all-or-nothing’ sampling of every bat in the hand and spontaneously shorten protocols in the field if workers find bats to be more sensitive than anticipated (they may be unexpectedly pregnant, or juvenile or underweight), all the while, achieving good science. If they are confident that they will re-catch bats, they might also dilute sampling across numerous visits, substantially easing the burden on the bat at any one time. In addition, our administrative framework also permits us to tightly integrate studies, maximising the scientific return on every bat caught.

PROJECT 22.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 virus and bacteria induced airway disease
Key Words	Virus, Bacteria, Asthma, COPD
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Asthma and chronic obstructive pulmonary disease (COPD) are chronic diseases of the lungs. 5.4 million people in the UK suffer from asthma whilst COPD is the third leading cause of death worldwide. How and why asthma and COPD develop and persist is still relatively poorly understood.
As well more mild illnesses such as the common cold, Viral and bacterial infections of the airways cause life-threatening attacks of asthma and COPD. Again, understanding of how these pathogens cause asthma and COPD attacks, and the range of other diseases associated with them is somewhat limited and treatments are in many cases not available or considered inadequate.
This project aims to increase our understanding of the mechanisms underlying asthma, COPD and other viral and bacterial respiratory disease. This will help us to identify, as well as test, more effective 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 potential benefits of the project will be in both advancement of science and development of new drugs. The project will enhance our understanding of immune responses to common respiratory virus and bacterial pathogens, improve knowledge of how these viruses and bacteria interact in co-infections of the airways and examine their roles in the development of asthma and in attacks of asthma and COPD. This information will help us to identify processes which can be targeted by new treatments and provide us with animal models of disease in which initial testing of such new treatments can be undertaken.

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

All experiments will be performed on mice. Over a 5 year period we expect to use no more than 24,000 mice (4,800 per year). This number includes breeding of up to

10,000 genetically altered mice and purchase of up to 14,000 non-genetically altered animals.

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

The maximum severity of procedures is considered moderate. Breeding protocols are considered of mild severity. Infection with some bacteria and viruses such as influenza and respiratory syncytial virus could cause some obvious signs of disease such as lethargy and weight loss. The administration of substances such as viruses, bacteria, or drugs to mice will be done by the least invasive method and where appropriate under anaesthesia. Because we are concerned with respiratory disease, some conscious animals will be made to breath substances which cause them to become breathless, in order to measure their lung function. This will however be transient and animals are expected to recover fully and rapidly from this. All experiments will result in mice being killed humanely, most commonly by overdose of anaesthetic. In the case of breeding procedures, mice may be either used for experimentation as discussed, used for further breeding, or transferred to other researchers for their studies.

Application of the 3Rs

Replacement

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

Replacement

In addition to animals, we perform studies on human volunteers and in cells and tissues donated by those volunteers. Wherever possible studies are performed on human samples. Questions such as those relating to clarification of complex immune system mechanisms often cannot be studied in cells from humans however and studies of people with disease often cannot for ethical reasons supply the types of sample or frequency of sampling required to answer a research question. In these cases we will use mice for our experiments, in which we can make genetic and drug interventions, or manipulate the immune system, allowing us to clarify disease mechanisms and demonstrate cause and effect, with the ultimate goal of developing new, more effective therapies for man.

Reduction

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

Reduction

We will always make sure by surveying the scientific literature that studies are not unnecessarily repeated. Statisticians will be consulted to ensure that the appropriate number of animals is used to ensure statistically, as well as biologically meaningful results. Appropriate negative and positive controls for a given treatment are essential to avoid unnecessary repetition of experiments, but where pilot experiments demonstrate that a control is redundant, study designs will be refined accordingly in future work. Finally, breeding strategies will be optimised to help reduce the number of animals used. All studies will be carried out in a way that enable publishing in adherence with 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 will always make sure by surveying the scientific literature that studies are not unnecessarily repeated. Statisticians will be consulted to ensure that the appropriate number of animals is used to ensure statistically, as well as biologically meaningful results. Appropriate negative and positive controls for a given treatment are essential to avoid unnecessary repetition of experiments, but where pilot experiments demonstrate that a control is redundant, study designs will be refined accordingly in future work. Finally, breeding strategies will be optimised to help reduce the number of animals used. All studies will be carried out in a way that enable publishing in adherence with the ARRIVE guidelines.

PROJECT 23.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 subtype specific tumour biology
Key Words	Tumour subtypes, Personalized therapy, Systems biology, Stratified medicine, Tumour progression, Metastasis
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Attempts to personalise therapy for patients with pancreatic, colorectal and breast cancers have yet to be successful, in part due to the fact that the biological differences between each patient's tumours have been difficult to define. This project aims to define groups of patients who have similar tumours – not only at the genetic (DNA) level, but also in the metabolites that are fundamental to how cells grow, change over time, respond to their environment, and die. This will help us to tailor specific therapies to individuals in each group and improve their treatment outcomes.

In this project, we will confirm the existence of previously defined subgroups and identify extra subgroups using tumour samples from patients, cell lines and mouse models. Using sophisticated biological and computational analyses, we will identify subgroup-specific genetic changes, metabolites, and their response to various therapies. The accurate and full characterisation of the tumours will lead to the treatment of patients with specific types of tumours with 'best-fit' drugs, and the tests that are needed for this to occur in the clinic. Overall, this proposal will help to identify novel personalized diagnostic and therapeutic strategies for patients with this devastating disease.

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

There is a clear clinical need to match specific patients from pancreatic, colorectal or breast cancer with specific therapies. Current treatments using chemotherapies provide only modest benefit at the cost of significant side effects. This is because: a) these therapies are administered to unselected patient populations irrespective of any other clinicopathological or genetic (DNA) characterisation; b) there is a lack of

clinical tests to match patients to therapies; and c) the cause of these cancers at the DNA levels are relatively poorly defined in pancreatic cancer. In this project, we will derive a more complete understanding of the genetic and other clinical differences in these tumour subsets using integrated computational and experimental biology approach to identify therapies. This approach will identify not only the additional tumour subtypes, but also their accompanying underlying biological abnormalities and the drugs that are likely to be effective. These data will be used clinically to: a) develop diagnostic tests for early detection and classification of tumours; b) develop diagnostics tests for matching appropriate therapies that benefit patients; and c) test promising leads in animal models and subsequent clinical trials.

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

We will use the mouse. The mice that we will use genetically altered to predispose them to various cancers. We will also use immune-compromised and normal mice. The approximate numbers of mice we expect to use during the 5-year project will be approximately between 15,000 to 32,500 that will be determined using statistical and computational methods that helps us to match mouse models closer to patient samples.

In the context of what you propose to do to 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 we do everything possible to minimise adverse effects, these can occur. Possible symptoms include loss of condition, weight loss and effects on organs, e.g. skin or liver. Mice will be checked regularly for signs of ill health. The mice are expected to develop tumours and we will test therapies find if the tumours would reduce. We will use pilot studies to determine any adverse effects if the study will be new. Also, we will use the pilot studies and computational methods to determine the number of animals and reduce them as appropriate.

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 suitable ways to properly model these tumours *in vitro* using cancer cell lines. In order to study tumours that form spontaneously, within a native host environment and at the appropriate time during development, genetically engineered mouse models are the only practical tools available to us. Genetically altered mouse models also allow us to model how the presence of the same mutations found in human cancers affect tumour development and response to therapies. To study the

effect of novel treatments on human tumours, we also use immune-compromised mice that allow the growth of tumour cells from patients. Together, these models are complementary and predict quite well the activity of drugs in the clinic.

Reduction

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

Reduction

Most drug development utilises rodents, so we have a considerable amount of information on existing drugs for comparison with novel therapies. Before any new agent is administered we first perform extensive *in vitro* laboratory experiments to establish the concentrations and exposure time required. Then we test that any novel agents are tolerated in animals at the doses required to give for a predicted efficacious exposure. In addition, we will use our computational methods to predetermine which mouse models match patient profiles, and thereby, minimise the number of animals. Mouse numbers will also be calculated to minimise usage while allowing robust 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

We operate within a very tightly regulated, clean and well administered facility that has an excellent track record for animal care and safety. In all cases we use appropriate anaesthetics, analgesics and procedures to avoid pain, suffering or lasting harm to the animals. When we need to cull them we do so by approved procedures. We have also implemented a real-time, networked database to monitor colony and determine the endpoints earlier to avoid the suffering of the mice. This way we get informed through the databses and get aware of issues with our animals and can respond to them quickly. Additionally, we are refining and replacing our current models with more sophisticated ones that incorporate non-invasive tumour imaging to enable early tumour detection. This will reduce animal numbers, shorten our experiments, and prevent large and potentially debilitating tumours. In this way we hope that efficient and compassionate use of our animals will help us to make a lasting impact on the treatment of these deadly cancers.

PROJECT 24.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-mesenchymal interaction in tissue regeneration and carcinogenesis
Key Words	colorectal tumours, cancer microenvironment, inflammation, 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):

Adult stem cells are contained within every tissue in the body and are critical for tissue development, maintenance and repair. It is vital that stem cell activity is carefully regulated in health and this is achieved through the action of chemical messages secreted by cells that surround the stem cell (the tumour microenvironment). Cancers arise from loss of control of stem cell division. It has long been considered that this loss of control occurred purely because of the accumulation of faulty genes in the stem cell itself, however recently it has become apparent that derangement in the regulating chemical messages can also result in loss of control of stem cell division resulting in tumour growth. Variation in the importance and influence of these chemical messages in different tissues may influence tumour site predilection

This project will explore the mechanisms involved in stem cell control in health, repair after injury and at the initiation and progression of tumours. We will generate mouse models that mimic the scenario of human cancer patients, including tumours that develop in different areas of the body (e.g in different regions of the intestine). We will also investigate combinations of therapies to see if we can enhance wound healing in the gut which can predispose to cancer formation and/or slow the growth of tumours. The work will focus on cancers of the gastrointestinal tract, which commonly affect human patients.

Specific objectives include

1. To examine the consequences of disrupting cell chemical messages on tissue development and daily maintenance

2. To investigate the supporting cell types and chemical messages important in regulating tissue repair following inflammation and injury, and assessing drug treatments that affect this

3. To investigate the role of chemical messages and the supporting microenvironment in driving cancer initiation and progression

4. To see whether we can use treatments that address the disrupted chemical messages from both the cancer cells themselves and the surrounding supportive cells

Mouse models are necessary as cell culture models cannot replicate the numerous interacting chemical messages that this project aims to study

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

Historically, research looking at the cause of cancer, particularly colorectal cancer, has focused on the accumulation of faulty genes in the cancer cells themselves. This has provided a wealth of information on some of the genes that can lead to cancer and has successfully guided the development of treatments. Recent work, (including our own), has shown that the tumour microenvironment can also have an important role in influencing the behaviour of cancer cells at the earliest stage of tumour formation and that this influence may continue in established tumours, contributing to some of the variable clinical behaviour seen in different tumours. We think this work will further our understanding of the interaction of the tumour cell, the stromal supporting cells and immune cells and establish how these elements interact in tumour initiation, progression and the development of metastasis. We believe this work will help to identify novel drug targets including targeting some signaling pathways that originate from supporting cells, rather than from the cancer cells themselves. By redressing disrupted chemical messages from the supporting cells we believe that this will help standard chemotherapy attack the cancer cells themselves more effectively

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

We expect to use approximately 17500 mice over the 5 years of the project. The majority of these animals will be used for breeding purposes. The numbers required are because the most realistic models of human cancers are those with accumulations of multiple faulty genes. To reproduce these combinations in mouse models requires several generations of breeding. Accurate models of human cancers are required to replicate the tumour biology seen in human patients and to understand the response of tumours to drugs or radiotherapy.

In the context of what you propose to do to 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 mice will develop benign tumours or cancers and these will usually cause predictable symptoms such as weight loss and general ill health. However, in the great majority of cases we can identify those cancers before they cause the

animal any distress and, after humanely killing the animal, we can gain enough information to answer the questions we are asking. For some mice in which the timing of cancer development is uncertain, we will undertake clinical monitoring for evidence of tumours and intervene early to prevent distress. Other mice will undergo induced inflammation or cell DNA damage and/or localised wounding to examine the mechanisms that induce wound regeneration. Daily monitoring of these animals for signs of anaemia and lethargy will prevent excessive morbidity from these interventions. Some interventions and monitoring will involve colonoscopic examination, which will be performed whilst the mice are asleep (anaesthetised) A key aim is to examine the effect of potential wound healing or cancer therapies to develop and test agents that might be used for treatment or prevention of human inflammatory bowel disease or cancer. Administration of these agents may require some mice to have injections, but that will only cause temporary discomfort. Overall, our breeding mice will experience mild severity with animals on experimental protocols experiencing disease of moderate severity. At the end of the work, all mice will be humanely killed.

Application of the 3Rs

Replacement

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

Replacement

Our work is all derived from studies of human cancer patients. We can do some experiments in cancer cell lines derived from human tumours, but these lack certain very important features. We know that the interactions between cancer cells and the supporting normal cells in a tumour are vital determinants of patient prognosis and how well the cancer responds to treatment, and these interactions are very hard to model realistically in cell lines as they lack the complex supporting microenvironmental tissues. Only animal models can faithfully recapitulate these aspects of human disease and mimic a human cancer and, even if not every aspect of tumour growth is the same in humans and mice, there are many similarities that we don’t find in lower animals. For example mice carrying mutations in genes that predispose to bowel cancer in humans also develop multiple bowel tumours that have provided many important insights into the human disease

Reduction

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

Reduction

Our studies are based on the minimum number of mice required to show differences between groups, such as response to a new drug or the effect of a specific gene

fault. These numbers have been generated based on statistics and optimised experimental design. Where possible, we perform initial studies in cell lines or other cell culture systems before turning to mouse models, and will aim to reduce animal numbers used by refining gene induction techniques

Refinement

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

Refinement

Compared with other animals, mice currently provide the only model of human disease that allows routine genetic manipulation, that has sufficient physiological similarity to humans, that comprises a large body of existing resources, and that can be bred with sufficient rapidity. The existing data on likely phenotypes and dosing of mice are far more comprehensive than those for other animals, preventing unnecessary animal use. Even when we cannot be certain of what tumours, if any, a mouse will develop, there are precedents that will allow us to anticipate when phenotypes will develop and whether they could cause the animal any distress. If necessary, pilot experiments will be perform on small numbers of mice in order to determine windows where research can be performed successfully before distress develops. All animals will be monitored daily and any mouse with evidence of distress will be humanely killed. Wherever possible, we shall humanely kill mice before they show any signs of distress.

We shall further refine our experiments using specific techniques, including

- use of anaesthesia for any procedure expected to cause more than momentary distress

- the use of aseptic technique for all procedures

- using targeted and/or inducible mutations so as to minimise ill effects outside the window of study

- use of the most relevant tumour induction methods to answer the experimental question including localised recombination methods

- minimising the delivery of agents to promote or retard cancer growth and using non-invasive methods wherever possible

PROJECT 25.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	Biological mechanisms of cardiovascular disease
Key Words	Cardiovascular disease, atherosclerosis, heart failure, GWAS
Expected duration of the project	5 year(s) 0 months

Purpose of 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 goal of the project is to investigate the mechanisms of cardiovascular disease such as heart failure, and the causes of heart attacks and blockages of blood vessels by fatty deposits. Cardiovascular disease is the largest cause of death in Western societies, and is also emerging as a major health problem in developing countries. New strategies to prevent or reverse cardiovascular disease are needed to help decrease death from this condition. In the last 20 years, much has been learnt about the mechanisms that regulate the health of blood vessels and how to decrease levels of bad fats in the body. Although fat lowering therapies have been very successful, there are still a significant number of people for whom these treatments are not effective. Hence, there remains a pressing need to identify novel key factors in cardiovascular disease. It is only with the identification of novel pathways which alter the progression of cardiovascular disease that we will be able to develop new treatments for this condition.

It has recently been established that multiple cardiovascular diseases have similar cellular mechanisms which drive the progression of these diseases. For example certain types of white blood cells are responsible for the progression of atherosclerosis, aortic aneurysm formation and poor recovery after a heart attack. In this licence we will investigate novel pathways in multiple cardiovascular models, and by using this methods we will greatly improve our understanding of common cellular pathways across multiple conditions. This is important as patients frequently present with more than one cardiovascular condition however, at the moment we have little information about how these conditions interact with each other. Increasing our understanding of these interactions will help pave the way for better treatment strategies.

It is now recognised that certain conditions such as diabetes worsen cardiovascular disease. In addition, other conditions such as the development high blood pressure during pregnancy (pre-eclampsia) have now been recognised to significantly increase the likelihood of both the mother and child developing cardiovascular

disease later in life. The mechanisms by which these co-morbidities alter the development of cardiovascular disease is not well understood. This lack of understanding of the mechanisms underlying these conditions makes it very difficult to develop treatments for them. In this program of work, we will also investigate how our novel pathways alter cardiovascular disease development in the presence of these additional risk factors.

The major aim of this project is to identify new and better targets for the treatment of cardiovascular diseases. Specifically, the objectives of the licence are:

1. To test the role of novel genes in the development of cardiovascular disease.
2. To characterise the cellular mechanisms by which these genes work
3. To investigate how these genes interact with factors which are known to increase the change of development heart disease such as diabetes
4. To investigate how these genes alter the risk of development complications during pregnancy such as pre-eclampsia and how this affects the cardiovascular health of both mum and offspring.

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

This project will advance our knowledge of factors which contribute to the initiation, progression and regression of cardiovascular diseases, and has the potential to identify new therapeutic targets for future prevention and treatment. Specifically this Project will address: (1) In the short/medium term, it will provide the information on how changes in the vasculature, inflammation and cardiac function are related to disease progression in cardiovascular disease. (2) In the longer term, it will pave the way for novel pharmacologic, genetic or molecular approaches to prevent or reduce cardiovascular disease (3) Help us to identify and manage people at risk from vascular disease complications such as preeclampsia A continued clinical need for prevention and treatment of cardiovascular disease may be addressed in future by identifying new therapeutic targets through better understanding of known biological pathways, and by identifying entirely novel pathways that were not hitherto recognised to contribute to cardiovascular disease pathogeneses.

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

All work will be carried out in mice. In order to ensure that the minimum number of animals are used in each experiment power calculations will be carried out prior to the start of the experiment to establish appropriate sample sizes to be set. For the majority of experiments a significance level of 5% with 80% power will be used to establish statistical significance. When possible experiments will have a factorial design to allow maximum information to be obtained for minimum input and good laboratory practice will be introduced to avoid bias such as randomisation of

treatment and blinded assessment of outcomes. It is estimated that approximately 30000 animals will be used in the life time 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?

Most protocols on the licence are of a moderate severity. There are five severe protocols on this licence. However, it is anticipated that the vast majority of the animals on this licence will experience mild procedures, with a small number undergoing more invasive procedures. We expect adverse effects to be minimal based on our extensive experience and our continuing commitment to animal welfare and application of the 3Rs. In order to answer the aims set out in this licence we will need to use genetic technologies to generate transgenic models of cardiovascular disease. A large proportion of the animals will be used to generate models of cardiovascular disease such as atherosclerosis and hypertension. As is typical with humans it is expected that this will be asymptomatic. Some mice will be generated to model more severe forms of heart disease such as heart failure. As with humans these models can cause adverse effects such as lethargy and discomfort. As we are interested in the processes that initiate cardiovascular disease we will aim to collect data from the majority of mice before these symptoms become apparent. Disease progression may be monitored using non-invasive imaging and measurements of cardiac function which may be conducted under anaesthesia. Some mice may be generated that are prone to sudden death due to rupture of a major blood vessel. As with humans this will result in a very fast death with the mouse losing consciousness within minutes of the rupture. As we are interested in the mechanisms which precede this event we will aim to collect data before this happens. If we observe this adverse effect in certain strains of mice we will use non-invasive imaging such as ultrasound to help us identify mice at risk of sudden death. At the end of these experiments the animals will be humanely killed and tissues collect for biochemical and histological analysis. During our studies, we will use blood samples collected from blood vessels close to the skin to look at factors circulating this blood. This will help us to monitor factors, which we know affect cardiovascular disease such as levels of fat or sugar in the blood. We will also look at the number of white blood cells in the blood, which we know have an impact on cardiovascular disease progression. In addition, we may look for other molecules, which we think, might be able to predict the progression of cardiovascular disease. We will be very careful to ensure that we only take very small blood samples for mice and when we need to take more than one blood sample for example in long term studies that leave enough time between samples for the mouse to fully recover. In addition, we will use non-invasive imaging methods such as ultrasound and MRI to monitor disease progression. Some animals will receive multiple imaging sessions in order for us to monitor disease progression over a long period. For example, mice may have an echocardiography to monitor heart function every month from 3-9 months of age. We will always ensure that all mice are fully recovered and back to normal for their first imaging session before they

have another imaging session. In some studies we may administer substances to alter certain functions which are known to effect the progression of cardiovascular disease such as drugs to alter the response to inflammatory cells. Where possible these drugs will be administered in the food, however, for some drugs we may use injections. When we need to give repeated doses of a drug for longer periods we will implant a small drug delivery device under the skin. As observed with humans consumption of a high fat and or high sugar diet leads to high levels of fat in the blood and can make the body less sensitive to certain hormones such as insulin. We will feed animals high fat and or sugar diets to mimic these changes. These diets are commercially prepared and are formulated to ensure that all other nutrients are balanced and at the correct proportions. Feeding these diets as with humans can result in obesity and can cause the development of type II diabetes. Mice are closely monitored to make sure these circulating sugar levels do not get to high and that obesity does not affect the normal activities of the animal. We will image the progression of cardiovascular disease in some mice using non-invasive imaging methods such as MRI and echo as is commonly done in humans. In almost all cases the animals will be carefully anesthetised to allow these recordings without distress or suffering, in order for us to monitor changes in the circulating factors such as levels of glucose or fats in the blood. This typically involves making a small puncture wound in a blood vessel close to the surface of the skin and the collection of a few drops of blood. Although a small wound is made the animal returns to normal function straightaway. A small number of animals will undergo surgical procedures to allow us to investigate the common causes of cardiovascular disease. One of these models is thoracic aortic banding, a well-established procedure which narrows the aorta and is similar to the problems associated with aortic valve stenosis in humans. To generate this model an incision is made on the chest and a small suture is tied around the aorta to decrease the diameter which results in a gradual thickening of the heart wall. In another model we will tie off a coronary artery on the surface of the heart which will result in a heart attack. As before this is carried out by making an incision in the chest of the mouse. After surgery all animals are given analgesia, are placed in a warm environment and are monitored closely. In most cases animals recover from the surgical procedure within 48h. After surgery the major adverse effect that could occur is heart failure which results in weight loss, reduced mobility and breathlessness. The combination of close monitoring and sensitive imaging techniques means we can identify mice which are entering heart failure before any symptoms become apparent, enabling us to keep distress to a minimum. We will also use surgery to model cardiovascular surgical intervention used in humans such as bypass grafting and angioplasty to look at how genetic interventions alter the outcomes of current surgical treatments for cardiovascular disease. In these studies a vein or artery is grafted into another blood vessel or the vessel has an angioplasty wire inserted to mimic the damage cause by this intervention in humans. Some discomfort is associated with the surgical interventions. Animals will be monitored regularly and surgical discomfort will be alleviated by analgesia. At the end of these

experiments all of the animals will be humanely killed and tissue collected for biochemical and histological analysis. In a very small number of studies, mice may have a combination of surgical models. In some mice, we may implant a device, which allows us to monitor the blood pressure and heart function of the mouse remotely 24h a day. Once this is implanted, the mouse is returned to its own cage This enables us to carry out very detailed analysis of cardiovascular function in a mouse, which is not restrained and critically allows us to look at cardiovascular function when the mouse is asleep and awake. Once the mouse is fully recovered it may under go a second surgery. For example implanted with a device which delivers a certain amount of drug throughout the day. This method means that we do not need to handle the mouse daily to administer the drug. We will use drugs which we think will be beneficial at lowing blood pressure or we may administer drugs which we know when produced by the body in large amounts cause cardiovascular disease such as angiotensin II. Some discomfort is associated with the surgical interventions. Animals will be monitored regularly and surgical discomfort will be alleviated by analgesia. At the end of these experiments all of the animals will be humanely killed and tissue collected for biochemical and histological analysis.

Application of the 3Rs

Replacement

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

Replacement

Cardiovascular disease is a complex interplay between metabolic and inflammatory mechanisms acting in numerous systems such as the vasculature, nervous system and the heart. Despite advancements in computer modelling, *In vitro* cell based systems and the use of clinical studies in patients with cardiovascular disease, these methods are still unable to fully model the complex biological processes in cardiovascular disease. Hence the use of animals is unavoidable if important biological questions about this condition are to be addressed.

We have strong collaborative links with clinicians and now have routine access to blood and tissue samples to isolate inflammatory cells, vascular tissue (e.g. sample of atherosclerotic plaques and aneurysms), and cardiac myocytes from patients with cardiovascular disease. This enables us to investigate the relevance of cellular pathways and individual genes in cardiovascular disease tissue. However, these samples cannot tell us how important these mechanisms are for the initiation and progression of the disease.

Where possible we have established cell based assays to test the role of genes implicated in cardiovascular disease and potential therapeutic strategies in place of in vivo models. We have created cell based models that have been altered so we

can change the expression of our genes of interest and we routinely utilize siRNA as a method to investigate consequence of loss of function of our genes of interest. Cell lines have been useful in establishing mechanism of action e.g. assays to establish interactions between inflammatory cells and endothelial cells. However, cell-based studies cannot address the impact of our manipulations on In vivo disease initiation, progression or regression.

Alternative animals such as Zebra fish have been considered and although these species have proven valuable in certain aspects of cardiovascular research such as angiogenesis and heart repair, they still have significant limitations as models of cardiovascular disease biology. For example, although Zebra fish can develop hyperlipidaemia at this time there are no Zebra fish models of atherosclerosis. Although Zebra fish are an excellent model system for heart regeneration post myocardial infarction this does not make them a suitable model for investigation the role of candidate genes in myocardial infarction pathology. In addition, although Zebra fish can model isolate pathologies associated with cardiovascular disease they cannot recapitulate the multi-factorial nature of cardiovascular disease

Reduction

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

Reduction

The majority of animals are used for breeding or in mild/moderate procedures; approximately 85% of animals. We will manage animal breeding carefully to reduce animal numbers to the minimum required for our phenotyping experiments. We hold weekly lab meetings where we critically review animal usage including the estimated need for animals in the coming months. This enables us to ensure that animal over breeding is kept to a minimum. We work as a team to ensure that maximum use of all available tissue is made from each animal. Power calculations will be carried out prior to the start of the experiment to establish appropriate sample sizes to be set.

We invest in new technologies which require smaller sample sizes and enable us to derive more data from one sample. We have established new technologies which enable us to reduce the use of animals. For example we have recently purchased a mitochondria analysis machine (Seahorse) which require much smaller sample sizes and allows the analysis of 96 samples in parallel. This assay was previously carried out using a Clark electrode where samples are analysed individually with large volumes of mitochondria per assay required. The increased number of replicates has decreased data variability and reduced sample size. It also enables us to investigate multiple conditions at once.

Recruitment and retention of inflammatory cells is a key step in multiple cardiovascular disease pathologies. We have previously used immunohistochemistry

to assess inflammatory cell infiltration in cardiovascular disease. Although immunohistochemistry can give valuable data about location and subtype of inflammatory cells present in that aorta it provides only a snap shot of the aorta and as such has large statistical variability. We have developed a method of aortic and heart tissue digestion, which allows quantification of inflammatory cell content in the whole aorta and heart using flow cytometry thus reducing the number of animals needed in each group.

We have optimised protocols to allow multiple tissues from one mouse to be utilized for multiple assays for example for the isolation of primary vascular smooth muscle cells, macrophages and endothelial cells which means cells, can now be collected from multiple tissue sites allowing multiple researchers to utilize the tissue from one 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

The disease models in this licence are already well established in our laboratory and we have worked hard to optimise animal welfare per- and post-operatively. Detailed protocols have been written in collaboration with other groups who use these techniques in order to ensure best practice. We constantly look for refinements and replacements that we can adopt in our studies. This includes literature searches to check for refinements and possible replacements. This also includes frequent communications with collaborators and other scientists to establish if they have any refinements that would be applicable in our models.

We continue to develop new imaging techniques in rodents to increase sensitivity and decrease variability in our models. We are currently optimising a new imaging method (high resolution μCT) to image atherosclerosis. Traditional methods only give a 2D analysis of atherosclerosis, giving only a snap short of atherosclerosis at a few anatomical locations. μCT enables us to image the volume of atherosclerosis in the whole of the aorta, decreasing the high variability associated with current quantification techniques. It also allows us to investigate location specific changes in atherosclerosis which allows us to look at how changes in blood flow patterns alters the development of atherosclerosis.

We are interested in the biology which causes heart disease as such we are most interested in what happens before animals develop symptoms of heart disease. We use non-invasive imaging such as ultrasound imaging of the heart to monitor the

progression of heart disease. This means the vast majority of our animals will never develop symptoms of heart disease.

As with humans certain strains of mice when fed a high fat diet will develop atherosclerosis. We frequently fed our mice a high fat diet to cause the development of atherosclerosis. Our extensive experience with the model enables us to feed mice for the minimal period of time to cause the required atherosclerotic plaque features. Unlike the diet the mice are normally given high fat diet is softer, hence mice are given wooden sticks and additional chewing material to keep their teeth in a good condition. In addition due to the grease nature of the diet cages are changed more frequently and different types of bedding used to make sure the mice do not develop grease coats.

We have many systems in place to minimise any suffering animal's experience during procedures. They are housed in a controlled environment with optimal lighting, heating, food and with appropriate companions. When we need to administer drugs or agents to mice we always pick the least invasive route, for instance in their food and drinking water or injections just under the skin. We will also use the lowest effective dose when this is known. If we are using substances for the first time we will carry out pilot tests on typically 5-6 animals, always starting with the lowest dose and only increasing when necessary. Anaesthesia and analgesia will be used for any procedures where the pain or discomfort could last longer than a few seconds. All surgery is carried out in a dedicated surgical room under sterile conditions. Animals are always given analgesia before and after surgery and are monitored closely after surgery.

PROJECT 26.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	Gut/Commensal Bacterial Interactions in Health and Disease
Key Words	Microbiota, Autoimmunity, Infammation, 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.
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 bacteria which inhabit the gastrointestinal (GI) tract, collectively referred to as the microbiota, control many aspects of human health and physiology. They assist in regulating many host metabolic and immunological processes that can either be beneficial or negative to their host. In this way altered bacterial communities in the GI tract have been associated with many human diseases especially those caused by activation of the immune system. In contrast, defined bacteria with beneficial characteristics have been proposed as therapeutics and have been employed successfully in animal models of disease.

A variety of *in vitro* (cell based) and *in silico* (computer modelling) techniques can also be employed to identify new bacterial species and strains with potential to prevent and treat human disease. Although this platform of techniques is useful and important, none can fully recapitulate the complex interplay of pathways responding to bacteria *in vivo* and therefore mouse models are critical for efficacy studies and further understanding of the mechanistic basis of bacterial therapeutic function.

The objectives of this project are as follows:

1. To treat mouse models with bacterial strains or the novel bioactive products they produce to develop new ways of treating human disease.

2. To understand the mechanisms by which these bacterial strains affect the host 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)?

The short term benefits of this licence are that our results will increase the understanding of how the immune system responds to particular strains of gut resident bacteria and enhance the understanding of how the presence of specific bacteria may influence the development and severity of autoimmune disease. Overall, our long term aim for this project is to identify new live biotherapeutic

products isolated from the human microbiome with the ability to treat a range of autoimmune and inflammatory diseases, either as a monotherapy or in combination with existing treatments. This work will also be beneficial to the work of other scientists in a number of disciplines and will hopefully be disseminated in peer reviewed journals and at conferences.

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

We will use mice for this project licence and have estimated that we will use approximately 1000 mice over the period of 5 years. This will allow us to assess how specific immune cells and bacteria influence and regulate a variety of disease states in these models.

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

To treat mouse models with bacterial strains or the novel bioactive products they produce to develop new ways of treating human disease. The majority of experiments will involve the treatment of rodents with bacteria or bacterial products by oral gavage. Oral gavage is the administration of a substance via the oral cavity, using a feeding needle or tube, into the lower oesophagus or stomach. Once introduced, the substance to be dosed is slowly expelled, and the tube is withdrawn slowly. Compared with other methods of oral administration, it is more invasive, difficult and stressful (handling, restraint and introduction of equipment into the animal is required). This is associated with a higher risk of complications, especially if repeated or chronic dosing is neces-sary. However, it is also the most accurate and reliable method for administering substances into the gastrointestinal tract. The mice used under these protocols will at most experience a mild to moderate level of severity and multiple steps are in place to ensure minimum discomfort is experienced by the an-imals. Acclimatisation of the animals to handling in advance, combined with animal facility staff training in competency for manual handling and restraint of animals in the procedure, itself minimises any stress experienced by the animal. Further experiments may involve treatment in a challenge setting e.g. administration of a stimulant to recreate an inflammatory setting. Administration of such substances can take place though a wide variety of routes including, subcutaneous, intravenous, in-tramuscular or intraperitoneal needle injection. Complications associated with these methods can include local irritation, pain, infection and damage to the surrounding tissue. Adverse effects can be minimised by choosing doses based on knowledge of the properties and toxicity of each compound, such that the minimum effective dose required to achieve the aim of the study is used. Generally administrating smaller volumes over multiple injection sites will also minimise adverse reactions. The number of animals used will always be the minimum compatible with sufficient statistical power to generate meaningful results. At the end of the procedures mice will be killed by a home office approved method.

Application of the 3Rs

Replacement

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

Replacement

We are committed to identifying and developing the latest *in vitro* and *in silico* systems for the identification and characterisation of human gut resident bacteria. However, at present these systems have significant limitations which prevent them from fully replacing animal models. In particular, they lack faithful recapitulation of the complex ecological niches represented by the microbiota *in vivo* or the interaction between bacteria and the host at the gastrointestinal interface. This project will provide mode of action insight into how bacteria exert beneficial or negative effects on mammalian systems, which will allow us to further refine our in vitro assays. We will continue to develop *in vitro* assays and maintain up to date knowledge of available technologies in an attempt to replace animal use wherever possible.

Reduction

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

Reduction

The numbers of mice used in experiments will be kept to a minimum by optimal experimental design and the animals used will be bought in from reputable breeding establishments. The size of experimental groups will be decided after consultation with a statistician and we will continue to consult with statisticians for optimisation of the experiments throughout the life of the licence.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 a model system in this project because they have an immune system which is sufficiently similar to humans. Mechanisms related to the bacterial interaction with the host have primarily been investigated already in rodents and they are the industry standard for route to clinical trial.A significant component of the work proposed in this project involves the identification of biomarkers indicative of both health and disease states. For example, we aim to identify markers with prognostic value, diagnostic value or indicative of protective/therapeutic responses. In this way,

we hope to offer refinements to current -methodology whereby endpoints can be reached prior to onset of clinical symptoms.

PROJECT 27.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 ecology of seabird migration and foraging
Key Words	stable isotope, migration, seabird, foraging, 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:
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):

Seabird populations are currently declining faster than any other comparable group of birds. Understanding factors influencing these declines requires a detailed knowledge of their foraging and migratory behaviour, which is at the core of my research interests. I use logging devices to track the movements and behaviour of seabirds, and use small samples of feathers and blood to provide information on their diet and sex.

The ratio of stable isotopes (chemical elements with different weight and therefore different properties) can be measured in a small sample of bird feathers or blood to provide important information about movement and foraging behaviour. For example, they can be used to quantify the amount of fish that some birds get by scavenging from fishing boats compared with fish caught naturally, which is important for understanding the impact of the Discard Ban, under reform of the EU Common Fisheries Policy.

Environmental change may not impact all members of the population equally. Sex-specific differences in foraging and migratory behaviour are well known but for species where males and females cannot be identified based on external characteristics, they can be sexed using genetic markers amplified from non-destructively sampled tissues.

The key scientific questions I will address are about seabird foraging and movement ecology, with the emphasis on implementing these in terms of conservation.

What are 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 current research is to inform conservation for seabird populations currently under severe threat – globally, seabirds have declined by ~70% over the period 1950 to 2010. This is of concern since these iconic animals provide an essential function in marine ecosystems – regulating plankton, cycling nutrients and

event helping to offset the impacts of climate change. They also have considerable intrinsic value for people. In the UK alone, we have approximately 8 million breeding seabirds of 25 species and for 8 of these species we have >30% of the global population, providing an important link with the marine realm. Moreover, at one small seabird colony in Scotland the local economy is boosted by ~£750,000 per annum because of visitors to this site. Without effective research-informed conservation, the future of seabird populations is bleak.

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

Northern gannet - ~500 individuals 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?

Birds will be taken from the wild, before being returned promptly following blood/feather sampling, measurements and (in some instances) attachment of a small logging device (s). This may cause short-term stress. To the potential for any deleterious effects, these mild protocols will only be performed on those animals considered suitable (based upon reproductive state, condition and qualitatively assessed stress levels).

Application of the 3Rs

Replacement

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

Replacement

Field-based applied research requires sampling of free-living animals – there are currently no viable alternative model systems available to address the questions at the core of my research.

Reduction

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

Reduction

Previous work reveals a degree of variation in strategies among individuals requiring that we sampling ~100 individuals per annum.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 research in largely confined to species that feed on a combination of anthropogenic and natural foods to enable us to understand better how changes in the availability of these foods might impact upon wild bird populations. We will use stable isotopes of non-destructively sampled tissues (i.e. feathers and blood) to avoid destructive sampling or the use of invasive dietary assessment methods such as stomach flushing.

PROJECT 28.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	Do fish have necks: measuring 3D motion of the vertebrae and axial muscle dynamics in suction-feeding fishes.
Key Words	Muscles, Bones, Spinal column, Fish, Biomechanics
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):

This project will study how muscles and bones work together to give humans and animals a flexible neck, by studying the hidden “neck” of fish. The neck’s importance in humans is starkly illustrated by the pain and discomfort caused by disorders of the neck. A neck allows the head to move three-dimensionally and independently of the limbs and body, and was a major turning point in the evolution of land animals. But currently we know relatively little about how the neck evolved, or how its bones and muscles interact to produce motion. Although fish lack a true neck, their backbone could function as a neck by bending to lift the head upwards as fish open their mouths during feeding. If fish do have a hidden “neck”, it is powered by their body muscles, which extend from head to tail. All muscles have a trade-off between how fast they can shorten and how much force they can produce. But by changing their shape as they shorten, muscles may be able to automatically adjust their speed to produce as much (or as little) force as necessary. I will use new imaging tools to study how these bones and muscles of fishes “necks” work by:

1. Measuring 3D motions of the backbone, head, and shoulder girdle

2. Comparing the shape of the vertebrae along the backbone

3. Measuring how the body muscles shorten and change shape

4. Creating a computer model to predict how muscle shortening controls neck motion.

What 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 change our perspective on the origin of the neck and provide insights into how muscles move our bodies. By linking the anatomy and motion of the backbone in living fish, this study will help us understand how the neck may have evolved in fish-like animals that are now extinct. It may also help fish farmers improve the growth and health of their fish. The information on the shape and

shortening of fish muscles can also be applied to understanding human muscles—and how changes in muscle shape during ageing may impact our health. The digital models, computer animations, and videos from this research will be used in outreach programs at a local museum and science clubs to teach and inspire young scientists.

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

I will use three fish species (temperate, tropical freshwater, and tropical marine) that each have differently shaped “neck” bones. Over the one-year project, I expect to use no more than 30 adult fish.

In the context of what you propose to do to 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 severity level of this study is moderate. Some fish will have a minor surgery, where tiny (1mm or smaller) metal spheres will be implanted under the skin in their muscles and bones. This short surgery will be done with general anaesthesia and pain-relief, and fish will be carefully monitored to ensure they recover quickly and go back to swimming and feeding as normal. These fish may experience brief stress when their tank is moved to a different laboratory to record their bone and muscle motion. To minimize stress, the transport will be short, we will keep all the tank conditions (e.g., water temperature, water cleanliness) the same, and fish will have a chance to rest after the move and get used to the new laboratory. At the end of the experiments, all fish 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 goal of this study is to measure how the spinal bones and muscles of a fish move as it lifts its head during feeding. Measurements of these backbone motions and muscle shortening have never been recorded before in any fish species, so this study could with information from published papers. And there are currently no non-animal models that can predict the complex, three-dimensional motions of the bones and muscles of a fish’s backbone. Larval fish do not have a fully developed skeleton, so I need to use adult animals to understand how the bones and muscles of the neck work to produce motion.

Reduction

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

Reduction

This project will observe bone and muscle motion directly in fish, and will not require statistical tests to answer the study questions. Because of this, I only need to use enough fish to judge how movements vary among fish of the same species (compared to how they vary among fish from different species). Based on previous experience, I will need a minimum of 3 fish from each species to do this. However, not all fish can be trained to feed reliably, which is essential for recording bone and muscle motion during feeding. Therefore, I need to get up to 10 fish of each species, and then select the 3 that feed the most often and can be well-trained. The remaining fish will not experience any suffering or ill 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

I have chosen the three species that represent a wide sample of the different “neck” shapes and motions found in fish: ranging from relatively simple backbones that are used to rotate the head upwards a little bit, to specially shaped backbones that are used for extremely large rotations of the head. For these three species, there is enough known about their feeding and anatomy to plan the study and ensure good measurements, and about their habitat and behavior to care for them in an aquarium environment. We have an excellent facility where these fish will be housed according to their specific needs (for example, temperature, water flow, hiding spots), and their health will be carefully monitored by trained aquarium and veterinary staff.

PROJECT 29.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	Identifying and characterising novel anti-schistosomals
Key Words	Schistosoma mansoni, helminth, vaccine, drug
Expected duration of the project	5 year(s) 0 months

Purpose of 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 goal of our research programme is to identify novel vaccine, chemotherapeutic and immunomodulatory agents useful in combating the neglected tropical disease schistosomiasis. By applying *in vitro*, *in vivo* and *ex vivo* models, we will characterise how selected schistosome biomolecules affect parasite development, mammalian cell phenotypes and host/parasite interactions.

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

There is currently no suitable anti-schistosome vaccine and existing control strategies rely on the effectiveness of a single drug, which has a currently unknown mechanism of action and is incapable of preventing reinfection in endemic areas. The identification and characterisation of immunomodulatory biomolecules provides information relative to the mechanisms schistosomes use to orchestrate long-term survival in infected hosts. This information could be used to direct more effective immune responses during our search for urgently-needed, novel anti-schistosomal drugs or vaccines.

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

The mouse model is the most easily adaptable and appropriate vertebrate definitive host for studying schistosomiasis in the laboratory as it is a fully permissive host, supports the full sexual development of the parasite and produces fully viable, infective larval stages. In addition, the mouse model can easily be genetically manipulated with a wide range of knockout and transgenic lines available. This allows for specific dissection of particular host factors that may or may not be involved in the proposed experimental procedures discussed herein. Finally, the mouse is the most highly tested animal model system (lowest vertebrate group) for determining levels of vaccine-induced protection or effects of chemotherapeutic

treatment in our field. We anticipate using 2450 mice in total throughout the 5-year 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?

Protocol (1) involves the percutaneous or intraperitoneal administration of S. mansoni larvae (obtained from snails) to mice (~1600 mice). In order to generate schistosome parasites of different lifecycle stages for our studies, these organisms must develop and mature in a suitable definitive mammalian host. The expected adverse effect of this protocol will be stress due to handling, irritation due to parasite infection and deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit. Protocol (2) will allow us to assess the immunoprophylactic potential of characterised schistosome biomolecules (~350 animals). Here, we will administer protein or DNA vaccines (intraperitoneal, subcutaneous/intradermal or intramuscular injections) to mice before percutaneously infecting them with schistosomes. At seven weeks after infection, we will assess the efficacy of vaccination when compared to control animals. The expected adverse effect of this protocol will involve stress due to handling, mild discomfort of vaccination/muscle electroporation, irritation due to parasite infection and deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit. Protocol (3) allows us to examine the function of schistosome biomolecules (~500 animals). By using RNA interference (RNAi) or targeted drug treatment, we will be able to generate information critical to our understanding of proteins necessary for intra-mammalian parasite development. Here, in vitro manipulated (using RNAi) parasites will be injected intraperitoneally into mice or infected mice will be treated (intraperitoneally, transdermally or orally) with an anti-schistosomal chemotherapeutic agent. Both of these in vivo manipulations can synergistically be used to assess the importance of key schistosome biomolecules. The expected adverse effects of this protocol include stress due to handling, irritation due to chemotherapy administration or parasite infection and deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit. In all protocols, mice will be subjected to a Schedule one method upon termination of experimental procedures.

Application of the 3Rs

Replacement

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

Replacement

Schistosomes are mammalian parasites and, thus, necessitate a suitable mammalian host for completing studies outlined in our research programme. While we can employ other non-mouse alternatives for some study objectives, this is not possible for *in vivo* determination of novel chemotherapies, vaccines or immunomodulatory agents.

Non-mouse alternatives include *in vitro* parasite culturing manipulations, appropriate *in vitro* and *ex vivo* cell model surrogates to assess how a schistosome biomolecule can affect a mammalian cell, molecular biology manipulation of schistosome genes, comparative genomics/bioinformatics to computationally interrogate schistosome biomolecules, functional genomics to suppress schistosome gene expression, epigenetics to assess schistosome development, biochemistry to assess enzyme activity and proteomics/lipidomics/glycomics to understand the biological nature of key schistosome molecules. All of these non-mouse alternatives will be synergistically used to prioritise schistosome biomolecules prior to performing *in vivo* experiments in the mouse model of schistosomiasis.

Reduction

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

Reduction

To minimise the number of mice necessary to obtain statistically relevant results, power calculations for determining optimal group sizes will be derived, inbred mice will be predominantly used for efficacy experiments and careful planning of all experiments will be performed in consultation with IBERS 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

All animal protocols employed in this license have been carefully developed over the last several decades in laboratories around the world and have been the subject of peer review. Our specific experiences utilizing animal models for the study of schistosome/host relationships have come about from ~25 years of practical experimentation. All procedures use animals obtained from a licensed supplier and all animals are housed in conditions that comply with the Animals Directive Code of Practice (2010/63/EU).

PROJECT 30.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 micro-environment of brain tumours.
Key Words	Brain tumour, immune cells, tumour microenvironment, 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:
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 project aims to study the role of various immune cells during tumour growth in the brain. The immune cells of interest include microglia, which are resident immune cells in the brain. Normally, these cells serve as guardians of the brain and respond immediately to infections and injuries for example. This reaction is essential to remove any abnormalities and to allow brain recovery afterwards. However, during tumour growth in the brain these defensive cells change their behaviour and begin to support the tumour causing it to grow and to spread. The reasons for this are not understood. Furthermore, other immune cells like peripheral macrophages, neutrophils and T cells infiltrate these tumours in addition, generating a very complex microenvironment which further supports tumour growth. Thus, understanding the reasons for this pro-tumoural behaviour of the different immune cells is the essential first step to develop therapeutic strategies to interfere with these cells to finally inhibit tumour growth.

We will study the interactions of immune cells and tumour cells using the zebrafish as a model. Due to the optic transparency of the zebrafish larvae we will be able to observe these interactions in the living animal as they occur in real-time. Work under the current project licence has already shown an immediate response of the brain resident immune cells (microglia) to tumour cells and a variety of direct cellular contacts between these immune cells and the cancer cells. Moreover, we were able to show that these immune cell responses have direct tumour promoting consequences. Using this model, we aim to understand the detailed interplay of different immune cells and the tumour now. Furthermore, due to the good accessibility of the zebrafish larvae for chemicals we can test which drugs influence the interactions of immune cells and tumour cells. Using this strategy, we aim to identify drugs that inhibit the pro-tumoural activity of the immune cells or even induce anti-tumoural activity.

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

Our studies will provide a comprehensive understanding of the different immune cells in the microenvironment of a brain tumour. To understand the regulation of these cells within a tumour provides the basis for the development of future therapeutic interference. Here the obvious aim is to find treatments that interfere with immune cell functions specifically within the tumour environment, instead of applying suppressive treatments for the entire immune systems. Such treatments could become an alternative treatment for brain tumours like glioblastoma, the most common malignant brain tumour, since these tumours still resist standard therapies. Furthermore, our studies will advance the scientific knowledge about the interplay of different immune cells in vivo (in the animal). This is important for the scientific community as the immune cells of investigation here are involved in a variety of other diseases including chronic infections/inflammations and multiple sclerosis for example. Our findings will be made available to other scientists in peer reviewed publications and presentations at scientific conferences.

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

We will use the zebrafish (Danio rerio) for our studies. Adult fish will mainly be kept for breeding whilst most experiments will be performed in larval fish that are between 3 days and 30 days old. A limited number adult fish will be used for experiments. For the duration of the project (5 years) we estimate to keep approximately 22500 adult fish and to use approximately 15000 larvae and 5000 adult fish for experiments.

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

Most adult fish kept for breeding will have a genetic alteration. Generally, this has no deleterious effect on fish health and well-being. Thus, keeping genetically modified zebrafish is does not induce any harm to them. In case a genetic alteration is observed to have a deleterious effect on fish health, these fish will be euthanised immediately and will not be used for our research. Tumour growth will be induced in larval zebrafish by two different methods. The first method is based on transplantation of a low number of human tumour cells into the brain of larval zebrafish and monitoring the responses of the immune cells over the next few days. The transplantation has been optimised over the past years under the current project licence. Nevertheless, additional injuries or infections might occur. Fish will be closely monitored and if abnormalities are observed fish will be euthanised immediately. Injected larvae will be kept alive for one week upon transplantation. During this time the tumour cells do not cause any apparent harm and larval fish behave normally. At the end of the week fish will be humanely euthanized. The second method is based on the activation of specific genes (onco-genes) that lead to tumour growth. These genes are activated in larval fish and tumours can be detected from 1 month of age. These fish might be followed for up to 12 months. The induced tumours appear to be slowly growing and no impact on the welfare of the fish has

been detected. Under the current project licence, fish were monitored until the age of 6 months and did not show any adverse effects. At the end of the experiment fish will be humanely euthanized. To observe the response of immune cells to the tumour and their interplay larval zebrafish might be “live imaged”. For this procedure larval fish will be anesthetised and imaged using a laser scanning microscope. This procedure does not cause any harm to the larval fish and upon completion fish develop normally. To understand the role of a specific immune cell type (T cell) during tumour growth some fish need to be injected into their brain with a substance that causes a mild inflammation. These injections can cause additional injuries of blood vessels for example. Fish will be closely observed upon injections and in case these problems occur fish will be humanly euthanized immediately.

Application of the 3Rs

Replacement

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

Replacement

We aim to study the interactions of cells of the immune system and brain tumours. Since immune cells are very sensitive to any alteration in their environment these studies can not be done in cell culture. The zebrafish larva offers the unique opportunity to observe interactions of the immune system and tumours in real time as they occur in the living animal. This is essential to understand these processes and to develop future therapies.

Importantly, these interactions can be observed in zebrafish larvae before day 5 of development. During these early time points of development, the larvae are not considered as sentient animals. A large number of our experiments will be performed in larval zebrafish below the age of 5 days and these replace the use of adults.

Furthermore, before doing experiments in zebrafish we will test the suitability in cell culture. Only treatments that show clear results in cell culture will be tested in the zebrafish larva.

Reduction

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

Reduction

To strongly reduce the number of animals we will do a large number of experiments between day 3 and day 5 of development before the larva is considered a protected animal. Nevertheless, a certain number of larvae will have to be studied post day 5 of development. Based on studies under the previous project licence on immune

cells in tumours, we have clear expectations of the variability within our experiments. Based on this, we will follow statistical guidelines for experimental design, which ensures that the minimal number of animals is being used to reach significant results. This will ensure that the scientific knowledge on immune cell function within a tumour is increased, without using excessive numbers of animals. In case of necessary drug treatments pilot studies will be performed *in vitro* (not in an animal) beforehand to further reduce the number of animals.

Refinement

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

Refinement

The optically transparent zebrafish larva is an excellent model for studying the interactions of immune cells and brain tumours in the living animal. In particular the opportunity to image cellular interactions as they occur in real time in the living animal, combined with the possibility of intervening genetically and pharmacologically, make the zebrafish the model of choice for our studies. Furthermore, a lot of tumour research is done on mice. Zebrafish represent an obvious refinement here as they are the vertebrate model of the lowest perceived sentience.

We will minimise animal suffering by performing the majority of experiments in larval zebrafish and only a limited number of adult fish will be used.

To minimise animal suffering zebrafish will be anaesthetised during experimental procedures and will be allowed to recover afterwards. One of our methods to induce tumour growth is the transplantation of human tumour cells into the larval zebrafish brain. Upon transplantation of human cells zebrafish larvae will be kept at 33-35 C to ensure survival and growth of the human cells. These temperatures do not affect welfare of the larvae and have also been found in natural habitats of zebrafish. Furthermore, our work under the previous project licence has confirmed that zebrafish tolerate these temperatures well. Transplantation protocols have been optimised under the previous project licence in a way that only the minimal number of tumour cells required for the experiments is transplanted. Thus, adverse effects due to the injection could be mitigated. In case the injections cause adverse effects (e.g. damage to membranes or vessels), these will become obvious within the first hours post transplantation and larvae will be humanely euthanized to prevent them from experiencing further harm.

To understand the role of a specific subset of immune cells (T cells) during tumour growth in the brain we need to use adult zebrafish as larval zebrafish don’t have

these cells. For these studies we need to inject specific substances into the brain to attract T cells. These injections might damage membranes or vessels in the brain and up to 16% of injected fish might not survive the procedure. In case these problems occur, it happens within the first hours post injection and no additional adverse effects have been detected in the following days upon injection. In order to minimise the harm to the animals, great care will be taken that only optimally fed animals go into experiments, e.g. fish will get extra food during the pre-experimental days. Fish will be checked by the experimenter at least three time a day during the first

three to five days after injection. After that, the wellbeing of fish will be checked at regular intervals during feeding (usually twice, but at least once per day). Fish will be closely monitored for changes in movement, feeding patterns, and other signs of distress.

All animals will be closely monitored during the course of experiments and in case unexpected signs of stress are observed fish will be presented to a Vet immediately.

PROJECT 31.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 normal and malignant haematopoietic cells
Key Words	Leukaemia, stem cells, haematopoiesis
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Acute leukaemia is a type of cancer of the blood forming cells. There are around 5000 cases each year in the United Kingdom and despite modern treatments the great majority of patients still die from their disease. There is therefore a large unmet need for new treatments for patients as well as a need for greater understanding of disease processes which enable the design of such treatments. In particular, acute leukaemias are driven by disease-causing stem cells which must be completely eliminated in order to cure patients. The purpose of this project is to develop greater understanding of these leukaemia stem cells and to determine how they differ from normal blood forming stem cells. Through understanding these differences we will be able to identify and develop new treatments to bring into the clinic for the benefit 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)?

Expected benefits include new knowledge about which genes and cellular pathways are most important in leukaemia and discovery of new treatment targets to develop into the clinic for patient benefit in the longer term.

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

2000 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, such as X-ray treatment of mice, blood or bone marrow sampling, or injection of cells and compounds are not associated with significant side effects and are of mild-to-moderate severity. Mice injected with blood cancer cells will, when the disease develops, exhibit signs of disease, such as hunched posture, poor levels of socialising and interaction. Under these circumstances, and whenever else a

mouse displays features of ill health, or at the end of each experiment, mice will be humanely killed using a Home Office sanctioned method.

Application of the 3Rs

Replacement

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

Replacement

Blood-forming stem cells are defined by their ability to make normal or leukaemic bone marrow cells following their transfer in to a second animal. Cell culture experimental systems are insufficient for this purpose because they do not provide the required environment to grow a new tumour or normal bone marrow system This is because blood forming tissue is very complex and involves lots of interactions between many different cell types which cannot be reproduced in cell culture. Without the use of mice, the biology of blood forming cells cannot be studied properly.

Use of mouse models of leukaemia is important for a second reason. One of the problems of experiments using human leukaemia cells is that the cells vary from person to person making it difficult to study of the effects of specific mutations. By contrast, mouse models of human leukaemia enable investigation of the biological effects of specific mutation in a controlled and informative way.

Reduction

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

Reduction

Use of mice will be minimised by (i) making use of cell culture model systems wherever possible, (ii) using imaging systems or bone marrow sampling to follow disease development in real time (rather than culling groups of mice at certain time points), (iii) careful experimental design with the help of a statistician, (iv) using pilot experiments before a full scale experiment, (v) using protocols for each experiment which include the objective, interventions, numbers of animals and analysis method (reviewed in every case by the licence holder REDACTED for experimental rigour and the 3Rs) and (vi) freezing down in multiple aliquots of leukaemia samples thus eliminating a requirement for continued production of cohorts 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

Mice have been chosen for the study because they represent the least sentient species from which meaningful experimental data can be generated, while exhibiting considerable genetic and biological similarities to humans with regard to their blood forming system. Only a mammalian blood cell generation model system has the potential to accurately mimic both the anatomy and complex cell biology, including microenvironmental interactions, of human normal and leukaemic blood cell generation. Furthermore, there is considerable experience in the wider scientific community regarding the use of mice as a model system for human diseases of the blood and many reagents exist for the phenotypic characterisation of mouse cells.

The techniques used have been carefully evaluated to minimise distress to the animals. Mice used in surgical procedures will be treated with anaesthesia, analgesia and post-operative rehydration by subcutaneous injection, followed by careful observation. In other areas, irradiation doses will be administered at a level sufficient to induce bone marrow suppression but no other long term impact; bone marrow injections and aspirates will be not be performed routinely, only where the scientific justification is high; and in studies that result in the initiation of leukaemia, mice will be closely monitored for health status and killed by a Home Office approved method when signs of ill health are displayed.

PROJECT 32.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 microenvironment in organ homeostasis and disease
Key Words	Liver regeneration, tissue repair, 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:
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):

Liver disease in the UK is increasing in prevalence. Currently the only treatment for end stage liver disease is liver transplantation. As a result, the number of livers available for transplantation is much lower than required. The aim of this work is to find out 1. How liver disease occurs, 2. How the liver repairs itself following injury and 3. What happens when regeneration goes wrong: does it lead to liver cancer formation? By understanding these processes we can design drugs to specifically target these processes with the ultimate goal of preventing disease and enhancing repair in the liver; thus eliminating the requirement for 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 benefits of this work primarily focus on liver disease and the treatment of such. These includes: 1) the understanding of the adaptive immune system during liver disease and regeneration. 2) Interventions that could promote regeneration. 3) Potential drug targets for chronic liver disease However, many of the processes which occur during liver disease also occur during disease in other tissues so our work in the liver will contribute to disease progression in a range of organs.

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

We will use mouse models of liver injury as our main models. These include genetic modified animals which enables the labelling of specific cell population or the specific ablation of T regulatory cells. Dietary and chemical models of liver injury will be used to simulate different aspects of human liver disease. The planned work will be conducted over five years and we will use a maximum of 8000 animals

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

As we are modelling liver disease, our animals will progressively demonstrate symptoms of the liver disease under the models that simulate chronic liver injury (with dietary or water). For example, weight loss, lack of mobility, become very thin, jaundice. These resembles the symptoms of human chronic liver disease such as liver fibrosis and fatty liver disease. The disease related symptoms will persist during the injury model regime, which range between 2- 6 weeks. In some situations, animals will experience interventions such as injections through intraperitoneal or intravenous, blood sampling from peripheral vein. Animals might experience temporary pain due to the injections but will return to normal behaviour rapidly. The expected level of severity in these liver injury model are categorised as moderate severity. However, we will closely manage these symptoms such as monitoring animal body weight, behaviours, and condition of fur coating to ensure that the animals do not undergo any undue suffering. For animals that undergo intraperitoneal or intravenous injections, extra caution will be taken when performing injections to prevent injury to other organs of the occurrence of haemorrhage. The animals that received liver injury models or ablation of immune cells may experience loss of weight, lack of mobility, and abnormal condition of coat such as piloerection. Animals weight and condition will be monitored frequently, such as: if weight loss exceed 20%, prolonged piloerection, hunched postures, animals will be humanely killed if exceeded the stated severity limit.

Application of the 3Rs

Replacement

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

Replacement

Regeneration in the liver is a complex, multi-staged process in which there are many different cell types interacting with one and other. It is impossible to model such complexity without using animal models. However, most experiments will be carried out in the laboratory at cell level before experimenting on live animals. Throughout the project, we will constantly, seek, review and incorporate alternatives to replace the need for animal studies. For example, in- co-culture experiments to confirm preliminary data, targets, and effect of small molecules/ recombinant protein before for the use *in vivo*.

Reduction

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

Reduction

It is possible to calculate the numbers of animals required for experimentation based on data from previous experiments and results. In all cases we ensure that we have

calculated the minimum number of animals required for the experiment to give us useful data. This approach reduces the animal numbers required, and also reduces the likelihood that the animal experiment would have to be repeated. We also try to develop new models (genetic altered and non-genetic altered) to reduce the number of animal used. For example, we have developed Cas9 expressing cell lines to enable CRISPR-Cas9 genetic screening in vitro to reduce the number of animals used for the programme of work.

For example, we have developed Cas9 expressing cell lines to enable CRISPR-Cas9 genetic screening in vitro to reduce the number of animals used for 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 objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

Our project aims to aim to investigate the effect of dysregulated immune system during liver disease using mouse models. The disruption of the immune system tends to occur during human chronic liver disease, and it is inevitable to use mouse models (both transgenic and non-transgenic) to simulate human chronic liver disease which takes several years to develop in human. We typically use mice for experiments as they model accurately human diseases and organ injuries. Genetic altered mouse model are widely available. We regularly refine the disease models we use to reduce animal symptoms and to improve the effectiveness of our models. For example we are proposing pilot studies with a small cohort to identify the optimal dose for Diphtheria Toxin (DT) administration for our studies to reduce the suffering of animals whilst providing useful information simulating the disease phenotype.

The animal models of chronic liver injury we used in this study (both dietary and transgenic) are well established and published across the field. However, we will perform small scale pilot studies for experiments such as altering the immune system with the FoxP3-GFP-DTR mice. The optimal regime to alter the immune system without triggering systemic autoimmunity will be determined before using this protocol in conjunction with other liver injury model, as we do not intend to trigger systemic autoimmune response in our study The behaviour of animals and signs of discomfort will be monitored throughout the injury regime, we will constant seek improvements on our current protocols through searching published protocols or exchanging knowledge with researchers within the field to reduce discomfort and establish models that are more relevant to human chronic liver disease.

PROJECT 33.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 antimicrobial agents for bacterial pathogens of livestock: light activated CO-releasing molecules
Key Words	Antimicrobial, E. coli
Expected duration of the project	5 year(s) 0 months

Purpose of 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 test the effectiveness of new antimicrobials (CORMs) against pathogens of importance to veterinary and human medicine.

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

The work conducted in this project will increase the knowledge of the mechanisms and effectiveness of these new antimicrobials. If effective, there is potential for a new class of antimicrobials to be taken forward for clinical trials. Due to antimicrobial resistance the list useful antimicrobials is reducing. These new antimicrobials, could increase our arsenal.

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

A pilot study will be undertaken using 2 groups (n = 18) of SPF chicks. Birds will be orally dosed within the first week of hatch with a defined E. coli strain. At 21 days old 1 group will be orally dosed with the antibacterial CORM. At 3 days, 2 weeks and 3 weeks post-treatment 6 birds/group will be killed by a Schedule 1 method and necropsies of the birds performed. Quantitative bacteriology for the dosed strain will be done on spleen, liver and caecal samples obtained post mortem. From previous projects, it is estimated that if pilot studies show promise, up to 300 birds may be used over the course of the project (5 yrs).

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

There is a risk of infection from the colonisation with avian pathogenic E. coli. For initial studies a strain will be used that has been used on numerous occasions in the past with no clinical disease observed. If the treatments appear effective, further studies may use strains with more potential to cause disease. However, birds will be regularly monitored and a score-sheet used to record any symptoms. In the event

where animal welfare appears to be compromised, animals will be euthanized early. At the end of the experiments, all chicks will be euthanized humanely.

Application of the 3Rs

Replacement

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

Replacement

Previous lab work using an insect model (*Galleria mellonella*) has led to a reduction in total protected animal numbers, to be used in this study e.g. toxicity studies were performed in the insect model and cell culture assays.

Reduction

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

Reduction

All experiments will be thoroughly planned in consultation with a qualified statistician to accurately determine the minimum number of animals required to give statistical significance.

Refinement

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

Refinement

Chickens have been selected as they are the prime host for the pathogen under investigation, avian pathogenic *E. coli*. For the initial study a bacterial strain and dose level will be used that has been used on numerous occasions in the past which colonises but is essentially commensal and non-pathogenic in birds. Animals will be monitored closely. In the event where animal welfare appears to be compromised, animals will be euthanized early.

PROJECT 34.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	Exploiting Immunological Tolerance for the Future of Regenerative Medicine
Key Words	Stem cells, Degenerative disease, Regenerative medicine, Allograft rejection, Dendritic 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):

Over the past 80 years, we have witnessed an unprecedented increase in life expectancy. While this may be considered a significant medical success story, extended longevity has brought with it a substantial increase in incidence of chronic and degenerative diseases, such as heart disease, diabetes and Parkinson's disease, which rob those affected of quality of life and productivity. Against this backdrop, stem cells offer the attractive possibility of providing a plentiful source of cell types and tissues to replace those that have become diseased or defective through the natural process of ageing. In particular, recent advances have created so-called 'pluripotent' stem cells that display the unique capacity to differentiate into any one of the ~200 cell types that make up the human body: a single line of induced pluripotent stem cells (iPSC) could, therefore, be used to treat numerous, unrelated diseases. The single greatest barrier to implementing this vision of regenerative medicine is, however, the immune system of the recipient which is poised to reject the replacement tissues. The aim of this project is, therefore, to investigate the viability of a new approach to intervening in rejection which exploits the properties of a cell type, called dendritic cells (DC), which have been shown to guide the immune system to tolerate foreign tissues, thereby establishing a state of 'immunological tolerance'. 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)?

If successful, our approach to tolerance induction may help facilitate the future use of iPSC in regenerative medicine as well as curtailing unwanted immune responses to therapeutic proteins. The lysosomal storage diseases, for example, constitute a significant unmet medical need caused by the absence of specific enzymes that normally clear waste products from cells. Those affected by such conditions rarely survive beyond childhood. Although administration of the missing enzyme can be curative, the immune system perceives the enzyme as foreign and mounts an immune response against it. We therefore propose to exploit our capacity to produce

abundant tolerogenic DCs to intervene in the induction of immune responses to the missing enzyme, thereby establishing a profound state of tolerance. REDACTED

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

Over the course of the next five years, we anticipate breeding a maximum of 17,000 mice in order to obtain all the necessary data to enable us to apply for regulatory approval to begin first-in-man trials. Of these mice, we estimate using approximately 1,000 per annum (5,000 mice in total) to demonstrate robust proof-of-concept.

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

The majority of mice used in this project will be either wild type or genetically altered in ways that will have no discernible effect on either their health or welfare. Future experiments may benefit from the importation of strains of mice in which the genetic modification impacts in predictable ways on their health status, however we currently have no plans to do so. The first experimental model we propose to use involves the grafting of a small portion of tail skin from a suitable donor to the flank of a recipient mouse. Skin grafting does not require an incision through the body wall or sutures and rarely results in any adverse effects beyond the pain and discomfort of the initial procedure which is carefully managed using standard pain relief until the wound has completely healed. The second model involves the implantation of tissues differentiated from iPSC under the fibrous capsule that surrounds the kidney. This site is highly vascularised and supports rapid engraftment whilst also providing a useful readout of rejection. Implantation under the kidney capsule is more invasive, requiring an incision through the body wall to expose the kidney which is subsequently repaired with sutures. Adverse events are likewise rare: occasionally the implanted tissue may grow more rapidly than expected and cause distension of the abdominal wall. The third model will only be used on rare occasions and will involve the immunization of mice with a defined protein antigen using an appropriate adjuvant. Adverse effects are typically transient, such as a localised inflammatory response. Any mice showing signs of pain, suffering or distress in response to any of the models used that cannot be resolved with the help of the vet, will be humanely killed, as will all animals, once the necessary data have been collected.

Application of the 3Rs

Replacement

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

Replacement

The rejection of foreign tissues is a complex physiological phenomenon that cannot be recreated *in vitro* due to the dynamic architecture of the lymphoid organs, where the immune response is first initiated. Although certain features of immune cells *in vitro* may be predictive of their involvement in rejection, *in vivo* models are ultimately required to provide unequivocal proof of our ability to intervene in this process by setting in place a state of immunological tolerance. Nevertheless, we shall continue to replace the use of mice where possible: we have, for instance, introduced *in vitro* techniques to demonstrate the pluripotency of any new iPSC lines we produce, so as to avoid the need for formation of teratomas in recipient mice.

Reduction

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

Reduction

We have identified the greatest source of variability between experiments to be variation between the batches of DC we produce. We shall, therefore, subject each batch to stringent characterisation before embarking on *in vivo* experiments in order to exclude any that do not comply with the expected profile, thereby reducing the need for repetition of experiments. Furthermore, we shall use appropriate power calculations and statistical blocking techniques to maximise the information we are able to gain from each experiment and ensure that the minimum number of mice is used to reach statistical significance.

Refinement

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

Refinement

Our choice of mice is guided by two principles: firstly they represent the lowest vertebrate species whose immune system is sufficiently similar to that of humans to enable us to derive predictive data, and secondly, genetically modified strains and molecular tools are available that permit us to answer fundamental questions about graft acceptance or rejection. We plan to refine our techniques during the course of this project to incorporate any amendments that are likely to be beneficial to the experimental mice, such as the introduction of inhalation anaesthesia, from which they recover
more rapidly, the replacement of Complete Freund's Adjuvant with next generation a djuvants such as TitreMax, known to reduce non-specific inflammation, and the use of appropriate pain relief following surgery.

PROJECT 35.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	Discovery DMPK Studies for New Chemical Entities
Key Words	DMPK, New Chemical Entities, Pharmacokinetics, ADMET, Drug discovery
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):

To provide a complete package of high quality, robust and incisive pre-clinical pharmacokinetic data (what the body does to the drug) using ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicology) studies that will facilitate the rapid identification and selection of the best candidate drugs for further development and avoid any unnecessary and wasteful *in-vivo* tests being applied to inappropriate compounds.

These approaches have been used in the past 20 years by most organisations involved in life-science R&D within UK, Europe and USA.

What are 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 is greater success in clinical trials from more carefully selected compounds and a long-term reduction in the lead-time for a new therapy to reach patients. This will deliver a greater potential to save lives, alleviate suffering and reduce the incidence of adverse effects experienced with existing therapies.

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

For the duration of this licence we will be using rodents only, specifically mice, rats and guinea pigs. For the duration of this licence it is anticipated that we will use 23450 mice, 10350 rats and 160 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?

From non-surgical context, the animals will be dosed with NCE's and sampled for DMPK data output to meet study objectives. From a surgical context, the animals will be surgical prepared to facilitate sampling (e.g. with Jugular Vein Cannula for blood sampling or Microdialysis probes for localised brain sampling) for DMPK data output post dosing with NCE's to meet specific study objectives. The expected adverse effects from the non-surgical and surgical models to be used under this licence are a) mild effects associated with the administration of novel compounds, such as hyperventilation and sedation that will not require further intervention or treatment, and b) moderate severity adverse effects due to the animals undergoing surgical procedures, such as pain and discomfort requiring pain relief and fluid replacement. If any adverse effect is observed, which is more than transient, the animals will firstly be treated to alleviate the unwanted symptoms. In cases where the symptoms persist, after consultation with NACWO (Named Animal Care and Welfare Officer) and Vet appropriate measures will be implemented that including termination by schedule 1 method. At the end of all protocols in this licence the animals will be killed humanely 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

Currently, there is still a prerequisite by governmental authorities for new chemical entities (NCE's) to be tested *in-vivo* ADMET studies before first-in-man studies can be started. Also, current *in-vitro* systems lack the complexity of *in-vivo* models and cannot be used as a predictive tool to fully replace *in-vivo* studies. Therefore, animals are still required to assess pre-clinical pharmacokinetics and are an essential part of drug discovery and development research.

Reduction

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

Reduction

In our aim to reduce the overall number of animals used for the duration of this licence, and as a good scientific principle, we always insist that there is a well established scientific rationale for undertaking any *in-vivo* study and that prior *in-vitro* data supports such studies. This careful selection of test compounds from *in-vitro* screening studies ensures that only those compounds with a positive profile for efficacy/potency, physic-chemical properties, metabolic profile and toxicity evaluation will be taken forward for use in regulated procedures in the species of the lowest acceptable order (i.e. rodents).

As part of our commitment to minimise animal numbers, we will design our studies in such a manner that the maximum information can be obtained and where possible serial samples are taken from the same animal (i.e. by vascular cannulation) so as to negate the need for extra animals or future additional or repeat studies.

The number of animals used for *in-vitro* assays in each experiment will be the minimum needed to provide sufficient cells or tissues. Additionally, we will use the minimum number of animals for the determination of pharmacokinetic parameters so as to achieve statistically relevant data.

In some cases, it may be possible to limit animal numbers by reducing the size of control groups, sharing control groups or using control groups for analysing multiple 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

Non-surgical and surgical rodent models are the research species of choice in drug discovery and development due to their size and substantial amount of literature data already available. These models have been used and validated extensively, and have provided much of our knowledge to date in ADMET studies that are requested for use on this licence to generate the package of high quality, robust and incisive pre-clinical pharmacokinetic data that we aim to provide. They can provide a correlation to humans, such as forming similar metabolites or exhibiting responses to a treatment are much more reflective of humans and in such cases we will ensure the most relevant species are always used.

The choice of animals are also dictated by the governmental bodies as they demand that prior to first-in-man studies, pharmacokinetic data is provided in two species, rodent and a non-rodent, to ensure that the drug is suitable for human use.

We will continuously monitor the literature to implement the latest animal husbandry and environmental enrichment legislation and practices. Furthermore, we will minimise animal suffering by using the most advanced technologies where possible, like non-invasive imaging, and by using appropriate anaesthetics, pain relief and infection controls.

PROJECT 36.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 Production of Antibodies
Key Words	Antibody, Polyclonal, Monoclonal, Immunogen, Antigen
Expected duration of the project	2 year(s) 3 months

Purpose of 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 provide a service for the production of antibodies for both the medical research community and diagnostics manufacturing industry within the UK and Europe. 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 disease and their use as critical tools in the areas of research and medicine, including basic research of cells and their function in disease, diagnostic technology and therapeutic medicine 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)?

Antibodies routinely help scientists to research the function of both healthy and abnormal cells in disease, by the detection of proteins within the cell 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 a clinical environment. This can allow for the rapid diagnosis of life threatening disease and assist in providing clinicians (clinical Scientists and Doctors) with specific information in terms of the most appropriate course of treatment to follow, thus preventing death. The use of antibodies in therapeutics is a fast developing area of medicine, with the use of antibodies as constituents of direct medicine in order to treat various diseases including cancer, auto-immune disorders and infection.

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

Rat 3,500 (5 Years) Mouse 6,500 (5 Years) Guinea Pig 2,500 (5 Years) Rabbit 12,500 (5 Years) Chicken 750 (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. When raising antibodies against DNA, special technology has been developed to do so. This technology involves the use of DNA coated gold particles which are introduced to the animal via bombardment of the skin with pressurised gas. This procedure is carried out under general anaesthesia and has minimal associated effects, which can include slight redness of the skin at the site of inoculation. The production of antibodies against bacteria requires the inoculation of a bacterial liquid(without adjuvant) direct into the blood stream. This methodology can result in the loss of animal body weight and (rarely) the onset of symptoms that appear similar to that of an allergic reaction e.g. laboured breathing, reduced mobility and redness of the eyes with associated light sensitivity. 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. All animals used for the production of antibodies 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 (monoclonal) antibody secreting cells.

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 and Chicken will be made available for the production of antibodies. Selection of a specific species , from one of the above 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 the production of antibodies. 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 the field of antibody production.

PROJECT 37.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 metabolic disease and its complications
Key Words	Diabetes, Obesity, Complications, Liver, Kidney
Expected duration of the project	5 year(s) 0 months

Purpose of 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 licence is to discover new drugs for the treatment of metabolic disease, which includes diabetes, obesity, non-alcoholic steatohepatitis and kidney disease which is caused by poorly controlled metabolic disease. There are currently 425 million people worldwide with diabetes, 1 in 2 remain undiagnosed, that equates to 1 in 11 people having diabetes. In 2016, it was estimated that 1.9 billon people are were over-weight, of which 650 million were obese. For kidney disease, over 70% of patients with diabetic kidney disease are dead within 5 years of diagnosis. There are no drugs available for chronic kidney disease, and drugs to treat diabetes and obesity are insufficient to adequately control the disease, as their prevalence in continually increasing. Therefore, new drugs are needed combat these debilitating 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 program of work is expected to identify and select approximately 6 potential new therapeutic agents with demonstrable efficacy in animal models of metabolic and kidney disease for clinical development. This will provide enormous potential future benefit for new treatment options above standard of care for patients with metabolic and kidney disease. In addition, we will be able to advance our understanding of the underlying cause of these diseases. Furthermore, with our intention to publish our research findings in publicly available journals will enable other external researchers to benefit from our studies.

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

We will use rats and mice, but predominantly mice, during this program of work. Some animals will be genetically altered to modulate a specific gene, or to produce a specific disease state such as diabetes, obesity or high blood pressure. We expect

to use approximately 7000 mice and 1000 rats for diabetes, obesity and non-alcoholic steatohepatitis work and 5400 mice and 1900 rats for chronic kidney disease work 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?

1. Healthy or diseased animals (including genetically altered animals) will be used to harvest cells from different organs for ex vivo studies and will experience mild or moderate severity, respectively. Healthy animals will not experience any adverse effects. Diseased animals will experience weight gain, high blood sugar, high blood pressure, excessive urination which will last for the duration of the experiment but will not reach a level that is likely to cause pain or death of the animals within the timeframe to the experiment. 2. In vivo healthy animal studies: young adult animals will undergo the following interventions: a) Administration of substances by either of the following routes i) Oral, under the skin, into the abdominal cavity, directly into the blood stream or into the brain. Some of these will require a surgery under general anaesthesia. ii)Under the skin administration might require placement of a mini-osmotic pump, typically this is done by making an incision approx. 1 cm into the skin and inserting the mini-pump under the skin and then the animal will be sutured and allowed to recover. iii)Administration directly into the blood stream may require placement of a permanent cannula into a blood vessel. This is typically done by a small incision approx. 1 cm in the skin over the vessel to be cannulated and the vessel cleaned of connective tissue. The vessel is then ligated, and small incision made in the vessel and the cannula then inserted into the vessel and secured by a ligatures and tissue glue. The animal will be sutured and allowed to recover. iv)Administration directly into the brain will require placement of permanent cannula into the brain. This will typically be done by making a small incision in the skin, precisely locating the cerebral ventricle and drilling a hole through the skull to insert a cannula directly into the brain. The cannula is maintained in place by use of surgical polymer and the animal is allowed to recover. b) No animals will undergo more than 2 surgeries. c) Measure of body function i)Blood pressure, kidney function and metabolism d) Imaging under single or repeated general anaesthesia e) Animals could be singly housed f) Body fluid collection i)Blood and urine. g) Animals will be killed by a humane method and tissues taken for analysis after death by highly trained and competent individuals. h) Impact on animal experience: i)The overall impact is moderate ii)Transient pain associated with blood withdrawal or substance administration iii)Pain associated with surgery iv)Stress associated with isolation from single housing v)Transient stress associated with restraint for blood pressure measurement vi)We do not expect any animal to die because of these procedures. I) Mitigation for impact on animal experience: i)Animals are expected to recover quickly from the surgeries. Post-surgical pain will be monitored at least daily and alleviated using painkillers ii) Use of environmental enrichment in housing to relieve stress of isolation iii) Acclimation to restraint before blood pressure measurement 3. In vivo

disease model studies: young adult animals will undergo the following interventions: a) Induction of disease using either genetic alterations, modified diets or surgical procedures under general anaesthesia (removal of one kidney, removal of 5/6 of kidney mass or obstruction of urine flow). Typically, for the removal of one kidney a 1cm incision will be made in the flank of the animal, and the kidney will be removed, and the animal will be sutured and allowed to recover. For the removal of 5/6 of kidney mass, a second surgery will be performed in which an incision will be made in either the abdomen or flank and part of the remaining kidney will be removed and the animal will be sutured and allowed to recover. For the obstruction of urine flow an incision will be made in the flank of the animal and ligatures will be placed around the ureter after which the animal will be sutured and allowed to recover. b) Control groups will contain healthy animals or sham-operated animals where appropriate c) Administration of substances by either of the following routes i) Oral, under the skin, into the abdominal cavity, directly into the blood stream or into the brain. Some of these will require a surgery under general anaesthesia (surgeries detailed in paragraph 2.) d) No animals will undergo more than 2 surgeries. e) Measure of body function i)Blood pressure, kidney function and metabolism f) Imaging under single or repeated general anaesthesia g) Animals could be singly housed h) Body fluid collection i) Blood and urine. g) Animals will be killed by a humane method and tissues taken for analysis after death by highly trained and competent individuals. h) Impact on animal experience: i) The overall impact is moderate. ii) Weight gain, high blood sugar, high blood pressure, excessive urination will last for the duration of the experiment and will not reach a level that is likely to cause pain or death of the animals within the timeframe to the experiment. iii)Transient pain associated with blood withdrawal or substance administration iv) Pain associated with surgery v) Stress associated with isolation from single housing vi) Stress associated with exposure to cold temperature vii) Transient stress associated with restraint for blood pressure measurement viii) We do not expect any animal to die because of these procedures. I) Mitigation for impact on animal experience: i) We will not mitigate the clinical signs of disease because we will test the ability of the drug substance to reverse them. However, diseased animals will be monitored closely for the inability to feed, groom, nest, walk or breathe normally. Presentation of these signs will result in animals being humanely killed. ii)Animals are expected to recover quickly from the surgeries. Post-surgical pain will be monitored at least daily and alleviated using painkillers iii)Use of environmental enrichment in housing to relieve stress of isolation iv) Exposure to cold temperature will not be mitigated because this is a required component the experimental design. v) Acclimation to restraint before blood pressure measurement

Application of the 3Rs

Replacement

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

Replacement

There is currently no in vitro or in silico system capable of simulating complex whole animal physiology and metabolism. Metabolic and kidney diseases have a complex pathophysiology with multiple components interacting to manifest the disease. Our therapeutic agents target specific biochemical responses or physiological mechanisms that in vitro systems cannot replicate.

Individual mechanisms can be probed in vitro, and we conduct extensive studies to characterise these as far as possible before conducting in vivo experiments. In this case we expect to access human tissues and cell lines and use these to understand at a basic level what mediators and mechanisms are involved.

Regulatory authorities such as the FDA and EMEA require compelling data packages to support the development of a new medicine in humans. In vitro potency data are seldom sufficient to provide confidence of efficacy in man, and demonstration of activity (and mechanism) in animal models is becoming increasingly important.

We are currently implementing the use of human kidney organoids which reflects the complexity of the human organ in a dish. We anticipate that characterising these and investing in this technology will enable less animals to be used in the future.

Reduction

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

Reduction

We will use relevant statistical tools (e.g. power analysis) to guide the design of our studies. Reference will be made to key texts (e.g. Festing, The Design of Animal Experiments, RSM Press 2002).

Study designs will be consistent with accepted scientific methods, and will include relevant positive and negative controls as applicable. For example, we will minimise unwanted sources of variability by ensuring that wherever possible experimental and control animals are studied side-by-side on the same day by the same person.

We have access to in house statisticians with whom we consult as necessary when planning in vivo studies.

Refinement

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

Refinement

Mice and rats are small and easily handled species with a highly characterised immune system and well-defined biology.

Mouse and rat models of metabolic and kidney disease have been established by other groups and reported in the literature.

The inclusion of mice enables us to use mutant or genetically modified animals for early hypothesis testing, target validation and humanization of target as necessary.

Our models will be the minimal severity possible to answer the scientific question being studied. Pilot studies will be conducted for new protocols to ensure the methods used provide for the maximum animal welfare in relation to the experimental objective. We will also aim to implement new ways, as technology evolves, to further improve the welfare of the animal during the course of these experiments (e.g by embracing non-invasive measurements).

Best practice, for example the use of analgesics after surgical implantation of continuous delivery devices, will be employed to minimise suffering.

PROJECT 38.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	Animal Models of Fibrotic Disease
Key Words	Fibrosis, Kidney, Scleroderma, Liver, Lung
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Fibrosis is a harmful process that occurs with many diseases, and in which normal healthy tissue is replaced with scar tissue, compromising function and ultimately leading to organ failure. Despite its low profile in public consciousness, fibrosis is associated annually with an estimated 45 percent of all deaths in the developed world. The organs that are mostly affected include the kidney, liver, lung and skin.

The overall objective of this project licence is to identify new medicines for the treatment fibrotic diseases such as idiopathic pulmonary fibrosis, diabetic kidney failure and fibrotic liver disease.

A large amount of animal models exist to replicate the multiple disease phenotypes that exist in the human population. This licence contains models that have been proven to be robust and replicate different aspects of human 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 expected to identify novel medicines and along with a better understanding of the fibrotic disease process in diseases such as idiopathic pulmonary fibrosis, diabetic kidney failure and fibrotic liver disease 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 and rats) along with 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, followed by 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 animals will undergo procedures that may involve injections and surgery after which they may experience moderate discomfort as they develop symptoms of disease. All animals will receive pain relief as specified by a veterinarian to alleviate their pain. Overall the level of severity for procedures in this project is moderate. Animals may undergo procedures that mimic kidney fibrosis such as a unilateral ureteral obstruction model or an Adriamycin induced model. They may display signs of kidney disease such as protein in the urine, a rise in serum creatinine and high blood pressure leading to kidney remodelling through the development of fibrosis. These aspects of disease may lead to weight loss and chronic kidney failure. Animals approaching end stage kidney function will be identified based on clinical indicators and the animals removed from protocol before suffering reaches the upper limits of the moderate severity banding. Animals undergoing lung fibrosis models, such as the Bleomycin induced model, may lose weight and display signs of laboured breathing which is consistent with the human condition. Animals will be monitored based on clinical indicators and the animals removed from protocol before suffering reaches the upper limits of the moderate severity banding In the liver fibrosis model following injection of CCl4 animals may experience transient drowsiness for a period of less than 10 minutes post-injection. Animals will be removed from protocol should they display any signs of jaundice. 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

At present, there is no alternative in vitro technology that replaces the need to use animals since there is a requirement for all components of the fibrotic pathway to be present to accurately model these chronic fibrotic diseases. Fibrosis is a complex interplay between many cell types which are heavily influenced by the immune system, loss of organ function, deposition of extracellular matrix and haemodynamic events. Currently this simply can’t be recreated in culture.

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. 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.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 and rabbits are the mammalian species of lowest neurophysiological sensitivity in which these fibrosis models have been developed.

Several 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 is achieved with minimum stress to animals. Humane endpoints are employed to limit suffering and disease burden.

Refinements to disease models are continuously assessed and applied where appropriate.

PROJECT 39.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 behind T-cell function and regulation
Key Words	T cells, Immune regulation, 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):

T cells are an important population of white blood cells that protect us from infections and cancer. T cells sense germs or damaged cancer cells through a specialised structure, called the T cell receptor (TCR). Each TCR is unique, enabling T cells to respond to any potential threat to the body. As there exists both a large number of T cells and many unique TCRs in the body, this presents a great challenge to study. Scientific approaches in the past have used techniques to label T cells with green proteins when their TCRs sensed a threat. However, these green proteins can remain in a cell for up to a week, many days after a T cell has responded. We have developed a new Tool, which labels T cells temporarily blue (within hours of TCR sensing a threat), meaning we can follow T cell behaviour with much greater precision. Using this system, we aim to reveal how T cells behave under normal healthy conditions, as well as in diseases such as allergy, infection and autoimmunity. In addition, we will look to understand how drugs may alter the function of T cells in autoimmune disease.

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

This project will advance scientists' understanding of how T cells respond during allergy, infection and autoimmunity. In addition, the breeding protocol will create new tools for use by others within the scientific community to take forward their own research. The project has the potential to identify new targets on T cells that we could design drugs to alter how T cells respond to threats in the body. This could help the development of future immunotherapies – which are drugs designed to alter how the immune system works. In addition, a key objective of the proposal is to investigate how a particular type of immunotherapy – called peptide therapy – works through altering the behaviour of T cells. This could better inform strategies for treatment of autoimmune diseases, such as multiple sclerosis, diabetes and arthritis.

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

The project will use both normal and genetically modified mice (which express the blue colour when T cells are activated). Over a period of five years, it is anticipated that a total of 3000 mice will be bred in order to address the aims and objectives of the project. In addition, of these mice, 2000 are anticipated to undergo direct procedures.

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

To study allergy, a refined model is used which results in a slight reddening and thickening of ear skin 24 hours after applying a substance to the skin. In addition, to test T cell responses during infection, we will use a well-established model that does not result in clinical symptoms for mice. Infection will be performed via infection with droplets in the nose, with the mouse anaesthetised for a short period of time to minimise suffering. Multiple rounds of anaesthesia will be avoided to reduce the cumulative harm to mice. For studying T cell responses during autoimmunity, it is necessary to use a mouse model of multiple sclerosis (mMS). These models require the injection of proteins immersed in oil containing heat killed bacteria under the skin of mice. The majority of these studies will look at the early stages of mMS, before more severe symptoms set in. These experiments will be restricted to no longer than 2 weeks duration to assess T cell responses during the initiation of mMS disease. However, to test drugs to potentially prevent mMS, a small group of mice may experience moderate symptoms (such as paralysis of tail and back legs). This is unfortunately necessary in order to reveal whether drugs are effective at preventing or improving disease. In some experiments, mice will receive substances in the diet, or be given orally, that can alter genes within T cells. All animals will be carefully monitored by trained staff throughout experiments, and all mice will be humanely killed following the end of experiments.

Application of the 3Rs

Replacement

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

Replacement

T cells are part of a very changeable and complex biological system, which involves numerous cells that migrate around the body and interact with each other. Whilst simple test tube models exist for investigating their behaviour in the lab, these do not always predict how T cells may behave in the actual body.

In order to study T cell responses that are relevant to human disease, we must use a species which shares all the major parts of the immune system. Amongst species that share the main components of the immune system with humans, mice are the least sentient option. Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use.

Reduction

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

Reduction

We will use a very refined model of MS, where each T cell has a TCR that makes them activate when they sense nervous system tissue. This model (called Tg4 MS model) is very reliable, and can induce disease in 100% of mice, which will mean experiments can be performed using only 3 mice as disease controls (compared to 5-8 using other MS mouse models). In addition, T cells from these modified MS model mice can be cultured in a dish in the lab and tested for how they response to nervous system tissues, reducing the number of mice that have to undergo experimentation.

Refinement

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

Refinement

REDACTED we can capture more information during the responses of T cells to infection, allergy and during autoimmunity. This will require fewer mice to be used. The Tg4 mMS model is very reliable, and protocols have been designed to induce moderate disease levels in the greatest number of mice. In order to make sure mice suffering is minimised, we have a dedicated scoring system for disease, which grades from 1 (mild) to 5 (most severe). Any mouse that develops grade 1 or 2 disease will be checked on by trained lab members twice a day. Mice that develop grade 3 disease will be humanely killed. Protocols have been refined to generate no greater than grade 3 disease. However, a balance is required that minimises disease whilst also maintaining a level and incidence of disease that is sufficient to enable whether a given treatment is effective.

Following potentially painful injections, mice will receive Sudocrem® to reduce irritation and prevent ulceration.

PROJECT 40.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 galectins and their binding ligands in cancer
Key Words	Galectin, tumour growth, carbohydrate, metastasis
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Cancer is the commonest cause of death in the UK, accounting for one out of every four deaths with a total of more than 150,000 deaths each year. Despite enormous effort and investment, effective treatment of this disease remains a big challenge. It is increasingly believed that therapies targeted at specific molecules rather than the classic chemotherapy approaches, hold the key for more effective cancer treatments in future. As a complex disease, cancer is regulated at multiple levels by a number of molecules during the process of tumour formation and development. One group of such molecules is a family of closely-related sugar binding proteins called galectins that promote cancer growth and spreading. Targeting the actions of galectins is increasingly considered an attractive therapeutic strategy to improve cancer survival. These novel galectin-binding inhibitors show to effectively inhibit galectin-mediated cancer cell behaviours in the lab in cultured cells. The overall aims of the proposed studies are 1) to test the influence of galectin family members, their interaction with binding partners on tumour growth and spreading and 2) to assess the effectiveness of the novel galectin binding inhibitors on inhibiting tumour growth and metastasis in mouse cancer models. These studies will help to increase our understanding of the mechanisms underpinning tumour growth and spread. They will also provide essential information for further development of these newly identified inhibitors as novel anti-cancer/anti-metastasis 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 programme will test the effectiveness of several novel galectin binding inhibitors recently identified in our lab on inhibiting tumour growth and spreading of several most common cancer types such as colorectal, breast and lung cancer and melanoma. The social and economic impact of finding new drugs that can effectively target these common cancers is huge. The information obtained from this

programme will also help the scientific community to develop new therapeutic strategies for more effective treatment of cancer.

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

All the experiments will be conducted in mice. We anticipate approximately 2000 mice will be used in experimental procedures 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?

All mice will be monitored closely throughout the experiment procedures. In some experiments, the mice will be subjected to injection of tumour cells and of the testing inhibitors, in drinking water or as food supplement. The mice will experience momentary needle stick pain at this stage. With the development of injected tumour, mice may experience weight loss or poor health such as failure to respond to gentle pinch, loss of consciousness, laboured breath, difficulty of movement, or diarrhoea when the mice will be killed humanely with the fully established standard protocols. In some other experiments, the injected tumours will be removed by surgery after the mice are put into sleep. A very small number of mice may suffer from wound infection. If this results in ill health such as the symptoms described above, and at the end of each experiment, all animals will be humanely killed with the standard protocols and the tissues will be taken for further analysis.

Application of the 3Rs

Replacement

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

Replacement

Whilst testing the cancer cells in petri dish in the lab helps to gain information in understanding the diseases, they are not able to recapitulate the complex tumour environment that has a huge impact on tumour development. Adequate testing in animals is essential to help us to understanding this fatal diseases. For development of new anti-cancer drugs, currently there is no alternatively but to first test them in animals, most commonly in mice, before any clinical trial in human.

Reduction

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

Reduction

We will maximally use non-animal methods such as culturing cells in petri dishes in the lab to identify the optimum dose of the testing inhibitors before conducting any test in animals. Our comprehensive use of non-animal methods will allow us to only test the most effective inhibitors at the optimum dose in animals to maximally reduce the use of animals. Furthermore, we will use mathematical models to first calculate and predict how many animals will be needed for each experiments. This will also help us to use a minimal number of animals to achieve the study objectives.

Refinement

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

Refinement

We have selected mice as our model specie in this programme because it is the most common and most established animal model in cancer research. The mouse model is also the gold standard in testing new anti-cancer drugs before they can be tested in human. We will constantly analyse our experimental data and also review new literature reports during the study in order to refine our protocols. This would help us to use the minimal concentrations of the testing drugs and the optimum methods to achieve the best anti-cancer effects with the use of minimal number of animals.

PROJECT 41.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 cellular mechanisms important in responding to, and repairing, joint surface damage in an ovine model.
Key Words	Joint, imaging, sheep, repair, osteoarthritis
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Osteoarthritis (OA), is an untreatable degenerative condition of joints that affects both man and animals and our research group is part of an international effort to understand the causes of the disease and to identify potential treatments. Damage to the surface of joints is known to cause OA. One of the key problems with developing effective treatments for OA is that the specific cellular processes that can respond to damage to the joint surface are unknown. The aim of this project is to indentify and investigate cellular mechanisms that are important in responding to, and repairing, joint damage in a sheep, an animal that has joints similar in size, shape and function to man, so that this information can be used to inform research into human and animal OA. We will also evaluate how proposed treatments for suface defects (for example drugs and cells) affect these cellular processes. As part of this project we will also develop new ways of imaging the cellular processes in the joints during the response to, and repair of, joint surface detects.

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

Osteoarthritis (OA) is a significant disease of man and animals, causing degeneration of joints, pain and reduced mobility. Studies suggest that up to 50% of adults developing symptomatic knee OA by the age of 85. At the current time, there is no cure for OA – treatment strategies revolve around providing pain relief with severe cases proceeding to joint replacement. This project will produce a detailed understanding of the cellular mechanisms in the joint during repair and in the development of early osteoarthritis and the response of these cells to a number of treatments. In order to achieve our aim we are developing novel, non-invasive, methods of imaging cells and cellular mechanisms within living animals using

labelled cells that will allow us to track cells within joints of sheep during joint damage and repair.

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

Sheep, total of 250 animals over 5 years.

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

All the animals will undergo a short (20 minute) surgery on one of their knee joints. During this surgery a small (less than 1cm) hole will be made in either the joint surface or the meniscus (shock-absorbing structure within the knee). This is done to cause direct (surface hole) and indirect (meniscus hole) damage to the joint surface to enable the cellular responses to this damage to be studied. In about half of the animals, having created the damage, a treatment, such as using 'stem cells' (cells with the capacity to repair tissues) or a 'scaffold' (an artificially created structural support that is compatible with biological tissues) will be applied into the damaged area to investigate how the cells of the joint respond to these therapies. The surgical procedure will cause the sheep to experience discomfort and pain immediately after surgery – in most cases this is of short duration (2 to 3 days) and this is minimised by pain relief in much that same way as for human patients who undergo joint surgery. A few animals (2%) may experience infections/ wound problems and, if so, the veterinary surgeon in charge of their care will treat them with antibiotics. Also a very few animals (<2%) may experience dislocation of the knee after surgery. This will be confirmed by taking X-rays and immediately repaired. As part of this work, we want to develop new methods of imaging joints and the cellular response to damage and repair. Therefore approximately 30% of the sheep in this study will also have anaesthetics to allow for non invasive (MRI imaging) and invasive (using a small 'needle' microscope). These animals may have up to 6 anaesthetics over the course of the study, which can last up to 52 weeks, although most studies within this project last between 3 and 6 months. Animals may also have blood samples taken during the course of the study – this procedure causes no more than transient, mild discomfort. Also approximately 10% of the animals will have cells harvested from their bone marrow anaesthesia that will cause transient, mild discomfort post-surgery. These cells are 'stem cells' that will be injected back into the operated joint in order to assess the response of the cells in the joint to the introduction of this different type of cell. The expected adverse effect of our studies is lameness. We do not expect lameness to exceed a mild lameness i.e. the animal will stand and walk normally but the lameness is apparent when the animal runs. Regular clinical monitoring, with particular attention to lameness is performed on all our animals. We also measure how much weight is being taken on the operated leg by walking the sheep over a pressure plate before and after surgery (performed at least monthly). In addition, animals will be fitted with individual activity trackers onto collars so that the

length of time they spend resting/lying down can be monitored and assessed. All of these methods of monitoring the animals will lead to early detection of lameness. Where lameness is detected, the veterinary surgeon in charge of their care will be informed and a course of treatment begun. If the animal does not respond to treatment or if it gets worse it will be humanely killed. At the end of the experiments all animals will be humanely killed 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

The project seeks to understand the interactions of cells in cartilage and bone with tissues over time and how this controls the repair of defects created directly and indirectly on the surface of joints. These interactions are complex and there are no adequate laboratory system to model this process. We also intend to determine the most suitable strategies for repair, for example using materials such as scaffolds, and the cellular mechanisms that underpin these repair strategies. Identifiying the most suitable strategies for repairing joint surface damage will allow us to progress towards clinical trials in humans. The use of live animals remains the only method to carry out these investigations as the joint/cartilage/body system combination cannot be adequately modelled with cells in a dish in a laboratory.

The two alternatives to work in animals are computer modelling and cell culture systems in the laboratory. Whilst both methods continue to become more sophisticated there is still a huge divide between what they can tell us and the actual events that take place in the body. We do conduct a large number of cell culture systems in the laboratory prior to conducting any animal experiments. For example we carry out preliminary studies of the interactions between cells including detailed studies of cartilage cells and bone cells to determine what the cells are capable of. We also carry out studies to ensure that the material used to make scaffolds used for repair are not toxic and support the growth of the cells which they will contact following implantation.

Finally, we carefully save all of the cartilage/bone samples taken from the sheep used in experiments and these samples are available for further studies in the laboratory as required. In this way we have built up a significant archived tissue bank of sheep joint tissues that is available to other researchers both in and outside our group.

Reduction

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

Reduction

We will always seek to use the minimum number of animals necessary to achieve the objectives of this project. Firstly, before beginning animal experiments, we will investigate the interactions between key cells in the laboratory. We will also identify suitable scaffolds and compounds that will affect repair and ensure that they are not toxic to joint tissues in laboratory experiments

We have considerable experience of the response of bone and cartilage in sheep using similar systems and we have conducted statistical analysis to suggest appropriate numbers. We also cross check these with studies published in the wider scientific literature. Our experiments are conducted in a randomized manner. For example we randomise which animals get which procedure/treatment and in what order they undergo procedures. In addition, we ensure that the experimentor is unaware to which group an animal is allocated such that analysis of data so that we do not introduce bias into our experimental interpretation. We use coding systems to anonymise data so that the person working on interpretation of data does not know the background of the data, which can lead to unintentional modification of the results. Indeed, in some instances, we use research workers outside our immediate group to analyse the final results of a study. We believe that these methods contribute to the robustness of our data interpretation.

The design of each experiment will be based on the specific research question in order to determine the experimental groups for comparison and the appropriate time points at which to determine outcome. Again our previous experience and those of other research groups will be used to inform this process. All experiments will be hypothesis driven with the comparisons to be made and the statistics used determined before the study. Statistical advice has been sought prior to starting these 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 sheep is considered to be one of the most suitable animal species for the evaluation of responses to joint damage and repair. It has joints of a size that are similar to man and the forces in and on the joints are more similar to humans than smaller experimental animals that could be used for these studies. In addition, the healing capability of the sheep articular joint surface is similar to humans i.e. poor. In contrast small animals, particularly rabbits, have excellent healing capacity,

likely indicating that the mechanisms that underlie the response of the joint to damage and repair is different to that seen in large animals such as sheep. There are also enough published studies using sheep joints which can be used to improve understanding of the responses seen and to allow good interpretation of the results gained in this study.

We are constantly seeking to refine our use of animals. We have pioneered the use of activity tracker monitoring, using small monitors attached to a collar (which allows us to track, for example, the distance moved and the speed travelled) in sheep used for orthopaedic research. This tracking is done 24/7 and allows us to evaluate this functional behavior of the sheep without the presence of humans. This is particularly important as the presence of humans may influence the way a sheep behaves and potentially influence study results. This tracking can be used both to monitor the welfare of the animals based on movement activity – we will carefully examine and evaluate any animal that is resting more than the others or than it used to before the experiment started.

Other examples of the work we have undertaken to refine procedures include working closely with animal husbandry staff to optimise the regime surrounding fluid and food availability in the run up to an anaesthetic to minimise the risk of regurgitation and associated complications as far as possible. Sheep are animals with 4 compartments to their 'stomach' and this means it can be be very difficult to achieve an empty stomach before an anaesthetic (as is routine for human patients). There is a risk of regurgitation of stomach contents when a sheep is under anaethesia, which can lead to possible development of pneumonia (or death) should fluid be inhaled into lungs, therefore the chances of this happening must be reduced as far as possible, without compromising the welfare of the animal.

Our sheep are handled routinely to ensure that they are familiar with the presence of humans and we train them to walk over the force plate for measuring weight bearing on the operated limb and thus data collection in this regard is minimally stressful for sheep. We also have a ‘tame’ sheep that lives with the experimental group, who reduces the stress amongst the other animals in the presence of humans and is used during weight bearing testing to ‘buddy’ the experimental animal i.e. trained sheep walks over force plate and experimental sheep follows.

Following surgery sheep are housed indoors in purpose built buildings with appropriate feed, bedding and companionship and checked for lameness and ill health at least twice a day. When they are assessed as fit to have more exercise they are turned out into fields. The sheep live for the majority of the experimental period, which is between 3 and 6 months, outside in the fields in one stable group of animals. This allows them to exhibit their natural behavior.

PROJECT 42.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	Haematopoietic stem/progenitor cell heterogeneity and its disruption in blood cancers
Key Words	Leukaemia, Stem cells, Transplantation, Blood Cells, Myelofibrosis
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Leukaemia and related conditions such as myelofibrosis are devastating blood cancers affecting all age groups with a major impact on morbidity and mortality. The vast majority of these blood cancers are currently incurable. Bone marrow transplantation can cure a minority of patients, but is associated with very significant mortality due to low blood counts following the transplant. The aim of this work is to improve approaches for bone marrow transplant by better understand the complex mixture of cells underlying the normal process by which bone marrow stem cells produce mature blood cells during adult and embryonic development. We also aim to improve treatments for patients with blood cancers by characterising the impact of damaged genes on the development of blood cancers.

To achieve our goals we will study mechanisms of how normal stem cells survive, self-renew and generate normal blood in normal or other mouse strain models. To unravel the processes leading to blood cancers, we will either induce leukaemias in mice or inject the mice with human cells from patients. This will enable us to mimic blood cancers in mice and study in detail the disease pathways.

What 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 two key areas where this research will have an impact on science and might improve treatment of patients with blood diseases: To help further refine and improve bone marrow transplantation approaches which are currently associated with very considerable morbidity and mortality. For example, we hope to be able to specifically identify drug targets that can enhance the speed of recovery of different types of blood cells, thereby reducing morbidity and mortality associated with this procedure. To improve our understanding of blood cancers with particular focus on “leukaemia stem cells”; the cell type which is responsible for causing relapse in patients. , the key benefit of this work will be to identify novel therapeutic agents that will be selected for further development clinically.

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

56000 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 protocols in this application are all of mild or moderate severity. The potential adverse events primarily relate to: • Immunesuppression and resulting infection due to low levels of white blood cells • Irradiation and consequent bone marrow failure, causing anaemia, risk of infection and increased risk of bleeding • Surgery, such as laparotomy, accessing the thymus gland, uterus and kidney • Leukaemia development and consequent bone marrow failure, causing anaemia, risk of infection and increased risk of bleeding • Administration of substances Welfare of animals at risk of adverse effects due to above procedures will be carefully and regularly checked as outlined in the proposal. If some animals are in pain or exhibit other adverse effects, pain-killers or other treatments may be given under veterinary direction and mice will receive wet food and will be more frequently monitored. Mouse strains showing any unexpected ill-health will be humanely killed. At the end of each protocol, animals will be killed by a schedule 1 method, in all cases ≤24 months of age.

Application of the 3Rs

Replacement

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

Replacement

Blood formation and leukaemia development are precisely controlled processes that require the living environment, such as bone marrow. HSCs are known to interact with other bone marrow cells, and when exposed to culture (non-living conditions), they change their properties. Therefore, these processes have to be investigated using animals. The mouse is the most widely used system to study the formation of normal blood and blood cancers. Mouse models have demonstrated to be highly relevant and essential for development of an understanding and clinical application of the blood forming system in man, not the least application of bone marrow transplantation and understanding of leukemia since mouse and human stem cells share similar properties. Other advantages of the mouse model (apart from it being mammalian) include the availability of laboratory reagents to study blood functions. Furthermore, availability of various mouse strains allows the study how genes of interest function in the blood system, including *in vivo* study of human blood cells in mice.

Reduction

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

Reduction

The laboratory has a number of systems in place to ensure minimal numbers of animals are used:

- Limit cage numbers through weekly checks
- Determining the use for animals in all cases prior to weaning
- Use of appropriate number of animals in each experiment with careful experimental planning and statistical considerations to maximise the amount of information obtained from each animal e.g. serial blood sampling
- Maximising yields of blood cells from each mouse for experimental use, for example, through optimal use of antibodies and nanofluidic molecular platforms developed in the laboratory

Cryopreservation to maintain smaller colonies; by collaborating with the embryo and sperm freezing service team in the institute.

Genome Editing

We will use this new and state of the art method to more rapidly generate complex genetic mouse models with the potential to dramatically reduce the numbers of animals required to study combinations of mutations.

Refinement

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

Refinement

We have carefully chosen the least severe procedures to minimise the pain and adverse effects for the animals. To minimise adverse effects such as infections, animals will in all cases be housed in individually ventilated cages. Cages, food, water and bedding will be sterilised.

Conditioning of haematopoietic cell transplant recipients with split radiation dosage: In order to detect the activity of transplanted blood cells, the host animal's own blood cells must first be depleted by irradiation, in the same way as is done with humans receiving bone marrow transplantation. In order to minimise the adverse effects of irradiation, the dose is split into two half doses of irradiation, rather than a single full

dose. In association with temperature and noise monitored housing, provision of moist food and extra bedding, and rigorous monitoring, this has resulted in further reduced and very low levels of adverse events caused by irradiation.

Analgesia: In order to minimise pain following operative procedures, analgesia will be administered post-operatively under the guidance of the NVS.

Administration routes: Drugs and biologically active agents will be administrated by the least invasive route for example orally in water or feed or gavage rather than by intraperitoneal route. For long term administration, osmotic minipumps will be used.

Aseptic Surgery: The principal aims of any surgical procedure are that it is carried out skillfully with the minimum of risk and disturbance to the animal and without infection, while producing quality scientific output. In order to achieve this aim we will ensure that all surgery is conducted according to Laboratory Animal Science Association standards.

Improved approaches to xenotransplantation: We have introduced a humanised “ossicle” model, that provides an accessible humanised bone marrow microenvironment. This enables the robust engraftment of healthy human blood cells in the humanised ossicles rather than mouse microenvironment. This approach improves our ability to accuarately study normal and cancerous human blood cells *in vivo*. We will also explore whether genetically modified humanised mouse strains might also enable more efficient engraftment of cancerous blood stem cells.

PROJECT 43.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 over 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 44.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 of poultry
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;
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;
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 objectives of this project are:

1. To determine the efficiency of utilisation of feedstuffs, including unconventional feedstuffs, by poultry species
2. To elucidate the effect of the use of feed additives in improving the utilisation of feedstuffs by poultry species
3. To examine the effect of different dietary interventions on growth, productivity and nutrient utilisation by poultry species
4. To understand the various factors that cause ill-health in foot and hocks of poultry species and dietary interventions to prevent such
5. To establish proper procedures for determining efficiency of nutrient utilisation by poultry, and
6. To understand the interactions between nutrition and poultry health, with particular emphasis on gut health, and ascertain how different dietary interventions influence ability of poultry species to resist 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)?

Some of the potential benefits from the project are: 1. Understanding of variability, and causes of such, in feedstuffs (conventional and unconventional) with the objective of reducing competition between man and animal for food resources 2. Development of strategies, using feed additives, to reduce possible negative effects of intensive animal agriculture on the environment, as for example the use of phytase to reduce phosphorus excretion to water bodies or in the manure 3. Improving efficiency of utilisation of finite resources by studying of alternatives that meet animal need without jeopardising animal growth and productivity 4. Understanding of alternatives to antimicrobial growth promoters to ensure optimum growth of birds and reduce subclinical growth performance issues

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

Species to use are broilers, ducks and turkey Nutrition efficacy studies: 21,000 Feed evaluation – gavage: 300 Feed evaluation – raised floor: 11,000 Foot and hock studies: 2,000 Standardised digestibility studies – 5,000 Nutrition and gut health studies – 11,000

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

Some of the birds in the project will receive diets that are not meeting nutrient requirement (vast majority of the birds will receive diets with adequate nutrients) and such birds will be expected to gain weight more slowly. In establishing the standardised digestibility, some of the birds in the experiment will be provided with diets with very little protein or mineral for a very short period, not exceeding five days. The potential negative effect is reduced growth, but in order to reduce this effect, the treatment will only be applied to birds that have received diets that are adequate in nutrients for at least 7 days. Oral gavage, administration via feed or water or oral inoculation of birds with campylobacter or coccidia may produce reduced growth rate. However, this is to mimic what may happen in a typical poultry farm, and the negative effect will be minimised by ensuring that birds that are challenged with the organisms are kept only up to the age at which relevant useable data can be obtained after induction. The maximum severity limit in the project is moderate but most of the birds to be used in the project will have mild severity level. At the end of the experiments, some of the birds will be euthanised using humane methods. Some of the procedures do not require euthanasia of birds as part of the experiment and such birds will be signed off the Act.

Application of the 3Rs

Replacement

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

Replacement

Studies that require effect of treatments on growth can not be done in non-animal substitute. Digestibility studies are usually preceded by in vitro proof of concepts but ultimately because the feed additives will be incorporated to feed for actual animals, it is a requirement that such products are fed to animals.

Reduction

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

Reduction

The number of replications and animal per replications are determined based on statistical analyses and previous experience. Each experiment is individually set up to maximise ability to test for treatment effect combined with every effort to use the minimum number of animals. Experimental protocols are reviewed before each experiment to ensure that animals are not used unnecessarily. In addition, there are monitoring exercises after experiment to see what lessons are learnt. The 3Rs is part of the monitoring exercises. Part of the review of the experiment include the input of expert 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

The objective of this project is to provide information that is relevant to the poultry industry, and hence poultry species are the choice of animals for the project. There are instances where nutritional effects studied are applicable across species (i.e. what is studied in broilers may be applicable to turkey) and in such cases, studied are not repeated for all the poultry species. Most of the protocols in this project are mild in severity level. Birds used in this project will be monitored on a daily basis to ensure that birds wellbeing is not compromised. There is also on-site specialist (avian) veterinary support who helps ensure birds wellbeing is maintained.

PROJECT 45.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	Breeding and maintenance of GA mice
Key Words	Breeding, Maintenance, Genetically-altered, Mice
Expected duration of the project	3 year(s) 6 months

Purpose of 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 aims and objectives of the project are to breed and maintain established colonies of Genetically Altered (GA) mice. These mice will be used for scientific research, which may require separate project licence authority. The project will allow us to acquire new lines of GA mice that are of interest to our researchers and it will allow us to cryopreserve embryo and sperm from GA mice when they are not being actively studied. It will also allow us to re-derive colonies to improve animal health and welfare.

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

This project is a continuation of a service licence to provide breeding and maintenance of genetically altered mice. A team of technicians with expertise and experience will care for these mice. Researchers will be provided with mice of a high quality that will allow for greater reproducibility in their results. The ability to cryopreserve embryos and sperm will lead to a reduction in the numbers of mice being bred. Rederivation will improve animal health and welfare and ensure that we provide high quality mice for our researchers. Results from studies undertaken on mice bred under this licence will be published in peer reviewed journals and lead to increase in the knowledge and understanding of the models being studied.

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

Mice (all developmental stages), 48,130 over 5 years (the current license is a continuation of a previous license; together the project is 5 years in duration).

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

There are no expected adverse effects from breeding of GA mice. Females of an appropriate size will be used and over vigorous males will be replaced. To identify the mice and to check the genetic make-up, a small tissue sample (normally from the ear) may be taken. This may cause transient pain, but trained and experienced people take the sample. Some female mice (less than 1% of the total animals requested) may be injected with small volumes hormones to increase the number of embryos they can produce, a procedure known as superovulation. The needle insertion may cause transient pain. Some mice (less than 1% of the total animals requested) may be anaesthetised for surgical procedures, this may by injection or by inhalation anaesthetic. This will be for relatively short periods of time. After being anaesthetised, one of two surgical procedures may be performed: either vasectomy in males or embryo transfer in females. For the vasectomy, a small cut is made in the scrotal wall and a small piece of the vas deferens cut away. The hole in the scrotal sac is then repaired. The mice are given analgesia before and after the surgery. For embryo transfer, a small cut is made on the back of the mouse, through the body wall. The uterus or oviduct is exposed and a small hole is made in the uterus or oviduct, embryos held in a very fine tipped pipette are then transferred into the uterus or oviduct. The incision is repaired and the mouse allowed to recover. The mice are given analgesia before and after the surgery. Animals are expected to make a rapid recovery after the anaesthetic. Mice with genetic alterations are affected in different ways depending on the gene/s affected, many look and behave as normal mice. In this project, some of the GA mice born may have balance problems making their walking unsteady. Other strains used in Alzheimer's research, may lose the structure or function of the cells in the brain, which may cause changes in their normal behaviour over time. Sometimes the mutation makes the mice smaller than their normal litter mates. All of these mice are monitored closely. To ensure that these animals can reach the food and water in their cage they will be provided with wet mash. The animals may be transferred to another authorised project, kept alive at the establishment or humanely culled. Trained and experienced personnel will carry out the procedures.

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 as although many research projects involve the use of in-vitro systems such as cell culture, human tissue assays and computer modelling, these cannot yet adequately model all aspects of the complex biological process involved.

The chosen species for this project is the mouse. Mice are biologically very similar to humans and mice can be genetically manipulated to mimic many human diseases.

Reduction

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

Reduction

The project licence holder and animal technicians have a good working knowledge of the colonies being bred and a good working relationship with the researchers. The breeding colonies will be managed efficiently to meet the research needs.

Each request for new GA mice will be reviewed by a committee and we will review the breeding colonies regularly and meet the research group to discuss their requirements.

Refinement

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

Refinement

Mice are the model of choice as their genetic, biological and behavioural characteristics closely resemble those of humans.

The mice will be cared for by animal technicians and veterinary staff who have experience of the husbandry and welfare relevant to their needs.

Experienced and competent personal licence holders will conduct the procedures and veterinary advice will be taken in respect to the use of anaesthesia, analgesia and aseptic technique.

PROJECT 46.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	Assessing efficacy of viral vaccine vectors in livestock species
Key Words	Vaccines, Livestock, virus vaccine vectors
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Presently, vaccines for many viral diseases of livestock are not available or when available are ineffective. The need for more and better vaccines is becoming more critical in the face of elimination of antibiotics for growth enhancement, as regulations are being put in place to control development of antimicrobial resistance (AMR). We will test new vaccine vectors delivering recombinant viral proteins for capacity to induce a protective and effective immune response. Testing will be across three important livestock species, cattle, sheep and swine. The aim of this project is to demonstrate that delivery of antigenic payload using vaccine vectors under study is more efficient and effective than classical delivery systems for protein antigens of pathogenic viruses.

What 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 vaccines resulting from this research program will result in improved animal welfare in the short term and makes the long term benefit of disease eradication possible. This will be a direct benefit to livestock farmers and the consumers that buy their products by keeping a safe and stable food supply readily available.

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

Over 5 years we anticipate using 50 cattle, 50 sheep and 75 pigs. Most experiments will last between one and three months and involve 10 to 15 animals in each experiment.

In the context of what you propose to do to 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 experience site reactions from the injection of the vaccines being tested as with any vaccination. There will be mild discomfort as a result of blood samples being taken. Once a vaccine trial is over, the animals will be kept for use in

a subsequent trial of a different vaccine. Alternatively, animals will be humanely euthanised and incinerated.

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 systems available for vaccine testing that do not involve the target species (testing a cattle vaccine in cattle). Given the complexity of the genetics and immune response of the livestock species involved, cattle, sheep and swine, no technology is available to give an accurate assessment of new vaccines as an alternative to testing in the animals.

Reduction

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

Reduction

Multiple new vaccines are to be tested in this research program. Animals used in these studies will be the minimum number of animals required (statistically) to achieve a reliable assessment of a new vaccine. In addition, animals will be kept and used in subsequent testing of a different vaccine in place of using new 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 of the vaccine testing in this research program is being conducted in the species that suffers disease when infected by the viral pathogen under study. As such, these are not models of disease, but these are the animals that suffer disease when exposed to the virus and a successful vaccine will protect these animals from the illness caused by the virus in question. In most cases, animals in these studies are anticipated to suffer mild discomfort from the process of vaccination and taking periodic blood samples, mostly from being restrained for the procedures. All studies will be terminated 8 weeks after vaccination (or boost vaccination), at which point, animals will be transferred to a new study or humanely euthanised and incinerated.

PROJECT 47.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 Biological Materials
Key Words	Blood, tissues, service, biological materials
Expected duration of the project	5 year(s) 0 months

Purpose of 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 aim of this project is to provide blood and biological products (including organs including brains, lungs and kidneys) from a range of animal species (mice, rats, hamsters rabbits, chickens, turkeys, primates and dogs) to support in research, diagnostic and regulatory work. This can include ensuring new medicines are safe before release for use and checking the calibration of diagnostic devices used in treatment of both humans and animals.

To do this we produce fresh bloods, plasmas and serums after assessing individual customer requests looking at the purpose of the work to be carried out by the customer and the benefits it may provide.

By storing frozen plasma, serum and organs we can then ship them internationally to customers, giving a consistent timely service across different end users working on similar work. This allows researchers to purchase the specific product required as opposed to animals having to travel.

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

The benefits from conducting this work are dependent on the research projects of the customers ordering: a large proportion of products produced under the previous licence supported regulatory work, which is required under government guidelines and ensures the safety of drugs. Other products go to support calibration of assays and equipment to ensure results from work conducted are validated and that drugs produced are free of viral contamination. Regulations which guide the choice of species selected by customers to perform this testing include: Food and drug administration, world health organisation and the ICH (International council of harmonisation). The data from the assays performed will be used in regulatory submissions to the appropriate regulatory authorities or is used to help form a picture of the potential of putative new drugs to be more efficacious with a better side effect profile than existing therapies in a wide variety of human and animal health

indications. These data may not always be positive, and hence, some of these tests may prevent the further development of such entities, preventing the un-necessary use of animals in efficacy and regulatory testing prior to testing in human or animal clinical trials. The scientific benefits directly linked to this licence are dependent on the research projects of our customers; but under previous licences the tissues have contributed to the knowledge of disease processes in man, animals and food crops, understanding of the development of the immune system and its regulation, and extension of the knowledge of neurobiology and associated neurological disease. By offering the different products and species from one location we can give consistency across the samples, allowing direct comparisons in the end work performed, even if this is at different locations by different customers. We are able to reduce the movement of animals by shipping blood products to end users across Europe who would otherwise have to transport animals increased distances to produce products themselves. We also can take organs after the death of the animal (for example brains and lungs) and store these until needed. The customers we supply have a preference to outsource this work so they can benefit from the high levels of specific experience and knowledge we provide.

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

Over the 5 year term of the PPL we expect to use up to 161,000 mice, 62,000 rats, 6250 rabbits, 5500 Hamsters, 180 dogs and 1060 birds (chickens and turkeys). The majority of animals used will undergo non-recovery procedures (collection of blood or organs and tissues); i.e. carried out under terminal general anaesthesia. However, approximately 250 rabbits, 80 dogs and 160 birds will be used for the repetitive collection of small blood samples. Dogs, birds and a small proportion of the rabbits (5%) would have blood withdrawn from a superficial vein at approximately fortnightly intervals resulting in each animal having approximately 24 samples taken per year. Most rabbits would only have one blood sample taken. Approximately 500 stock primates may also be used for the provision of blood products and tissues, and very occasionally other samples such as urine/faeces and hair.

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

The majority of the animals under this licence will only undergo procedures under non-recovery anaesthetic; these animals will only experience mild discomfort, due to being held still as the anaesthetic is introduced, such as experienced by human patients undergoing surgery. The only difference is that they will not awaken from the anaesthetic and will have death confirmed or be humanely killed at the end of the procedure. Anaesthetic will be introduced either by injection into the veins or by inhalation of gas. For rabbits and dogs sedation may be used before hand to reduce the need for longer periods of restraint. Chickens, Turkeys, Dogs, Primates and Rabbits will be kept as blood donors, and will have approximately 2 blood samples

taken a month. These are small volumes that are under 10% of blood circulating volume and will be collected from superficial veins, similar to human blood donations. These animals will only experience minimal restraint during the period of sampling and it is not expected to cause any adverse effects. Where appropriate topical local anaesthetic will be applied to the area before sampling.

Application of the 3Rs

Replacement

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

Replacement

The work performed, that uses products produced under this licence, is required under safety and regulatory guidelines; these include testing of drugs (both medical and veterinary) prior to their release to market, as well as ongoing calibration and quality checks of equipment and processes to ensure accuracy of the results that are published.

Currently there are no methods to generate animal specific blood products (cells, plasma, serum) without the use of animals.

Reduction

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

Reduction

By keeping donor colonies of dogs, rabbits and birds we are able to take small blood samples across a period of time from the same animals. This reduces the number of animals needed overall and provides a consistent product decreasing the need for retesting.

By collecting blood under non-recovery anaesthetic we are able to collect a higher volume of blood per animal compared to collection after the death of the animal. This reduces the numbers of animals used overall.

Our customer services department provides a central point to order blood and other biological products from, for a range of customers from small university groups to large contract research companies. This means we can collect different products (blood and organs, including brains, heart, liver and lungs) from the same animal and provide to multiple end users. This is frequently done with blood products from birds. All tissues are collected after death from all species.

Refinement

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

Refinement

The choice of animal is determined by the customers' and regulatory requirements, with species such as dogs only used where non-rodent species are required and it is the best model, due to either the research being related to dogs, or due to the similarities in systems that they share with humans.

The methods used for the collection of blood samples are based on guidelines of volume and frequency that will cause the least harm to the animals. The processing after collection is designed to get the highest quality and quantity of product so sample sizes can be kept as small as possible; we consider storage methods from across multiple fields including human transfusion services to ensure that we can maintain the quality of stored product.

Dogs and birds kept as donor animals are held in group living conditions, with dogs having access to both inside and outside areas as part of their housing; all donors are assessed individually and both their behavioural and physiological condition is monitored throughout the time they are a donor. Rabbits are only kept as repeat donors if the end use requires it, for example we work with a customer who uses fresh rabbit blood cells in human medical diagnostic work and before using the cells from any rabbit they have to validate it in line with ISO 15189 (International standards for medical laboratories). By keeping a donor rabbit they can complete the validation once and then only take small volumes thereafter.

For donor dogs, chickens and turkeys a peripheral vein such as the jugular vein in the neck is used for collection of blood samples, this is a superficial, easily accessible, larger vein which means the time the animal is held for the procedure can be kept to a minimum and adverse effects, even for larger samples are rarely seen. For rabbits the marginal ear vein or artery will be used, with the vein mostly used as the samples taken are small and the vein has less chance of bruising. This is an accessible blood vessel that means the rabbit can be held in a natural position for the duration of the sample. For all the animals used as blood donors the sample time and experience of feeling is similar to a human blood donation or blood test performed medically. For primates, samples are usually taken from the femoral vein, as this is easy to access, and reduces the amount of restraint needed for the animal.

Dogs and primates will only be used when the product is required for work that cannot be done without using dog and primate specific materials, currently there is a requirement under EU legislation that drugs are tested in a non-rodent species before release into the medical and veterinary markets. Dogs and primates are used in cases where they have similarities with humans in how they deal with the drugs at

a cellular level. Worldwide legislation also requires the use of the same type of species specific plasma or serum product as the test species to support regulatory toxicology work, hence dogs and primates must be used to supply blood products to support toxicology studies in these species.

PROJECT 48.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 new radiotherapy and drug treatments for primary and secondary brain tumours, lung cancer and mesothelioma
Key Words	glioblastoma, mesothelioma, radiotherapy, toxicity, molecular targeted 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 overall aim of this project is to develop relevant mouse models of brain and lung cancer, and use these models to identify novel therapies that can increase the effectiveness of conventional cancer treatments (i.e. chemo and radiotherapy). Both glioblastoma (brain cancer) and mesothelioma (lung cancer) have a dismal prognosis with treatment resistance playing a key role in tumour recurrence and progression. The work out lined in this project will aim to investigate the biological mechanisms underlying treatment resistance with a view to develop more effective treatment strategies. Our plan to achieve this (1) to test what tumour cell lines grow tumours in mice (this will involve intracranial surgery for the glioblastoma cell lines), (2) characterise tumour biology using MRI scans and histology to identify pathological features and expression of drug targets in the tissue, (3) use the best characterised tumour models to test novel radiotherapy-drug combinations (this will involve administration of drugs to mice, irradiation of mice and MRI scanning), (4) investigate the adverse effects of radiotherapy on normal tissues using behavioural testing, MRI and histology to determine changes in tissue physiology and structure.

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

• This project will increase our understanding of glioblastoma and mesothelioma biology, which could identify mechanisms of treatment resistance and how to overcome these. • Clinically relevant radiotherapy and drug combination treatments will be tested in the models being developed. • REDACTED Ultimately our scientific findings with mice could make an impact on cancer sufferers by supporting these trials.

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

• We predict to use approximately 3600 mice over 5 years for studies involving tumour bearing mice, whereas normal tissue toxicity studies are predicted to use 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?

Injection of tumour cells in to the mouse brain in order to grow brain tumours, or creation of a window on the mouse skull to look at the behaviour of tumour cells in the brain (under a microscope) requires cranial surgery. The mice are under anaesthesia throughout the surgery and given pre and post-operative pain relief. After surgery mice are kept warm using a heated cage rack and returned to their cage once fully awake and mobile. Mice are regularly health checked by competently trained staff using monitoring charts that list symptoms that could be experienced by the mice under procedure. The most common symptoms expected with regards to intracranial tumour growth are weight loss, subdued behaviour and in some cases seizures. Mice displaying such symptoms are humanely killed. Mice treated with radiation may experience anaemia, diarrhoea, paralysis or abnormal behaviour patterns. Where possible, we will use non-invasive imaging techniques as a method of monitoring tumour growth and response to therapy. All imaging techniques require anaesthesia and may be performed on more than one occasion. At the end of each experiment mice are humanely killed and the tissues harvest for further testing. The described procedures have moderate levels of severity.

Application of the 3Rs

Replacement

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

Replacement

The failure of cell culture models to recapitulate key features of brain tissue is a likely factor explaining why most new treatments developed in the laboratory have failed to provide benefits for patients with glioblastoma. To investigate this further, we have developed a novel cell culture system that contains biological components and mimics structures found in the brain. However, despite the advancements in our cell culture models, these assays cannot fully model the complexities of cancer development in the living organism. It has been well-documented that both immune system and tissue components play an important role in disease progression and these factors cannot be fully recapitulated in the test-tube, thus the requirement of animal studies remains.

Reduction

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

Reduction

Non-invasive imaging methods will be used to monitor tumour growth and responses to therapy. This will avoid unnecessary killing of animals at different time points, and allow for longitudinal studies that are more statistically powerful. Testing of cells in conventional and novel culture models on plastic will be undertaken to identify promising agents prior to testing in mice. Pilot studies with fewer numbers of mice will always be performed when using new cell lines and therapies. Data from pilot studies will be used towards power calculations of sample size for larger quantitative 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

This licence uses immunocompromised mice to grow tumours of human origin with procedures that are no greater than moderate severity. It is essential that we use immunocompromised mice in order for non-mouse cell lines to grow tumours successfully without rejection by the host. Wild-type mice (which have a full, uncompromised immune system) may also be used in studies were the immune response is thought to play a key role. Pilot studies will be performed when using new tumour cell lines to determine the take rate and for characterisation of tumours. This will determine if a full-scale experiment is merited and will help answer scientific questions efficiently.

To minimise suffering, all mice on procedure will be frequently monitored and humanely killed when exhibiting signs of altered health status and/or tumour burden. All users will be fully trained in monitoring tumour development for each model and will be signed as competent prior to initiating their own studies. Dedicated monitoring sheets list clinical signs and give classifications of mild, moderate or severe to clarify to users when mice should be killed. Our animal unit is proactive in environmental enrichment and provides fun tunnels and nesting materials in cages.

All surgeries will be performed in a dedicated surgical suite and always using aseptic techniques. Pre and post-operative analgesia, and anti-microbial therapy (where required) will be administered routinely to animals under-going surgery as advised by the vet and in line with current guidelines.

PROJECT 49.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	Neurobiology underpinning obesity and comorbidities
Key Words	appetite, brain, obesity, type 2 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):

Obesity and related diseases such as type 2 diabetes, heart disease, stroke, cancer and dementia represent key medical and economic challenges for this century. However, effective obesity prevention strategies and treatments are limited. For the past 15 years, most obesity medications have worked by influencing appetite through action in the brain, but have produced unwanted side effects. Our strategy is to identify how these drugs produce the beneficial effect so that we can devise new drugs without the unwanted 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 work is expected to provide new information about the way chemicals and hormones made in our bodies regulate what we eat and our blood sugar. It will advance our knowledge of how these detailed processes work in the normal state and how they ”go wrong” in obesity to increase our risk of developing disease and shortening lifespan. This work could lead to the discovery of new ways for treating these common world wide conditions.

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

Up to 4,800 mice and 400 rats will be used over 5 years.

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

We do not expect animals to experience adverse effects. Most mice/rats will be given different diets and/or drinks. Some animals will also be treated with medications that are expected to reduce appetite, improve blood sugar and improve learning/memory. Surgery will be carried out to a very high standard by fully trained and competent staff on a subset of mice/rats. For example, some mice/rats will

undergo a 20-30 minute surgery to provide a way to deliver medications rapidly. Mice/rats may also undergo a minor (typically 5 minute) surgery to implant a device under the skin that can release a medicine slowly. We do not anticipate that adverse effects will be observed. Animals will be given painkillers and post-operative care just like people recovering in the hospital. At the end of the protocol, all 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

At present, there is no alternative to animals for studying the complex processes of appetite, food choice, amount of food consumed, body weight and the regulation of metabolism. For example, cells can't chose a biscuit over broccoli and can't gain or lose body fat. Humans are not suitable for this work because the current technology is not sufficient to identify specific cells regulating energy balance. For example, fMRI can only identify gross brain regions that are activated in response to meals, but cannot provide any further information. We are already aware of the general brain regions regulating energy balance. What is now required is an identification of specific neurons within these broad regions so that we can identify specific targets for obesity and type 2 diabetes treatment.

Reduction

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

Reduction

When designing the experiments, we perform statistical analysis to ensure that we use the minimum number of mice or rats per group that will be informative using power analysis and consultation with a statistician, where necessary. We use the most strigent and rigourous techniques to make sure that our experiments are performed to give us clear answers to ensure against unjustified duplication of procedures. We also consult the literature and manufacturers of products to guide us to optimal experimental conditions before embarking on any experimentation. We also strive to reduce the numbers of rats and mice through breeding techniques to give us the experimental genotype every time, as long as this genotype isn't harmful. We also use in vitro and ex vivo techniques where possible to study specific cells in detail.

Refinement

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

Refinement

The mouse has been selected for most of the experimental work as it is the simplest species in which appetite and body weight has been extensively characterised using the latest technological advances. Where rats are a better model for a specific components of human metabolic disease, they will be used.

Experimentation used will be rigorously assessed by qualified people outside REDACTED and carefully planned to ensure against unjustified duplication of procedures. All staff will demonstrate and have documented competence prior to independent experimentation. We will only use well-established reagents and protocols to induce expression, deletion, activation or inhibition of the candidate gene/cells and evaluate health/behaviour. Where the target modulation produces a metabolic condition, we will apply strategies and medications that treat this. Where possible, we will administer medications or restrict foods that cause/restore the same levels of hormones or chemicals that are naturally made in our bodies.

Different types of animal housing (single, pair, group) will be considered in advance of each experiment, on a case by case basis, depending on the scientific outcome required. Unless experimentally required, animals will be group housed in recommended husbandry and care conditions.

Where experimentally required, surgery will be carried out to a very high standard by fully trained and competent staff. Pain relief and anaesthesia are given following advice from the vet, just like people in the hospital would receive.

The work in this project will be undertaken in accordance with the surgical procedures will be undertaken adhering to the guidelines described in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010).

PROJECT 50.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 physiology of mammalian eggs and early embryos.
Key Words	Fertilisation, Eggs, Sperm, Embryo
Expected duration of the project	5 year(s) 0 months

Purpose of 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 the basic mechanisms that operate at fertilization in mammals and to establish new ways of diagnosing and treating human infertility.

Infertility affects about 1 in 6 couples and the major form of treating human infertility is *in vitro* fertilization (IVF). However, this technique is not always successful for reasons that often remain unknown. One major potential reason why fertilization does not occur is because the egg lacks a stimulus from the sperm. It has been previously shown that this stimulus is provided by a specific protein in the sperm. Our research will investigate how a lack or deficiency in this protein could explain these currently unresolved cases of infertility. We will find ways of making this sperm protein artificially so that it could be used in future to help couples conceive. We will use mouse eggs and mouse embryos as models for human eggs and embryos.

Another major problem with current IVF treatments is that when fertilization is successful, there may be several embryos generated. It is common practice to re-implant 2 of these embryos into the uterus of the prospective mother. However, this can often lead to twins, and it also increases the chances of triplets. If a mother has twins or triplets there is a greater risk of problems developing during the pregnancy. It would be best to only transfer one embryo but the problem then remains of how to choose which is the 'best' embryo. In our research we will also be studying the biochemical responses in the mouse egg and embryo in the first few days of development. In some cases we make use of specialist imaging methods that do not harm the embryo. At a later stage these could be developed for use in IVF clinics to select the best quality 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)?

We will advance our knowledge of how a sperm stimulates an egg to develop into an embryo by finding the factors in the egg that allow it to respond to the sperm protein that triggers development. By making a stabilized version of this sperm protein we will provide IVF clinics with a new way of treating couples whose eggs have failed to fertilize. We will also improve our understanding of how we can assess the ability of a human embryo to undergo successful development. We will investigate how eggs use substrates such as fats for energy, and whether their metabolism is an indicative factor of developmental viability. This could eventually lead to better methods of selecting which single embryo to re-implant into a prospective mother undergoing IVF, and this will in turn help reduce the additional problems in pregnancies associated with twins and triplets.

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

We expect to use up to 3000 female mice and about 200 male 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?

We will inject mice with hormones so that they make more eggs than normal. The mice are injected twice, two days apart. Then after a further delay of about 15 hours (to allow ovulation) the mice are humanely killed. This procedure involves injecting the same hormones that women use as part of IVF. We will also keep some genetically modified male mice but they are not expected to have any adverse health problems. These male mice will also be humanely killed and sperm collected post-mortem.

Application of the 3Rs

Replacement

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

Replacement

There is no 'animal-free' cell culture system for making eggs that can develop into embryos. The way in which a mammalian egg develops into a healthy baby is very complex and there are many factors that can affect the formation and health of a baby that go way beyond our basic knowledge. The timing and complexity of the signals in mammalian embryos make them rather different from invertebrate embryos. Hence, the only way to study how factors affect a human embryo is to use another mammalian embryo.

Reduction

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

Reduction

The key factor in our work is the number of eggs we can obtain for experiments. We use the minimum number of mice for each experiment by using hormones to induce superovulation and hence obtain the maximum number of eggs per female.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 their eggs are most similar to human eggs in terms of their metabolism and response at fertilization. With mice we also have the ability to use genetically modified mice that lack particular proteins involved in the events of fertilization. This mutation only affects the ability of the mice to reproduce. The procedure that is carried out under a licence involves injecting female mice in the abdomen with a hormone using a sharp needle, on two separate occasions. The mice rarely, if ever, suffer any side effects or other consequences as a result of this injection other than the production of more eggs than usual.

PROJECT 51.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	Manufacture and Development of a Coccidiosis Vaccine
Key Words	Chicken, Vaccine, Coccidiosis
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 licence will enable the continuing production of vaccines for the immunisation of chickens against coccidiosis, a very important and significant disease of poultry worldwide.

The techniques and processes have been refined and are well established. The production systems used have been established for several decades, successfully producing billions of doses of vaccine for worldwide use.

Production of vaccine is achieved by infecting ‘donor’ chickens with a controlled low dose of modified coccidial organisms. Infection doses have no significant impact on the health or well-being of the ‘donor’ birds and by natural multiplication these organisms; many more are shed in the birds’ droppings. Vaccine is produced by washing and cleaning this material to yield high-quality organisms that can be dosed to commercial poultry as a vaccine. One ‘donor’ chicken will produce enough vaccine for many thousands of vaccine doses.

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

The vaccine reduces the impact and effect of this potentially fatal disease globally, and eliminates the need for antimicrobial administration to food-producing poultry for coccidiosis control. Vaccinated birds are protected against the effects of coccidiosis, improving their well-being and reducing suffering.

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

Chickens, approximately 140,000 per annum

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

Chickens may be dosed in their feed or administered orally by trained technicians, this may cause minimal discomfort. Vaccine ‘seeds’ are modified coccidial organisms given as a single controlled dose to each bird and this method has no obvious effect on their health or well-being. All birds are humanely euthanased 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

Despite extensive research and investigation it has proven very difficult to provide the coccidial parasite with ideal conditions to multiply in artificial systems. Alternative methods of production or producing a different type of effective vaccine are being actively explored.

Reduction

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

Reduction

Yield improvements have been improved to reduce the numbers of birds used and further improvement opportunities for increasing vaccinal material output per bird are being researched.

Refinement

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

Refinement

Coccidial organisms can only be grown in chickens to produce high enough levels of vaccinal material for commercial production. Birds used in this production technique are from a disease-free source and are hatched specifically for this programme of production.

The birds are housed in a bespoke unit within a controlled environment and droppings are automatically collected without handling the birds.

Welfare conditions are closely monitored and any potential impacts reduced.

PROJECT 52.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 coding in the rodent hippocampus and cortex
Key Words	Memory, hippocampus, sensory cortex, electrophysiology, 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:
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):

Despite great advances in the field, several questions about how we store memories and how our brain processes information from our surroundings remain unanswered. This project aims to better understand how neocortical and hippocampal regions – two areas known to be involved in memory formation and storage- encode information, how this information is initially stored in memory, and how this memory is subsequently consolidated. Our experiments generally focus on how sensory information is processed by neocortex during behaviour, and subsequently consolidated by interactions between the hippocampus and sensory cortex, during sleep and wakefulness.

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

These experiments will be conducted in rodents since there is plenty of evidence showing that many of the brain structures involved in processing information are preserved through mammalian evolution and are similar in humans. Understanding what are the mechanisms for memory formation and consolidation could be of great value to prevent -or keep to a minimum- the effects that several neurodegenerative diseases, such as Alzheimer’s disease, have on memory. Our studies will also provide information about how the brain processes information coming from different sensory modalities (i.e. audition, vision, etc.). Understanding this might contribute to the development of technology designed to help auditory and visually impaired persons.

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

Mouse (2500 / 5 years) Rat (1000 / 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 of the expected adverse effects relate to the surgical implants, and problems derived from the surgeries, such as anaesthetic death, post-operative pain, post-operative infection and occasionally, as a result of infection, post-op implant failure. These potential effects will be mitigated by careful anaesthetic and aseptic surgical techniques, careful monitoring daily in the post-surgical period and prompt treatment of any infections that might develop. We are also constantly working to develop light-weight devices to measure neural activity, which have three main advantages: reduction of the adverse effects, increase of the amount of data we can obtain from each animal (i.e. reduction of the number of animals), and, most importantly, reduction of discomfort for the animals. Generally, these procedures are well tolerated by the animals, and do not impede their natural or trained behaviours or their health. At the end of the experiment the animals will be either put to sleep with anaesthetic gas and then killed with an overdose of a sedative drug or killed with rising concentration of CO2.

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 awake and behaving animals is essential to test memory, learning, and other cognitive processes.

Non-animal alternatives such as computational modelling will be used where possible.

Reduction

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

Reduction

Numbers of animals will be minimized by collecting as much data as possible from a single animal, by means of multi-electrode implants and by advances in recording technology. Appropriate statistical analysis will also reduce the number of animals needed.

Refinement

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

Refinement

Rats are proposed because it is a very well established model for studying memory formation and consolidation and sensory information processing. In addition, rats are able to perform a great variety of tasks after short periods of training.

Mice are proposed because of their similarity with rats, and because of the available genetic technologies that enable exploration of the underlying molecular processes and brain activity manipulation.

We are constantly working on refining our techniques (such as the design of the devices used to measure neural activity) so that they mean as less discomfort for the animals as possible. Also, gentle handling of the animals from an early age by the experimenters help the animals to feel safe during the experiments or training sessions, which reduces the adverse effects of stress.

PROJECT 53.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 poultry production and welfare
Key Words	Welfare, Eggs, Reproduction, Genetics, 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;
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 project aims to improve reproduction, product quality, welfare and sustainability in meat and egg type poultry. The combination of egg and meat production make the chicken the foremost source of animal protein in the world and in the case of eggs an almost perfect balance of protein for human nutrition. The widespread availability of this high quality protein has been the result of improved genetics and systems of management. However, some of this progress has had unintended consequences on both welfare of the bird or quality of the product.

In this project we are addressing issues which influence both quality of eggs and the welfare of the hens that lay them.

We seek to understand the control of food intake in chickens. This is because food restriction is currently required to maintain egg laying in meat type birds and to ensure their health. However despite keeping the birds healthy the caloric restriction is considered a welfare problem and it presents management problems.

In egg laying birds, bone weakness during the egg laying period can lead to a higher risk of fractures, particularly when chickens have more room to move around.

Poor egg quality is a major reason for the culling of egg laying hens and increases the risk of microbial contamination of the eggs, which could be a problem for consumers or allow pathogens to reach hatched chicks. Also in egg laying birds and broiler breeders injurious pecking can cause harm to birds, sometimes death. We seek to ameliorate these problems by improving the hens using traditional or DNA enabled genetic selection and identify management solutions.

The duration of lighting is one of the major tools available to poultry farmers to control reproduction. We are trying to understand the mechanism by which light works to stimulate the reproductive system with the ultimate aim of improving the management of poultry reproduction.

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

By understanding the mechanism of feed intake we can devise strategies which can control growth whilst maintaining reproductive output and reduce feed seeking behaviour of the birds. By measuring and understanding the genetics and physiology of bone weakness we can help select hens or derive nutritional strategies which will reduce the chances of bone breakage. By measuring and understanding the genetics and physiology of beak morphology we can help select hens which will inflict less damage on one another. By devising new measurement strategies and understanding egg formation we can improve the selection of hens to reduce waste, increase biosecurity and protect consumers. By understanding the physiology of the perception of light by birds we may be able to devise novel lighting systems to improve reproduction.

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

Chicken and Quail, 4500 over 5 years

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

It would be expected that for around 70% of the animals only blood sampling, typically for genotyping, will be performed with a mild severity, and this only when a sample from the choriallantoic membrane in the hatched egg cannot be used. A further 20% may additionally experience an injection or change of environment such as altered lighting. Apart from minor discomfort or mild stress no adverse effects are expected from these changes of environment. For a small proportion (~10%) a surgical procedure with a moderate severity for the purpose of substance administration will be performed. The birds are expected to recover quickly from the surgical procedures.

Application of the 3Rs

Replacement

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

Replacement

We are studying complex interactions of environment and multiple organ systems, e.g. bone and oviduct in the production of eggs or the brain in control of feeding and reproduction, which cannot be studied *in vitro.*

However where possible we do find assays that can be carried out on tissues rather than animals. This is particularly the case for situations were causative genes have been identified.

Reduction

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

Reduction

The numbers used are determined by a calculation which takes account of the size of differences we expect to observe. This ensures the numbers are appropriate.

By having the best possible housing we reduce variability to reduce numbers and where possible we use cadavers or animals which would be slaughtered as part of the normal agricultural practice.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 study chickens because they are the species which we need to understand. Quail are used for studies where their responses are more robust than chickens and smaller numbers of animals can be used.

Most experimentation will involve mild stress or discomfort for a very short period during restraint. In most cases only a drop of blood is taken or the animals are housed in cages to measure food eaten or egg production. Wherever possible we will use administration methods which minimise handling.

In the case of surgery, drugs will be used to control pain.

PROJECT 54.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	Testing therapies for neuromuscular diseases
Key Words	Muscular dystrophy, mdx mouse, gene therapy, oligonucleotides, muscle 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;
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):

There are currently no treatments for neuromuscular diseases that stop or reverse the decline in muscle function. Using mouse models we aim to develop clinically applicable therapies for these conditions via gene therapy or the use of drugs targeting disease symptoms.

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

Neuromuscular diseases are an area of high unmet medical need that affect not only the individual patient but also their relatives and others involved in caring for the patient. Many cases of Duchenne muscular dystrophy (DMD) appear spontaneously with no family history, hence they cannot be effectively controlled by genetic counselling. DMD is debilitating and fatal and reducing or stopping the progression of the disease would be life-changing for patients and their carers.

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

All work will be done in cell culture or in mice. Many of the assays in mice will be carried out under terminal anaesthesia or post-mortem. We expect to use less than 3,000 mice over the 5 project 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 aim of the project is to develop clinically applicable therapies. Thus we seek to be as minimally invasive as possible. Most of the studies involve injections or addition of potentially therapeutic drugs to food or water and no surgical procedures except under terminal general anaesthesia (when the mouse does not feel pain or wake up at the end). As such, most of the procedures are of only mild severity. However, some of the work with cell based therapies (less than 200 mice) will require recovery surgery and this will be 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

In the vast majority of cases the drugs and genetic constructs under test will have been evaluated in cell culture. However, it is not possible to fully evaluate the treatment effects without testing in an intact whole animal with functional nervous and hormonal regulation of cellular processes and the complex inter-relationship of the muscle or brain and spinal cord and the blood supply which may act to limit drug effectiveness.

Reduction

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

Reduction

We will use the standard operating procedures (currently located on the treat-NMD website: http://www.treat-nmd.eu/research/preclinical/preclinical-efficacy-standards/). We have considerable experience with the mdx mouse model which provides knowledge of the variation in each measure and therefore accurate calculations of the required sample size. Experiments will use a randomised block design in most cases where mice in the same litter are assigned to different treatments at random to compensate for any litter to litter effects. Where the effect of a specific intervention is unpredictable and the potential variation is uncertain, pilot trials using 3 animals per group with a limited number of groups will be used to assess the variation before larger scale experiment using a range of doses with group sizes determined using power calculations. In some cases it may be possible to use a special method (factorial design) that reduces group sizes to 3-4 per 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

All experiments will be conducted in mice as this species has been the most widely used for genetic manipulation and has the greatest number of spontaneous and induced mutants and genetically modified strains. It is also the lowest vertebrate group for which there are models of the most common neuromuscular disorders.

The main model for the initial in vivo assessment of therapies for DMD will be the mdx mouse and this will be used for the majority of the studies: the mdx mouse model of DMD is a relatively mild model that shows no obvious signs of the disease. Most of the studies involve injections or addition of potentially therapeutic drugs to food or water and no surgical procedures except under terminal general anaesthesia (when the mouse does not feel pain). We will use anaesthesia and appropriate analgesia where a procedure may cause pain.

PROJECT 55.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	Immunological defence against bacterial pathogens
Key Words	Immunity, Inflammation, Lung, tuberculosis, 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):

This project aims to:

1. Define the function of specific cellular and molecular pathways in mediating the protective and pathogenic immune response following infection with bacteria (such as bacteria that cause tuberculosis).

2. To manipulate specific cellular and molecular pathways to determine the impact on protective and damaging outcomes.

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

To reduce the burden of infectious disease on the worlds human and animal populations we need to design specific interventions to block these diseases. The development of these interventions requires in-depth definition of the molecular mechanisms of infection, disease development and protective and damaging immune responses. This project uses the mouse to determine the molecular pathways of the immune response to bacterial infection to rationally design new interventions such as vaccines. Tuberculosis is a disease of significant public health importance for which usual vaccination strategies have not been successful. By defining how tuberculosis manipulates the immune response we can design better interventions. We use the mouse model in the context of human and animal disease. The work performed in this project is integrated with human experimental medicine studies. In this way, progress made in understanding of disease pathways is linked directly to human data. The information from this work will be disseminated to other scientists within disease focused networks. The work will be published in scientific journals and used by ourselves and others to develop effective human and animal health interventions.

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

We will use male and female mice of up to 12 months of age and will obtain them by in-house breeding and by supply from accredited mouse breeding facilities. There will be approximately 9000 mice used 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 mice will experience no adverse effect apart from momentary disturbance due to handling, injection through the skin and into veins or body cavities. Some animals will experience mild to moderate suffering as a result of infection with bacteria or immune intervention. This will include weight loss and apparent discomfort which will be indicated by changes in the behaviour of the mice. Mice will be monitored by weighing and observation and suffering will be reduced by delivery of analgesia or anaesthetic under the direction of veterinary staff. Close monitoring of bodily signs of discomfort will be used to determine when to humanely kill the mice. Mice will not undergo anything more than moderate suffering. All animals will be humanely killed at the end of the experimental protocol 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

We need to use animals with air breathing lungs in order to define the role of each part of the immune response in the expression of immunity to infection in the lung, the site of tuberculosis disease. Modelling this type of interaction using cells in the laboratory cannot be done in an unbiased manner as there are many factors which may be important but which we are unaware of. Use of animal cells in the laboratory is not a primary aspect of the proposed work as the modelling we are doing is of the complex interaction between multiple cell types within the lung. We will use cell culture in the laboratory as a tool to define some function but this is not really a tool to replace animal use in this proposal.

Reduction

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

Reduction

We generate and test our experimental designs with our on-staff statistician and by use of the NC3R's Experimental Design Assistant.

The number of mice used for these experiments will be the minimum number needed to achieve the answers required. We calculate group sizes using information from previous studies to predict the number of mice in each experiment required to determine the answer to the important health question using as few animals as possible. Group sizes will be continuously monitored and adjusted in the light of results obtained. Control groups of mice are included in all stages of experiments and can be used in answering several questions at the same time thereby reducing the number of animals required.

Refinement

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

Refinement

New technology allows us to determine how each component of the immune response contributes to the protective and damaging response to infection. Many of these pathways work together and use of the whole animal allows us to find out how each component impacts the response. The current state of the art in mouse experiments allows for the function of specific cell types to be compared under identical conditions. The use of genetically altered mice which express on/off switches allows for the role of specific molecules on specific cell types to be assessed. The delivery of specific cell populations into other mice allows for the function of these cells to be defined. These highly manipulated models are required to make sure we are clear about the function of specific cells and molecules within the animal as a whole.

The integration of the work within a program of experimental medicine and unbiased data collection means that specific experiments with defined working hypotheses are performed.

A key refinement in the use of animals is to ensure relief of stress which not only harms the mouse but also compromises experimental data interpretation. Any observed stress will be relieved by analgesic delivery where appropriate and by monitoring and euthanasia where required. We will monitor the animals to ensure they undergo only moderate suffering. This refinement is based on the use of signs of mouse behaviour which reflect discomfort. Our protocols allow all trained employees to treat the animals in a similar manner thereby improving reproducibility and allowing refinement of numbers.

PROJECT 56.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 Pathways in Neuroregeneration
Key Words	spinal cord injury, nerve injury, axonal 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):

Traumatic injuries to spinal tissue and nerves have a high incidence and prevalence that lead to severe long-term disabilities as a result of nerve damage. To date, there is still a lack of effective treatment that may limit long-term nervous system disorders and disability, mainly due to a lack of understanding of the functional changes that accompany these disorders. It is therefore an urgent scientific priority that new therapies are developed. The success of this will depend upon both a better understanding of the biology of the nervous system and upon the development of new technologies. However, our understanding of how nerve cells respond to nerve and spinal injury is still incomplete and this limits the design of effective therapies.

Using animal models that allow us to study the mechanisms of human nerve and spinal cord injury, where all nerve injuries will be conducted under general anaesthesia and animals will be provided with post-operative pain relief, we would like to determine at the molecular level the reasons for the imperfect or failed ability of the nervous system to regenerate and recover after 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)?

By generating new knowledge we expect to improve our understanding of human diseases of high importance such as nerve and spinal cord injury and stroke. Ultimately our work with animal models may provide novel therapeutic opportunities to enhance regeneration and functional recovery in these human diseases.

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

About 8000 mice and 1200 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?

The expected adverse effects will be impaired nervous system function resulting in muscle weakness. Where the optic nerve is damaged, blindness will result in one eye but this will not impair the animal's activity or independence. In all the injury models used, mice recover quite quickly and enough for them to eat and drink independently. Intermittent fasting is used in one of our studies, and this generally results in extended life span and decreased risk for diseases and disorders associated with ageing. The expected level of severity is moderate. If any unusual symptoms develop, the veterinary surgeons in the facility will be promptly consulted. At the end of the 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

The goal to develop novel therapies for human disorders of the nervous system must be tested in animals as cell systems do not provide the biological complexity and interaction occurring in human organs and diseases. There is no specific advance in this context. We will however limit the use of animals to regenerative treatments that have been screened first in cell culture in order to minimise the number of animals needed for in vivo work.

Reduction

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

Reduction

In vitro testing in cell culture will be extensively used to verify initial hypothesis before investigation in animals. In addition, only the necessary number of animals needed to reach statistical significance for each given experimental question will be employed. A common sense approach will also be adopted whereby if our treatment strategies are clearly having no effect, those particular experiments would cease and our strategy revised or revoked.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 used extensively in nervous system disease research, which includes regrowth or repair of nervous tissues, cells or cell products in models of nervous system injury. They allow reliable data for comparison in humans. These rodents are relatively low order sentient animals (compared with non-human primates, cats, dogs, etc.), and as such, are widely used by the scientific community for the type of research we intend to carry out. Mice are preferred for genetic studies because of the similarity of their genomes to that of humans, and also because of their availability, ease of handling and high reproductive rates. Mice are also our species of choice because a large number of genetically modified mouse types are available which enables data cross comparison in our studies. Rat models, however, are most widely used to study spinal cord injury as they exhibit similar responses at the injury site as humans. Rats are therefore preferable in preclinical studies for novel therapies where mimicking the human pathology is crucial. Our experiments will include moderate but not substantial severity protocols.

We carefully chose animal models that resemble the human diseases we study and are good predictors of effectiveness of treatment under investigation without having to resort to a more substantial severity level.

Animal suffering will be principally minimised by optimal operating technique and providing adequate analgesia and good post-operative care. Where there is a choice of route of administration of substances the least invasive will be adopted. LASA guidelines will be followed during administration of substances and dosing procedures 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. Pain and distress will be carefully monitored after procedures and appropriate measures will be taken if signs of pain/distress are observed.

There is evidence that the positive effects associated with intermitted fasting include increased protection of the nervous system and unchanged body weight. Therefore, choosing intermitted fasting for our nutritional confinement study describes a method of refinement in animal welfare.

PROJECT 57.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	Redox Regulation of Pulmonary Hypertension and its Preclinical Therapies
Key Words	Cardiovascular, pulmonary hypertension, hypoxia, oxidants, 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):

Blood vessels which connect the heart with lungs reside at a higher oxygen tension compared to other vessels. These pulmonary vessels narrow their cavities in response to the low body oxygen (known as hypoxia). During short-term exposure to low oxygen, or hypoxia, this mechanism is reversible and helps better oxygen delivery to major organs. However, prolonged hypoxia results in the vessels becoming fibrous and continuously narrowed. This requires the right side of the heart harder to pump leading to it not working properly and leading to the disease called pulmonary hypertension (PH). PH is a disease where severely increased pressure develops in the blood vessels connecting the heart and lungs (pulmonary vessels), which results in shortness of breath and other symptoms that are exacerbated by exercise. Patients with PH have a very limited life expectancy despite existing drug treatments. Current treatments for PH are not entirely effective because they can only widen pulmonary vessels a small amount, and for a short while. This does not really relieve the pressure as the walls of these narrow vessels are already too thick, fibrous and structurally changed. The goal of this research programme to investigate how oxidants help to maintain the healthy pulmonary vessels state. Proteins that can “sense” the oxidants level in the lung vessels during low oxygen are to be identified. This project will provide new insight into the processes that regulate hypoxic narrowing of pulmonary vessels and how it can become dysregulated and cause injury to the lungs and heart. By understanding these processes better a novel therapy may be developed for patients with pulmonary problems.

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

The new studies proposed here will provide further valuable information on how oxidant can be involved in development or prevention of PH. Our research therefore will increase the knowledge of how PH occurs and progresses. The proposed studies will guide us to test new drug targets and new drugs to treat or prevent PH in patients. Even with currently available treatments, 3-year survival is only 60-70%.

We aim to develop treatments that treat the main cause of the disease, rather than its symptoms. There is a high likelihood that these studies will significantly advance our understanding of PH, and open new therapeutic avenues, which might ultimately help improve the symptoms and survival of patients with PH.

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

This project will use rats and mice, including genetically modified mice. We anticipate the use of a maximum of 17,100 mice and 3,400 rats over a 5 year period.

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

The majority of our protocols involve the use of genetically modified mice and require the exposure to certain agents or environmental factors that stimulate the development of PH. These could be low oxygen tension (i.e. hypoxia-induced PH), or drug compounds that cause structural changes and thickening of pulmonary vessels. We then use drugs or other substances to try to prevent or reverse the disease. In order to monitor the disease progression and recovery, the animals may be subjected to general anaesthesia, injected with drugs, or implanted with catheters enabling drug delivery, or small radio-telemetric devices for remote blood pressure measurements. Implanted animals may be single-housed for some period of time. Most of our measurements are carried out at the end of any protocol once the animal is anaesthetised and the animal is not allowed to recover from the anaesthetic. In some protocols we make measurements in anesthetised animals using non-invasive imaging techniques (i.e. echocardiography) so that we can follow the course of PH in each animal. During the course of experiments animals may lose weight, breath faster and become less physically active. They may become huddled and feel unwell. Overall the expected level of severity in our protocols is mild-to-moderate. At the end of each protocol animals are humanely killed, usually 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

PH is a complicated disease which affects more than one vital organ, and in which the disease-related changes develop over a period of several weeks to several months and years. We cannot “mimic” PH in a dish, as this will fail to provide information that is useful in terms of how therapies may be subsequently used in humans. Animal research and models are therefore required to understand PH

development and its effects on body health, as well to test any potential therapeutic strategies in the intact animal. We will however use human cells in the laboratory dish to provide important information before embarking on animal experiments. Studies of human tissues from patients who died of PH are not ideal as not only such tissue difficult to obtain, available specimens are often not suitable for research purposes in terms of quality and often comes from uncontrolled populations. It is not currently possible to use computational models, although our findings may guide future programs. In the future, artificially constructed data networks might help to predict the likely course of a disease and its response to treatment.

Reduction

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

Reduction

We will only test treatments in animals for which there is a sound scientific basis, based on our finding from experiments in a dish (cells), perfused animal organs and human tissues from patients that died. All efforts will be made to restrict animal numbers. Measuring blood pressure in animals with implantable radiotelemetric devices and employing imaging techniques such as echocardiography or MRI, permit direct comparison of the data before, during and after the treatment or intervention. Many of the animals that we plan to use will be anaesthetised and killed under anaesthetic without waking up (which does not cause any additional harm to the animals). This will increase the amount if research data from each animal, and inform further studies. Thus data is maximised whilst animal numbers are minimised. We will define the number of animals required by calculating sample size according to statistical principles. In addition, we will do power analysis and relevant statistical analysis to analyse our data, and care will be taken to minimize animal usage wherever possible. We will also utilise state-of-the-art imaging tools whenever possible, to enable us to track progression within an individual animal and thus maximise the data collected from each animal 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

Rats and mice are the lowest vertebrate group in which models of PH have been developed and are thus the most appropriate species to study. Although we have chosen to primarily use a mouse model of PH, we require the use of both rats and mice because rats, but not mice, develop pulmonary hypertension in response to monocrotaline. Monocrotaline is a plant alkaloid, which when injected to mice,

becomes metabolically activated in the liver and causes selective damage of inner layer cells (i.e. endothelial cells) of pulmonary artery. This in turn causes pulmonary arteries wall thickening.

Rats may also be used as it is necessary to have a second rodent model for pharmacological studies proposed here, i.e. to test the most promising therapies, after they have been showed to be working in mice and before work can progress towards clinical trials in man. On the other hand, mice provide better genetic models of disease as we have the ability to breed animals with specific defects in their genes which are involved in specific pathways underlying disease and are thus useful to screen for drug effects quickly and efficiently. The introduction of sophisticated imaging techniques (e.g. echocardiography) and measurements similar to those used in patients with PH means that the number of groups of animals can be reduced because we can track progression within an individual animal and thus maximise the data collected from each animal used. Animals are monitored at least daily. If an animal shows any predetermined signs of illness or discomfort such that the humane endpoint of the experiment has been reached it will be humanely killed. At the end of the experiments animals are humanely killed and tissues harvested for further analysis. We work closely with the veterinary surgeon who advises on methods to maximise animal welfare for animals on study in our experiments.

PROJECT 58.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 survival and connectivity of developing neurons
Key Words	Neuronal development. Axon. Dendrite. Survival.
Expected duration of the project	5 year(s) 0 months

Purpose of 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 this project is to further our understanding of how the vertebrate nervous system develops. We will elucidate the functions of factors that control the survival and death of nerve cells and factors that control the growth and morphology of neural processes (axons and dendrites). Our studies will largely focus on the roles of members of a large family of proteins, the tumor necrosis factor superfamily that have well-recognised roles in the 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)?

The main benefit of our research will be to extend our knowledge and understanding of basic developmental processes in nervous system. Such knowledge may be relevant for neurological diseases. For example, our demonstration that the TNF superfamily member APRIL plays an important role in establishing the midbrain dopaminergic projection which degenerates in Parkinson’s disease.

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

Approximately 24,000 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?

Mild severity. Licence required for breeding mice with genetic mutations that cause the animals minimal suffering. Other than this, no experiments are carried out on live animals. Licence also required to kill neonatal mice by a humane method of killing to harvest tissues.

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 to study neurons cultured to address fundamental issues relating to growth factor biology developing nervous system. Cell lines are not available and cannot be generated for the various kinds of neurons at different stages of development we need to study. Studies of genetically modified mice provide the most relevant and convincing evidence for gene function.

Reduction

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

Reduction

Several lines of transgenic mice will be required for our research. Reduction strategies will include the following: (i), when particular mouse lines are not in current use for experimental studies, the number of mice will be reduced to a core breeding colony; (ii), when particular lines are not going to be used for an extended period of time, we will store frozen embryos so that the breeding colonies do not have to be maintained for these mice; (iii), we will cross new strains into a stain that typically breeds very well and have relatively large litters, thereby reducing the numbers of mice that have to be maintained and bred to provide sufficient numbers of mice to obtain material for experimental studies.

Refinement

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

Refinement

Mice are the most appropriate animals for these studies because the necessary descriptive developmental data on the neuronal systems used are available for these animals and because of the availability of mice with targeted mutations in the genes of interest.

At the end of experiments animals will be killed humanely killed..

PROJECT 59.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	Polyclonal Antibody Development
Key Words	Polyclonal, Blood, Antiserum, Rabbit, Sheep
Expected duration of the project	5 year(s) 0 months

Purpose of 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 objective of this project is to provide biological materials (blood and antibodies) that will be used for detection, diagnosis, research or therapeutic purposes. Antibodies are produced by the body's immune system in response to substances which are recognised as foreign, such as bacteria, viruses, and foreign proteins (known as antigens). Antibodies bind specifically to the antigens and therefore be used to identify them in a range of applications (plant, medical and animal science) from fundamental and applied research through to the development of diagnostic and therapeutic devices

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

This licence will allow biological materials required to support specific research and commercial development projects in human, animal, plant and pathogen research to be available when required. Antibodies can be used in a wide range of research and commercial development techniques. In fundamental research they can be used to find new cellular targets and pathways involved in normal and abnormal cell function. This may lead to the identification of new approaches for treatment of diseases e.g. cancer. In applied research and development, antibodies will be used to produce tests that allow identification of specific substances present in disease. This allows early detection and diagnosis of diseases and close monitoring of progression so that the most appropriate course of treatment can be administered. Antibodies are increasingly used as a therapy to allow specific targeting of the treatment within the body/plant. This maximises the treatment effect whilst reducing any associated side effects.

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

The target species are sheep, rabbits and chickens. The numbers projected to be used over the next 5 years are based upon those used over the last 3 years. Sheep - 1540; Rabbits - 2290; Chickens - 210, Mice - 315, Hamsters - 40, guinea-pigs - 50.

In the context of what you propose to do to 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 currently not possible to replicate an intact immune system to generate antibodies in vitro. The production of custom antibodies requires the 'foreign' substance (antigen) is given to live animals to elicit an immune response. To promote the development of this response and antibody production, a substance known as an adjuvant may be used together the antigen. An emulsion is made with these substances so that there is a sustained slow release of the antigen further enhancing the antibody response. Due to this slow release, a localised swelling with mild inflammation may be seen at the injection site as a consequence of the injection. This resolves with a short timeline. The injection site will be monitored and animals will be checked before any subsequent boosts are carried out. Blood samples will be collected from animals in order to test the levels of antibodies within the blood. They will be collected from an appropriate site on the animals e.g. veins and arteries. This can very occasionally lead to formation of bruises. For chickens, eggs will also be collected. The expected severity limit for antibody production is mild although where animals are retained on protocol for a longer period of time as they have produced a good antibody response, the cumulative severity may be moderate. After use on this procedure animals, if deemed fit and healthy by the veterinary surgeon, may be kept alive for reuse on a separate project or entry into a breeding group. Alternatively, animals will be killed 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

The licence will only be used to provide biological materials where no other alternatives are available. Despite advances in molecular biology, it is currently not possible to generate antibodies against all antigens that can be used in research and development applications. An intact immune system is needed to generate these molecules. In addition, with the current shift in scientific research towards looking to identify new targets within cells, there is a growing need for highly specific antibody molecules within scientific research and product development. The justification for the use of animals will be addressed for each antigen tested.

Reduction

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

Reduction

The number of animals and protocol to be used will justified for each antigen tested. The number of animals employed in Protocols has been minimised through experience using this protocol over the last 30 years. The number of animals will be minimised with appropriate selection of species dependent on the requirement for the antibody by the client – e.g. a sheep may be used rather than a number of rabbits when a defined volume of antiserum is required. Our extensive experience within this field allows us to provide guidance on best practice for species and number of animals to be used. Where poor responses are seen to antigens, sample size calculations may be applied using data from the initial immunisation programmes to determine the number of animals to be used in further testing.

As part of this programme of work, the ability of the immune system to produce antibodies in response to immunisation with multiple antigens will be investigated. If successful, this will reduce the number of animals used under protocol.

Refinement

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

Refinement

Cross bred animals will mainly be used in the protocols. They have a strong immune system that produces high affinity antibodies. The sheep are housed in pasture/sheds in keeping with standard housing of these animals within farms. The rabbits are bred in house (both pure and cross-bred). They are housed in floor pens with added environmental enrichments (e.g. raised areas, toys) and are fed both standard diet, fresh vegetables and hay. They are maintained in a stress-free environment as close to natural as possible to maintain health. These environments promote a strong immune response within the animals.

Close working and handling of animals by staff in an open social group allows prompt detection of any ill health. Highly qualified technicians and veterinary support will ensure that any deviation from health will be picked up immediately.

For all procedures, the best practices of the NC3Rs will be implemented as required.

PROJECT 60.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 Substances
Key Words	Pharmacokinetics, Tolerability
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

There are many common and rare human diseases where treatment is sub-optimal or there is no effective treatment available. To develop effective treatments new drug need to have no side effects after dosing and be effective in controlling the disease for which it was developed.

This licence will enable us to provide information on whether a proposed drug has any side effects following dosing and whether it is taken up into the body in correct amount and at the place in the body required to control 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)?

This project will provide first in-animal data that will be used to rank compounds for tolerability and drug exposure that is a critical step in drug development. These studies will allow substances to be ranked for safety and exposure so the best examples can progress into further drug development and eventually to clinical trials and into the clinic for patient and animal use.

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

This project licence will only use rats and mice and of these approximately 80% will be mice and ~20% rats. The number of animals used will be dependent on the service requirements but is likely to be about 100,000 over the 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?

A safe dose will be identified starting with a low dose that will be given two animals and if it is tolerated the next two animals will be given a higher dose or lower dose if the first dose was not tolerated. This will be repeated until a safe clinically relevant

dose is found. In majority of studies the drug will be given to the animals by a route that does not require any surgery (oral dose, injection into skin, vein etc) which will cause brief discomfort or pain. However, in some cases (<10%) animal will have lines implanted to access the circulation or under the skin. These will be carried out surgically and animals will be given pain relief to mitigate any pain from the procedure. All animals taking part in a study will be regularly monitored for signs of ill-health (loss of weight, change in coat condition, reduced response to stimulation or lack of interaction with cage mates) and will be humanely killed if their condition does not change. At the end of the study, typically 24h in duration, it is necessary for the animals to be humanely killed and post-mortem carried out to look for any damage or change to internal organs. These studies will be followed by drug distribution in the blood and tissues but using the safe dose identified above. These studies are typically only 24h in duration with several blood sampling time points during this time, which will cause brief discomfort or pain. At the end of the study tissue samples are taken for bioanalysis after the animal has been humanely killed. The majority of animals (~80%) in these studies will undergo experimental procedures classified as mild, and ~20% 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

Use an extensive panel of non-animal alternatives and computer modelling to identify and select the most suitable compounds before testing in animals.

Whilst non-animal and computer models are highly predictive and stop the development of many compounds before they are tested on animals, currently there are no non-animal models that can fully replace animals due to the complexity of a complete animals with multiple interacting systems.

Reduction

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

Reduction

The experimental designs and analytical methods used in this project will be scrutinised by a statistician to ensure good quality data is obtained with the minimum number of animals and appropriate statistical tests are applied.

Extensive literature searches will be carried out prior to any study to ensure best study design and method are being used as well as check the work has not already been carried out.

Where historical or published data is not available, a small pilot study before a full study will be carried out and data used to statistically calculate the minimum number of animals required in each group to ensure 80% chance of finding a meaningful result.

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.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 first choice for studies but there are likely to be instances where this species is not relevant such as due to drug interactions or clearance is not the same as in humans or where larger volume of blood might be necessary. In these case rats will be used.

We will use the following to minimise harm to animals:

- The most appropriate species will be used
- 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.
- Collect the minimum volume of blood on the fewest occasions
- Where pain is likely then prophylactic analgesic agreed with the named vet is used.
- Use rigorous monitoring of clinical condition to ensure animals are euthanized within agreed severity bandings.
- Continually assess published literature to ensure latest refinements are used and avoid duplicating work.

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	Therapeutic control of African trypanosomiasis
Key Words	infectious disease, vaccine, drug target
Expected duration of the project	5 year(s) 0 months

Purpose of 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 test of human and animal trypanosome parasite molecules that are potentially therapeutically exploitable.

What 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 will carry out pre-clinical trials towards the development of vaccines and drug targets for an infectious disease, African trypanosomiasis, that affects humans and animals.

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

Mice (620)

In the context of what you propose to do to 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 remain conscious during blood sampling or immunisation and will experience the skilled insertion of a needle or the minor puncture of a superficial blood vessel. Transient inflammation or irritation may be experienced around the injection site. During vaccine development, animals will be immunized with prepared vaccine candidates, then later challenged with parasite infection. Constant assessment of animal welfare will take place throughout vaccine tests. Animals may transiently experience mild symptoms of disease in rodents (e.g. poor coat condition, dull eyes-score, temporary shivering). In the case of clinical progression to moderate symptoms (e.g. hunched animals with reduced mobility and/or responsiveness), this will result in the humane killing so to minimise harm to animals (and prevent disease progression to a stage of distress).

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 experimental animals is essential to address questions related to a human infectious agent, particularly those related to the development of vaccines and novel medical treatment. All preparatory work will be carried out *in vitro*.

Reduction

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

Reduction

To meet **the Objectives** of this licence, it is important to have a high coverage of exploitable candidates. When screening for potential drug targets, it is crucial that one is able to identify the true positives within the testable set, those that will then be exploited in a clinical setting in the future. To this end, it is appropriate to set a reasonably high power that only misses 1 in every 10 positive candidates. Equally, when screening for potential vaccine candidates against a lethal disease, vaccination efficacy must be set high, at 90% or more, such that to significantly extend an individual's longevity/reduce risk of infection. Therefore the proposed study uses the minimum number of animals possible in order to detect effects as little as 10%, and with confidence power of 90%. Performing the proposed work with any less power would represent unnecessary and wasteful 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

Rodents are the smallest animals with human-like characteristic immune system. Research over many decades has shown that rodents can support the growth of infectious agents without significant discomfort to the animals when the disease is monitored closely and animals are humanely killed at the appropriate time.

Animals will typically be housed in groups and monitored by trained and competent animal technicians and research staff. 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. These will be conducted according to best practice guidelines by trained and competent staff. Any concerns regarding the health or welfare of an

animal will be discussed with the Named Veterinary Surgeon. Early humane endpoints associated with clear clinical symptoms will be used to minimise the impact of the disease process once experimental data is obtained. At the end of the procedures animals will be killed using a recognised humane method.

PROJECT 62.NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Regulating glial function to promote regeneration and remyelination in the central nervous system
Key Words	CNS (Central Nervous System), Glia, Neurone, Regeneration, 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:
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 brain and spinal cord – or central nervous system (CNS) - contains two main kinds of cells – ‘neurones’ and ‘glia’. Neurones are the signalling cells and glia are specialised to support neurones. Without support from glia, neurones start to degenerate. Glia are essential for maintaining healthy brain function across the life-course and protect against pathological changes that underlie normal brain ageing and neurological diseases, such as Multiple Sclerosis, cerebral palsy, trauma, stroke and Alzheimer’s disease. The aim of this project is to provide new insights into fundamental biology of glial cells that is essential for maintaining a healthy brain across the life course and to inform on the processes that underpin neurodegenerative changes in normal ageing and pathology.

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

The lack of knowledge of how glial cells perform their many support functions and how this goes wrong in the ageing brain and in CNS diseases is a major impediment to our ability to successfully treat neurodegenerative changes. This project will identify new potential therapeutic targets to promote brain repair and regeneration.

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

Mice will be used. Mostly, we will use transgenic mice and breed and maintain 10 or more strains of transgenic mice, totalling 10000 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?

There are four main procedures, with different levels of potential harm to the animals. At the end of all experiments, animals will be killed by a humane procedure.
1. In Protocol 1, we will breed genetically altered mice that have no adverse effects.
2. In Protocol 2, we will breed so-called ‘knock-out’ mice, in which turning off a

specific gene may have potential adverse effects, including minor fits or possibly mild problems with walking. We have designed the experiments to avoid these adverse effects by inducing partial reduction in the genes of interest. 3. In Protocol 3, we will maintain ageing mice, 12-18 months old, at which age mice begin to display early changes in brain function that are similar to those observed in healthy humans, aged 50-65 years old, which may have potential adverse effects such as minor problems with locomotion. 4. In Protocol 4, we will administer agents that alter glial cell function, by administration into the body fluids of the animal, nasally, or directly into the fluid compartment of the brain (ventricles) by injection. Administration of agents may have transient harmful effects, such as minor fits or possibly mild problems with walking. We will design the experiments to minimise these possibilities, by using agents with known pharmacology and toxicology, testing these agents in a test tube, and starting with low doses in animals to induce partial effects that are sufficient for us to determine their functional importance, whilst minimising harm to the animals.

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 number of models to replace animal research, including cell lines and the vast majority of experiments to be performed in this programme of research will be on isolated cells and tissue. However, a major task that we face is to translate our results in the test tube to new potential therapies for maintaining healthy brain function in humans and slowing and possibly curing neurodegenerative diseases. Currently, animal experiments are indispensable for preclinical drug development. For this project, we will identify new ways to promote regeneration in a test tube and confirm their importance in isolated tissue, prior to testing their therapeutic significance in animals, by pharmacological and/or genetic manipulation.

Reduction

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

Reduction

The experiments are designed to use the number of animals required to achieve statistical significance, in accordance with established guidelines for animal research. The use of genetically modified mice reduces the total number of animal experiments required overall, since we will be able to identify neural cells by their expression of fluorescent markers, and identification would otherwise require much greater amounts of tissue for complex histological and cell biological 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 will use transgenic mice because they enable us to specifically affect potential drug targets in specific cells. Currently, these mouse models are the primary preclinical models for the development of novel therapies and wherever possible we use in vitro methods. A number of lower vertebrate models - Xenopus and Zebrafish - are available for screening novel compounds in vivo, but these do not currently preclude the use of mice for preclinical experiments and require much longer duration projects than is feasible for the current project. Instead, we will screen compounds *in silico* and *in vitro*, then test the most promising *ex vivo*, and only those proven to be effective will be examined *in vivo*. In this way, we have refined the experiments to use protocols where the potential risks of harm to the animals are known and minimised. In cases where there are known harmful effects that are part of the experiment, we have selected methods where the effects are highly localised and transient and should not cause clinical harm to the animal. In the case where we are developing pharmacological and genetic modulation of glial therapeutic targets, we have designed the experiments so that the function of these molecules can be altered incrementally, to cause effects on the cells without clinical harm to the animal.

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.

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Word limit; 1000 words

Project Title	Dissecting resolution mechanism for clearance of infections and tissue regeneration
Key Words	Inflammation, Tissue Protection, Organ Repair and Regeneration, Clearance of infections, 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;
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 the experiments conducted in our laboratory is to gain an understanding into the ways our body defends itself from invaders such as bacteria. We are also interested in understanding how our body also repairs itself after damage. The reason for this is because understanding these processes will shed light into the causes of inflammatory diseases such as those that afflict modern societies including rheumatoid arthritis and cardiovascular disease. This may also help us develop new treatments that would not carry as many side effects as those found with the majority of medicines that are used today. Therefore, the experiments conducted in our laboratory will help address some very fundamental, and therefore critical, aspects of why certain diseases such as arthritis as well as those affecting the heart and blood vessels occur and identify potential new leads for treating such diseases. The will also help us find new ways to treat infections that do not solely rely on the use of antibiotics, also in light of the recent increase in bacteria that are resistant to antibiotics such as MRSA.

In studies conducted over the last years we have identified several molecules that are involved in orchestrating the body’s defence system that combat bacterial and viral infections as well as promote the repair and regeneration of damaged tissues. These molecules are produced by a number of cells including white blood cells, where these cells converting fish oil derived omega-3 fatty acids to these protective molecules known as specialized pro-resolving mediators (SPM). More recently we found the brain and central nervous system are very important at controlling the production of these factors. Our studies indicate that in conditions where the production and actions of these molecules is dsyregulated, there is an impaired

ability of the body to clear invaders (e.g. bacterial) as well as to repair and regenerate itself leading to disease and pain.

In the present studies our aim is to obtain a better understanding of the way the mammalian body:

i) responds to different types of damage and infection in different organs and tissues such as blood vessels, the spleen and the heart

and

ii) to determine whether these processes can be harnessed to develop new medicines that can treat these inflammatory disorders without the unwanted side effects associated with current 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 research conducted in our laboratory is at the cutting edge of the Resolution Pharmacology field. We have discovered a large proportion of the molecules that we will be investigating during our proposed studies and therefore our aim to continue conducting research that will elucidate innovative and fundamental mechanisms active in mammalian systems. We also envision that mechanisms uncovered by these studies will provide new leads for therapeutic development as has been the case for some of our previous discoveries, which are now under clinical development. Our aim as pioneers in the resolution pharmacology arena is to continue contributing to the discovery and development of novel/better therapeutics.

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

We will make use of rodents, over 90-95% of which will be mice. As such we plan to use – over the 5 year licence - ~13,850 mice and ~1,400 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 employed in the experimental protocols described in the present project will be employed to further our understanding on diseases that affect both western societies (e.g. rheumatoid arthritis and cardiovascular diseases) and mammals at large such as bacterial infections. These models will be employed to mimic specific aspects of these conditions and therefore provide us with a detailed understanding of the complex possesses engaged in these conditions. This will allow us to identify more efficient treatments approaches that do not carry many of the side effects associated with available treatments. We aim to achieve this by using models that are already well established and characterised within our group. For example we will inject mice with a specific bacterium or a mix of bacteria and assess how the

immune response deals with this when the mice are given specific treatments. We will also measure the production of factors that are predictive of how well the immune response is dealing with the infection. The majority of animals will be used in models that provoke mild to moderate discomfort to the animals in our experience. In models where discomfort may be somewhat higher such as of arthritis and heart disease we will take additional steps to mitigate the pain. For example in experiments assessing heart disease where the heart tissue is damaged it is expected that in a small proportion of the animals the heart will fail. If this is to happen in our experience this happens within the first few hours after the procedure therefore during this time we will maintain the mice under anaesthesia to minimise pain. Subsequently, where appropriate, mice will also be given painkillers to minimise any pain. Even under these conditions in less then 6% of animals death may occur after recovery from anaesthesia, in these events this usually occurs due to heart failure resulting the sudden death of the animals, therefore they are not anticipated to suffer extensively. However in most cases the anticipated harms are expected to be only temporary, e.g. pain following injection, and should resolve with 24-48h. In some cases, the pain may be longer lasting as will be the case when in arthritis. As in humans, these mice are anticipated to have joint pain and as a result they may not be able to feed well. Where ever possible we will administer painkillers to mitigate the pain however this may not always be possible and in this case we will establish clear endpoints and monitor the animals regularly and if animals reach such predetermined points they will be euthanised 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 aim to conduct the majority of our studies using isolated cells from humans or tissues to minimise animal use. However, since our current understanding of processes occurring within the body is still limited it is not always possible to study disease processes in isolated cells alone therefore animals need to be use to recapitulate some of these aspects as well as to test putative treatment in animal systems which is a fundamental aspect to medicinal development. When animal experiments will be used these will be conducted to address specific scientific questions that we would have identified using in vitro systems or human tissues.

Reduction

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

Reduction

In order to ensure that only the appropriate numbers of mice are used for the predetermined experiments we conducted statistical analysis using results from preliminary experiments as well as from published experiments. Furthermore to further reduce numbers whenever possible (i.e. when it does not compromise the outcomes being measured) control mice will be utilized to measure different outcomes.

We are also employing imaging techniques and assessing multiple parameters in each animal to further reduce the number of mice. Results obtained with animal systems will be immediately validated with primary human cells and where possible also with either patient or healthy volunteer tissues.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 the described experiments we intend to recapitulate specific aspects of mammalian/human disease. Therefore the experiments here will be conducted to address specific aspects of a particular disease for which we have obtained results from either humans or *in vitro* systems. For example we recently found that there are diurnal changes in the levels of specialized pro-resolving mediators that also correlate with increased risk of myocardial infarct in patients with cardiovascular disease. In order to better understand the mechanisms that lead to this increase risk and identify potential treatments we will use a combination of transgenic animals and high fat diet to mimic some of aspects leading to cardiovascular disease and then imaging to dissect the cellular events leading to myocardial infract. Where appropriate we will also administer analgesics and anaesthetics to minimize pain and distress to the animals and replicate aspects of the human condition. For example, when studying mechanisms in infection, animals will be given anaesthetics during the surgical procedure and fluid resuscitation together with analgesics for pain relief during the recovery phase to mimic the human scenario. In models that are anticipated to lead to joint pain and therefore may impact the ability of mice to move comfortably we will provide an appropriate environment, including nesting material and soft bedding to reduce stress and adopt measures such as providing gel feeds for animals that cannot feed well. For all experiments animals will be monitored according to a rigorous monitoring schedule using pre-established criteria that identify early endpoints where treatment/advice from the NVS will be sought.

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	CNS white matter development, plasticity and disease
Key Words	Myelin, Regeneration, Stem cell, Glia, Neuronal circuit
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Half of the human brain is white matter, which rapidly transmits information between the ~100 billion neurons (the nerves or 'wires' of the brain) situated in the grey matter (the other half). Unlike the grey matter, the white matter continues to develop long into adulthood. The function of white matter depends on oligodendrocytes, a brain cell type called a glial cell, that wrap myelin (a fatty insulating substance) around neurons to provide fast transmission of impulses or 'signals' to coordinate neuronal activity and maintain neuron function - similar to the insulation around an electrical wire. Alterations in myelination are increasingly being implicated as a mechanism for learning, and when myelin is damaged in disease it leads to both cognitive (conscious intellectual activities like memory recall, learning or thinking) and physical disability.

A large proportion of the human brain is made up of glial cells (astrocytes, oligodendrocytes and microglia cells), which are the ‘supporting cells’ of the brain, supporting neuronal function. The understanding of their role in the function of neuronal networks within the central nervous system is limited. Conventionally, research into neurological diseases has been extensively grey matter-focused, due to the clear loss of neurons and loss of their function. However, current knowledge on glial cells, and myelin within the brain implies a more systemic failure in the brain as a source of neurological disease than solely a neuronal problem. Thus, to untangle neurological diseases, focused research into neuron-glia interactions is needed.

The overall aim of this project is to gain insight into how myelination is regulated throughout life and in disease. We will focus on how glia cells affect formation of neuronal networks in the brain, how they are involved in memory and learning, the effect of ageing and how myelin regeneration can be enhanced in neurological diseases.

The work set out in this license ranges from fundamental questions about the biology of glia-neuron interactions, neuronal circuit development, and myelin damage, to studies more focused on finding new potential drug targets for medicines. For example, testing whether new approaches emerging from these fundamental discoveries have pro-regenerative efficacy (i.e. can new myelin be formed? or can loss of brain function be saved?) in suitable 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)?

Multiple sclerosis (MS), a disease where the primary cause is damaged myelin, affects around 2.5 million worldwide, with 130,000 people affected in the UK, costing the UK society nearly £4 billion per year. Currently there are no fully effective treatments available. In recent years progress has been made in modifying the immunological aspects of the disease, but as of yet no myelin regenerative treatments exist. In addition to MS there are a number of other white matter diseases, such as cerebral palsy or post-infectious encephalomyelitis (inflammation of the brain and spinal cord), where myelin regeneration therapy could be suitable. Moreover, white matter damage can occur either as an unwanted side effect of other treatments, such as cancer (i.e. 'chemo brain'), post-vaccination encephalomyelitis or secondary to neurological damage (spinal cord injury and stroke) or HIV-1 infection (which if untreated can lead to AIDS). Therefore, the direct benefit of understanding the mechanisms regulating myelin regeneration is enormous and could lead to the identification of novel ways to augment myelin regeneration therapeutically for a number of white matter diseases. Recent data indicated that myelin, and glia cells, are involved in multiple diseases that were previously considered 'neuronal' such as dementia, schizophrenia, attention deficit disorder, autism and bipolar disorder. Thus, understanding how glia affect development and changes occurring throughout life will have a significant effect on understanding the development, and mechanisms underlying, a number of brain diseases. This work will advance our knowledge of how the brain develops and functions throughout life and the mechanisms underlying the spontaneous regeneration process that can occur in the central nervous system white matter. New insights may be gained that could lead to new strategies for treating neurological disorders: (i) developmental brain disorders; such as cerebral palsy (~17 million affected in the world), and leukodystrophies (genetic diseases which affect formation or maintenance of the white matter, often appearing in early childhood); (ii) acquired disorders such as schizophrenia and multiple sclerosis (~2.5 million affected in the world); and (iii) age-related disorders such as dementia (~50 million affected worldwide; including Alzheimer's Disease, frontal-temporal dementia and vascular dementia).

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

This project licence covers use of rats and mice. Over a 5 year period we expect to use approximately: (1) 12,640 mice (these include genetically altered mice bred

under this licence). (2) 5,450 rats (these include genetically altered rats bred under 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?

A variety of different procedures will be performed as part of this license, in most cases animals will recover quickly and without incident (mild severity) or occasionally will be non-recovery, where animals are anaesthetised for the procedure and are killed under anaesthesia. Procedures that fall under the mild category include; injections, drug administration in food or water, blood sampling, breeding and maintenance of genetically altered animals where the alteration is not harmful, behavioural tests, or labelling (with dyes or fluorescent markers) the central nervous system under non recovery anaesthesia. We use drugs or dyes, either injected or fed to the animals, for several different reasons; to activate genetic modifications, to provide pain relief before or after procedures, to label certain cells so that we can identify them, or to change the responses of certain cells to different stimuli e.g. so that we can boost or inhibit myelin formation or maintenance. Once procedures are completed animals will be humanely killed, the methods used to do this will vary but will be carefully considered to balance the experience of the animal with the usefulness of the tissues that we can obtain after death. Some of the work under this license will lead to moderate adverse effects, these include genetic alterations which can lead to brief seizures or tremors, maintaining animals as they age, eye injections (under anaesthesia) which could cause irritation, brain injections (under anaesthesia) and neural implants (under anaesthesia) which can lead to temporary functional difficulties such as low level problems with walking or head tilts. Injections into the eye are used because the optic nerve is normally fully myelinated with a well known timeline of myelination and this makes it an ideal model system that allows us to investigate how myelin is normally made and ways in which it might go wrong. Eye injections are safe and are routinely used in human patients. The eye injections take place under general anaesthesia (human patients receiving eye injections generally just have local anaesthesia) to ensure that the distress of the animals is kept to a minimum. We use neural implants to either shine light into the brain to activate certain cells, to take pictures of the brain, or to have a slow release of drugs into the brain over a long period of time so that we don't have to do lots of repeated injections into the brain. We use injections into the brain for 3 main purposes; to change the activity of the brain cells, to label specific cells, or to generate a small demyelinated injury (or lesion, like in multiple sclerosis). The most severe work involves causing damage to the brain so that we can see how it heals, damage is initiated by injecting a chemical into the area of the brain that we want to have the injury; in most cases the worst clinical sign is a head tilt and some difficulties in balance when moving around that resolves in 72 hours or less. In less than 10% of cases this damage to the brain can lead to spontaneous rolling of the animal or rolling when stressed, or the animal being temporarily unable to right itself. Animals that continuously roll will

be humanely killed immediately, and those that cannot right themselves and show consistent reactive rolling will be killed if they do not improve within 8 hours. Painkillers will be provided to animals so that they are not in pain from any of these procedures, and during the recovery period they will be kept warm and in a low stress environment, we will also help them to eat and drink if they need it by giving them easily accessible soft food and long nozzled water bottles. Animals will be closely monitored at all times and will be checked on with increased frequency during the recovery period. Surgical procedures will be performed under general anaesthesia and sometimes multiple separate general anaesthesias will need to be used, typically after anaesthesia animals are somewhat disorientated but will be receiving painkillers to minimise any post-operative pain. Animals will only undergo multiple general anaesthesias if there is a scientific need for a second surgery, for example, in one surgery we may need to inject a substance that makes certain types of cells in the area respond to light, allow the animals to recover and the substance time to work, then perform a second surgery in which we make an injury then shine light onto the cells to see if that leads to faster healing. Animals will only be used for multiple surgical procedures if they recovered quickly and completely from the first procedure. In general recovery from anaesthesia should be quick and animals can return to normal behaviour without much intervention. The cumulative effect of any procedures will be considered and kept to the minimum required to yield statistically meaningful data. All animals used in this license are cared for by dedicated staff who follow detailed instructions regarding their care. Humane endpoints have been set throughout this license to minimise suffering, ultimately all animals will be humanely killed and their tissues harvested for further analysis. Procedures are continually reviewed and refined whenever possible to improve the overall welfare of the animals.

Application of the 3Rs

Replacement

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

Replacement

The experiments proposed are to advance our understanding of how the brain develops during normal life and in disease and how glia affect this process, including in repair. Whenever possible culture experiments, using human central nervous system cells derived from skin cells, will be conducted in specific cell culture petri dishes in the laboratory to reduce animal use. We will also use tissue from dead animals for culture assays to reduce the invasive procedures as much as possible. However, cell culture based assays do not always represent an appropriate alternative to animal use, thus animal models are needed, especially to study complex environments with different cell types interacting, or to study behaviour, and

are essential to understand complex disease mechanisms such as myelin regeneration and changes in the central nervous system with ageing. Existing data show that for most purposes the rodent nervous system is a good model of the human one, both for normal function and for the disease processes that we are investigating in this project. Thus, making it our best choice to investigate these complex interactions

Reduction

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

Reduction

In all experiments, we will ensure that the minimum number of animals will be used by optimising the experimental design and by using appropriate statistical methods compatible with obtaining scientifically and statistically significant results. We will ensure that researchers receive extensive training before they start experiments on animals and will share tissues between researchers wherever possible thus minimising animal numbers used overall. Sample sizes are based on statistical calculations using pilot experiments (where a small version of an experiment is run to test whether a full experiment is likely to work) and data from the literature. All experimental analysis will be carried out 'blinded ' i.e. the person analysing the data does not know what animal a specific data set belongs to, so there is no 'bias' or influencing of results based on the researcher anticipating what they expect the data to show; we will randomly allocate animals to different experimental conditions and for analysis, this reduces bias in our analysis and ensures that our conclusions will be 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

Myelination only occurs in vertebrates and so non-vertebrate species are inappropriate for understanding myelination and myelin regeneration. There are no myelin regenerative models that have been developed in birds, reptiles, amphibians, or fish. Thus, we use rats and mice because they are the least sentient species that can model glia-neuron interaction, myelination, cognition and behaviour in neurological disorders. The brain circuitry implicated in many neurological disorders is highly conserved between rodents and humans.

The brain lesion models proposed for inducing myelin damage have been chosen in part because of the minimal behavioural and locomotor (movement deficits that they

induce. For example, our primary model does not result in functional deficits, i.e. the animals are able to move freely and perform normal physiological functions, like feeding, drinking, grooming and movement after the initial surgical recovery period. Our brain lesion model is carried out by performing an injection under anaesthesia into a specific area of the brain where a lesion does not cause any lasting symptoms, animals get better over time and recover back to their pre-lesion state of fitness within a couple of days, though the symptoms immediately post lesion surgery can be severe and distressing to the animal. Causing lesions in other parts of the brain could cause lasting symptoms either to behaviour such as social interaction, or to normal physiological functions such as movement - e.g. causing paralysis.

In undertaking these procedures, we are continually assessing how the procedures can be refined in order to minimise the discomfort that the animals may experience and seeking to replace *in vivo* procedures with *ex vivo* procedures where possible. Some of our previous refinements have included: the administration of post-surgical pain relief in edible jelly rather than by injection, thus reducing handling stress in recovering animals; numbing cream on the ear bars of stereotactic frames (used to keep the head still during surgery) to further minimise discomfort for animals during their recovery; and improved monitoring/grading systems so that any signs of distress can be readily and consistently recognised and recorded by all researchers and animal unit staff and appropriate action carried out to reduce that distress at the earliest available opportunity. We take the welfare of the animals very seriously and routinely use pain relief and provide appropriate supportive care during surgical procedures, we familiarise animals to handling for procedures where handling is necessary, and increase monitoring of animals that have undergone invasive procedures so that we can quickly identify and treat or minimise any adverse effects arising.

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	Mechanistic Analysis of Memory in Mice
Key Words	Learning, Memory, Mechanisms, 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):

A fundamental biological principle is that animals and humans can make, store, and retrieve memory. Memory processes occur in the brain and several brain disorders impair memory. Among the known disorders are Alzheimer's disease and other dementias, intellectual disability and post-traumatic stress disorder where a traumatic memory is frequently retrieved. Current knowledge is not sufficient to understand the precise nature of memory processes, a lack of information that precludes the development of treatments of memory impairment. The main objective of this project is to advance the basic understanding of memory processes. To this end animal experiments are essential, allowing for sophisticated manipulations, such as altering the function of particular proteins in discrete brain regions that impact on brain plasticity, using surgery and genetically altered viruses, , which cannot be done in humans for ethical reasons, as such treatment can lead to lasting memory impairment . Memory, which is a property of the intact brain, will need to be studied in animals undergoing behavioural testing. For this project the mouse is chosen as experimental animal, because for this species a large number of techniques have been developed to study the function of particular genes and to model dementia and intellectual disability. Thus, mice are the ideal species to investigate the nature of memory processes. The specific objectives of this project are: 1) To identify new proteins or protein modulations that contribute to memory and to test whether these proteins/modulations could contribute to memory deficits in mouse models of dementia, intellectual disability, or other memory impairment. 2) To find proteins or drugs to enhance memory formation and/or to prevent memory deficits in mouse models of dementia, intellectual disability or other memory impairment. 3) To identify new proteins or protein modulations that are induced by retrieval of memory and may therefore be important for updating of memory.

What are 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 insights into the molecular and cellular basis of learning and memory. These insights will advance the understanding how the brain acquires, stores, retrieves and maintains information. The project will also address how learning and memory abilities changes with normal ageing and in mouse models of dementia, such as Alzheimer's disease, and intellectual disability, such as autsim. Our project will also investigate mechanisms of memory erasure. Memory erasure is an important way of adaptation and if impaired it can lead to disorders such as post-traumatic stress disorder, a disease where repeated retrieval of a traumatic memory incapacitates the patient. In mice a fear memory can be pharmacologically erased at the time of retrieval. However, current phamarcological memory erasure cannot be applied to humans due to bad side effects of the drugs. Our project will provide more mechanistic understanding of inducing specific memory erasure. The pharmacological part of our Project will contribute to the future development of treatments of memory disorders, including post-traumatic stress disorder, that are currently not available. Findings will be made available to other scientists through publications in peer-reviewed journals and presentations at scientific conferences and meetings. Under the previous project we published 6 papers as lead authors (and 2 more are currently written) and we presented findings at 7 international scientific meetings. Likelihood of achieving these benefits: Breeding of genetically modified mice, administration of substances including injection of substances into discrete brain areas i and behavioural testing of mice are well established methods and I have used these methods successfully under my current and previous project licences.

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

Over a 5-year period I expect to use about 11,000 mice, some of which are genetically altered.

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

The majority of the studies will behavioural investigations with genetically altered and non-genetically altered mice. The genetically altered mice will not have a visible impairment (such as impaired movement), as this would confound the specificity of our analysis of memory mechanisms. For some manipulations the mice will have to undergo surgery under general anaesthesia. In these cases, substances will be injected into discrete brain regions to specifically interfere with protein signalling that is involved in memory processes. During an approximately 1 hour-surgery we will typically make very small windows into the skull to get access to the brain. These small windows will be made only once, and they are made to insert a fine capillary/needle into the brain to inject substances, causing only minimal damage to the brain. In some cases a capillary will be cemented onto the skull to allow for flexible administration of substances. After recovery from surgery the mice will

undergo behavioural testing. After behavioural testing the mice will be killed by a humane method and brain tissue will be taken after death for analysis.

Application of the 3Rs

Replacement

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

Replacement

Animal experiments are essential to study memory processes, which are properties of an intact brain in a behaving animal. The animal work allows for sophisticated manipulations, which cannot be done in humans for ethical reasons. Memory, which is a property of the intact brain, will need to be studied in animals undergoing behavioural testing. Consequently, experiments in a laboratory using for example cells in a dish or computer simulations are not suitable replacements for the studies with animals undergoing behavioural testing, as the latter provide an indication of how the brain/memory is working in response to different manipulations.

Reduction

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

Reduction

Prior experiments in a laboratory using, for example, cells in a dish will be used to optimise the treatment conditions to affect the function of a protein of interest. For example, laboratory experiments will identify the most suited virus among a group of designed viruses to affect the function of a protein of interest that impacts on plasticity in the brain.

Advice on the proposed experimental design and methods of analysis of the results will be taken from the Statistical Services Unit. Where relevant, factorial experimental designs will be used, rather than the one-thing-at-a-time approach, to maximise the information obtained from the minimum resource.

When multiple treatments will be studied only one control group 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

Work with mice is proposed because there is a large repertoire of techniques to manipulate genes and their products meaning that study of the impact of these manipulation on behavioural memory can provide detailed understanding of the underlying brain processes . Further, a large number of mutant mouse lines including models of dementia and intellectual disability are currently available. This means that, in combination with the above, we can use mice that have been specifically bred to model specific dysfunctions of the brain makeup such that we readily manipulate and study specific genes of interest. Such a large repertoire of manipulations cannot readily be applied to rats or more evolved organisms both on a practical and ethical basis so the mouse remains the closest species to humans that is fully modifiable and thus relevant to delivering the scientific aims of the project.

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	The coordination of translational control in nuclear receptor signalling in Xenopus development
Key Words	Xenopus, translation, retinoid, nuclear receptor
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Genes, made of DNA, contain all the information for making the functional proteins of a cell. This information is decoded in two steps. In the first, an intermediate is made, called mRNA, which then directs the formation of a protein in a process known as translation. Whilst protein formation is often controlled at the first step through production of the mRNA, it can also be controlled at the second step – making the protein. Retinoid receptors are proteins responsible for many events in the embryo and their production seems to be controlled at the second step. We want to identify the signals that regulate this control and ask whether they act on other genes also involved in retinoid signaling.

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

Little is known about the coordinated control of protein production by regulated translation. The first benefit will be to advance our basic knowledge of this process. The second potential benefit relates to clinical applications for the retinoid receptors. These include the formation of differentiated cells from basic stem cells, to the activation of proteins to clear the amyloid plaque that are a causative agent of Alzheimer's Disease. These applications rely on the retinoid receptor proteins being present in the cell at a certain level and will be less effective if the protein is in short supply. Understanding the regulated translation of retinoid receptors will optimise protein production and be very likely to make the use of retinoids more effective.

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

The amphibians, Xenopus laevis and X. tropicalis have the same set of retinoid receptors as mammals that perform many of the same functions as they do in mammals. The advantage of Xenopus is that they produce lots of tadpoles in a short space of time from a single female using a very mild procedure that induces egg

laying. We expect to do around 350 procedures over 5 years but this will use fewer animals as often the female frogs will be re-used several times for egg-laying.

In the context of what you propose to do to 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 protocol is to produce embryos (frog-spawn) from female frogs by injecting a hormone that induces egg-laying. This is a mild procedure, an injection with a hypodermic needle causing a momentary discomfort that many of us will have experienced with a 'flu jab. The frogs rarely have any adverse side effects (< 1% of frogs). The eggs are fertilised by testes removed from a humanely killed frog. Because the procedure is mild, the female frogs are often re-used after a rest period of at least 3 months. In a second protocol, we will generate mutations in specific genes using an efficient, widely-used method called gene editing. This is an accurate way of introducing specific changes into the DNA of a cell. The mutated embryos will be analysed at the early stages of development before they are independent feeders. If both copies of the gene are mutated it is likely that the mutation would be lethal. If this is the case, the remaining embryos will be humanely culled. We will maintain some heterozygotes and grow these to adults if, as expected, the individuals are unaffected by carrying just one mutated copy. When these lines of genetically altered frogs are established and characterised they will be preserved by cryopreservation of sperm 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

The retinoid receptors and related proteins underpin many aspects of animal development. It would be difficult to identify the components within the embryo that contribute to retinoid signalling if the process was analysed in single cell lines that do not reflect the complexity of interactions seen in an embryo. The processes examined are essentially the same across all the vertebrates, so by using frogs we can examine many more embryos from fewer animals (one female frog can produce several hundred eggs at a time) than is possible with mammalian models, such as the mouse.

Reduction

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

Reduction

The experiments will be carefully designed to extract the maximum amount of information from the minimum number of procedures. In addition, we will re-use female frogs, since the procedure to stimulate egg-laying is mild and in the vast majority of cases (>99%) the frog shows no adverse effects as determined by a systematic and careful assessment of the health of each individual female frog. Xenopus embryos produce many embryos at a time, so we follow good practice and share the embryos between projects to limit the number adult females required and maximise the output.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 a fundamental vertebrate process such as retinoid signalling, the mechanism of action is the same in all species from fish to mammals. We can therefore use a more amenable model system such as the frog, Xenopus, that produces many hundreds of accessible embryos at a time. However, the Xenopus model also has extensive and sophisticated support. Like humans, the complete genome sequence of two species of Xenopus is known and publicly available, which facilitates the design of genetic experiments. In addition, research in Xenopus is supported by a worldwide network of research labs that share their knowledge, resources and expertise.

Both protocols in the project are mild and include a number of checks to minimise the chance of harmful effects. The injection of hormone to induce egg-laying in female frogs rarely has an adverse effect, allowing their re-use following a series of health checks. There is also an upper limit to the number of times a frog is used. In between use the animals are housed in a modern tank system with carefully monitored environmental controls (temperature, water quality and light-dark cycles) and a regular feeding routine that has been optimised over many years to produce healthy frogs that generate healthy eggs and embryos.

When genetically altered animals are produced they are carefully monitored across the embryonic stages. Those showing a phenotype are analysed at these stages and culled before the free-feeding stage. If lines are produced from embryos in which just one of the two copies of a gene is mutated, the tadpoles and froglets are monitored at regular intervals and those showing abnormal traits and behaviours are humanely killed.

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	Development and plasticity of synapses and networks
Key Words	Genetically modified, nervous system, model
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:
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 successfully breed and maintain animal model strains specifically for the study of the development, plasticity and pathology of the nervous system.

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

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

The benefits are that the future use of these animal model strains will contribute to the understanding of the basic mechanisms of brain function and to nervous system disease processes which could ultimately lead to the development of new therapeutic methods. Previous work with these and similar lines has directly lead to important new knowledge of the function and dysfunction of the nervous system.

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

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

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

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

Application of the 3Rs

Replacement

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

Replacement

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

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

Reduction

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

Reduction

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

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

Refinement

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

Refinement

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

PROJECT 68.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	Sea lice and amoebic gill disease in Atlantic salmon
Key Words	Salmon, Aquaculture, Veterinary medicines
Expected duration of the project	5 year(s) 0 months

Purpose of 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.
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 licence will allow us to develop treatments for two of the most significant health problems for salmon farming. Sea lice are a damaging parasite that are showing increasing resistance to current drugs, meaning costs are increasing and lice on farmed fish can threaten wild salmon. Amoebic gill disease (AGD) is an increasing problem as sea temperatures rise, and current treatments are either difficult or can be harmful to the salmon themselves.

To develop more effective treatments, such as new drugs or physical methods, we need to be able to test them on infected fish in the laboratory, as we cannot grow sea lice without using fish we maintain colonies using infected 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)?

Improving the health of farmed salmon reduces suffering and increase the supply of a healthy food. Salmon is a good source of marine omega-3 oils that help maintain human cardiac and brain health.

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

We will use Atlantic salmon because data from salmon will be required to gain approval of new drugs for use in salmon farms. We will only use young farmed salmon that have adapted to their seawater life stage. We expect to use 60,000 fish over the 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?

Most fish used for sea lice research will be infected with such low numbers that there is only mild or no damage to the skin. As infection is due to many factors including the frequency of encounters with sea lice, and the susceptibility of the fish to

infection, some fish will be more heavily infected and suffer minor skin damage. We observe all fish carefully, and any fish showing deeper skin damage will be killed immediately by a humane method. Fish affected by AGD show thickening of the gills causing them to breathe more quickly. We observe all fish carefully, and any fish showing moderate respiratory distress will be killed immediately by a humane method. For any new drug we may not know the safe dose in salmon and will have to expose fish to a range of doses. The potential toxic effects vary with the kind of drug tested but we will try to anticipate them and include any potential side effects in observations. Alternative treatments may have varied side effects during their development. For example, fast jets of water or a laser system for example that may dislodge scales causing minor irritation. All fish will be humanely killed, for example with an overdose of anaesthetic and sent for incineration.

Application of the 3Rs

Replacement

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

Replacement

The sea lice we will use can only be grown on fish. Some of the pathogens involved in gill disease can grow but not cause disease on invertebrates such as crabs, but we need to use fish because as with sea lice the complex interactions between parasite and host determine how well any intervention will work.

We are sometimes able to identify candidate medicines on sea lice without using fish, but all veterinary medicines must be proven effective and safe in the intended species before approval.

Reduction

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

Reduction

Sea lice produce large numbers of larvae that can be tested used without using fish to screen out molecules which are unlikely to be useful as medicines.

It takes fewer fish to grow enough lice for a test on the lice themselves than it does by counting the lice on groups of treated and untreated fish. Checking sea lice from farms for drug resistance will be done as far as possible without exposing the fish to drugs.

We follow standard protocols for testing on fish. The number of fish used to grow the required sea lice is adjusted based on the size of fish available, and we aim to use as few fish as possible without causing excessive skin damage.

We will only grow the minimum lice needed for each strain to maintain it and supply planned 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 only keep sea lice species known to infect salmon, and only strains that will be useful for developing new medicines or investigating resistance to current treatments.

We have control over the numbers of lice infecting fish because we add them to a tank directly. This means we can take into account the size of the fish, keeping infections lighter on smaller fish.

We check the level of lice on fish after infection and will observe the fish at least twice daily throughout. Any fish found to have an unexpectedly high level of infection or deeper skin damage will be killed by a humane method such as an overdose of anaesthetic and sent for incineration.

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	Clonal architecture in normal and neoplastic intestine
Key Words	stem cell, clone dynamics, colon 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):

In a tissue like the colon most cells will only live a short time, a few days, and a very few will continue to live and grow to replace the cells that are lost. These surviving cells, called stem cells, must continue to do this throughout life. However the mutations that can cause cancer can change the way stem cells behave. For example a higher percentage of the mutated cells may be stem cells and they may start replacing normal stem cells. We want to measure this effect for different kinds of mutation and understand how it happens and how it affects the chances of cancer developing

There are many different kinds of cells in a tissue like the colon but by focussing on the stem cells and measuring how they change after mutation we can work out when a medicine is most likely to stop cancer developing in the first place by preventing the normal stem cells being replaced.

What 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 being able to measure how normal stem cells get replaced by stem cells with cancer causing mutations we can describe the effects of different mutations, and also of different risk factors such as diet in a simple way. The extent of "overgrowing" can be measured for different kinds of mutation and related to the risk of developing cancer. This means that the different mutations that drive the development of colon cancers can be compared to each other. Importantly it also allows the effect of potential medicines to be tested as a good therapy might directly reduce advantage of some mutated cells that will go on to develop cancer or that are causing it to grow.

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

We will use up to 30, 000 mice over five years. Many of these will be used in breeding programmes to generate genetically altered mice with several different genetic alterations. It takes several generations to obtain the mice needed because

we need to introduce the genetic events required to drive cancer, or increase the probability of it happening as well as introducing the molecular tools that allow us to monitor clonal growth of individual mutant 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?

Many of the animals bred will receive no interventions and will develop no symptoms but are required to act as breeders to generate the appropriate genetically altered animals for investigation. Procedures are designed to be minimal and cause only transient discomfort. In those animals developing symptoms associated with inflammation (that predisposes to cancer) or cancer itself a number of humane end points will be employed to ensure that animals do not suffer and are maintained for the shortest possible time. Some animals will receive longer periods of intervention (e.g. daily injections over weeks) as treatments applied to patients are mimicked to alter or restrict the growth of advantaged clones (e.g. using radiation). Again this will be kept to the minimum required to observe if the intervention is effective and animals will not be maintained (or expected to develop) ill health associated with the radiation. Some animals will develop intestinal tumours and some inflammation that may also lead to intestinal tumours. These animals might develop symptoms such as diarrhoea but will be carefully managed to ensure they are killed before they suffer from the consequences of these conditions. Some animals will have human cancer cells or tissues implanted under the skin which will be allowed to grow to form tumours. This is not expected to cause any change in the animals overall health or behaviour and will be managed to ensure that this is the case with any affected animal being killed.

Application of the 3Rs

Replacement

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

Replacement

Both normal and cancer stem cells have to live and grow in complicated tissues within the body. To understand what allows them to survive and to grow, depends on lot of different cells "talking" to each other in ways that only happen in a whole body. Tissue culture systems cannot mimic the interactions of cancer cells with all these other cells including immune cells and nerve cells and blood cells. We do use cells grown outside the animal to determine what allows them to grow (or prevents them growing) and this helps to decide on the most appropriate treatments that are applied to the mice.

There is also the issue of time for clones and cancers to form and for their fate to be established. This can take many months - a timescale not compatible with tissue culture based approaches.

Reduction

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

Reduction

Many of the models we plan to use are composed of complicated genetic modifications. To reduce animal numbers breeding strategies are designed to be as efficient as possible. In many cases animals not inheriting the combination of modified genes required for experiments are appropriate to serve as controls. We have an ongoing programme of cryopreservation of frozen sperm/embryos to allow lines that are not required for many months to be retained without unnecessary breeding.

Many experiments are planned ahead of time with statistical advice available locally. Additionally with those models which are already well characterised we have a baseline in which prior experience has shown that 6-10 mice per group (control and test) give sufficient power to detect changes in tumour numbers and changes in the time taken for them to appear.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 form of mammal that can be used to study normal and diseased tissues. The structure and function of the gut in, for example, fish is very different to that of mice and people. That is separate structures identifiable as stomach, small intestine and colon cannot be discriminated. Of the different cell types (6 in mouse and human) fish have only two. Importantly they also lack the glands that contain the stem cells.

An enhanced regime of heath checks is applied to those animals showing any sign of disease to ensure that they are properly monitored and humanely culled if they start to suffer pain or distress.

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	Analysing Gene Function in Xenopus Development
Key Words	Xenopus leaves, Nervous System, Embryology, 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:
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 this project is to understand how the vertebrate nervous system is formed, using frog embryos as our model system. Development of the nervous system results from tissue interactions that begin during the first 24 hours of frog development. These tissue interactions continue throughout development, to generate the many different regions and cell types of the adult nervous system. One of our aims is to study these interactions and to identify the genes that collaborate to generate the nervous system. Defects in the development of the nervous system are amongst the most common causes of congenital abnormalities in the neural tube. A second aim, is to study the genes that regulate migration of the cells that form the peripheral nervous system (such as that lining the gut). Migration of these cells shares many similarities with migrating cancer cells and may provide insights into the latter 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 project will increase our understanding of fundamental questions of how anatomy is built during embryonic development. A good understanding of normal development can help us understand how defects arise that lead to congenital abnormalities. Since embryonic development is similar in all vertebrates, the knowledge gained in this project will be applicable to humans. The project will also help our understanding of diseases such as cancer and may therefore provide future health benefits.

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

We will use the frog Xenopus laevis as our model organism. Approximately 400 frogs will be required over the 5 year duration of this project, with females being re-used no more than 12 times (with at least a 3 month gap between re-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?

Female frogs will be injected with hormones that will cause them to lay eggs 12-24 hours later. Eggs will be collected and fertilized with sperm, generating embryos that will be the subject of our experiments on the development of the nervous system. The injection procedure is very mild and usually results in no adverse effects. Occasionally, the injection leads to redness of the skin and small sores on the nose, but these frogs usually recover with a few days.

Application of the 3Rs

Replacement

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

Replacement

Embryonic development is a complex process that requires multiple tissue interactions. These cannot yet be reproduced using cultured cells, we therefore require male and female frogs to provide the embryos needed for this project.

Reduction

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

Reduction

Female frogs can lay thousands of eggs, which are sufficient for many experiments. We therefore organise our work schedules so that as many as 6-7 scientists may use these eggs. Sharing resources minimises the number of procedures that we need to perform.

Refinement

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

Refinement

Xenopus is our animal model of because it has numerous advantages over other model vertebrates (such as the mouse and chick). They produce large numbers of eggs that can be observed at all stages of development. The large embryos are ideal for cell and tissue transplantation studies and have been instrumental in demonstrating the key roles of cell-cell signalling in vertebrate development and in identifying the relevant signalling molecules. Adult *Xenopus laevis* can be maintained

in the laboratory and induced to ovulate at all times of the year. Genome and RNA sequencing data means it is easy to design reagents for disrupting gene function and these reagents are easily injected into the large egg. Since large aspects of development is conserved in all vertebrate embryos, data obtained in this project will be applicable to other vertebrates, including humans.

The injection procedure causes no more than mild stress in the majority of cases and animals show no long term adverse effects. Handling is kept to a minimum and animals are returned to the holding tanks as soon as possible. Animals displaying adverse effects receive veterinary care and if necessary killed by terminal anaesthesia.

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	Neuromuscular and neurodegenerative disorders: pathogenesis and therapy
Key Words	Neuromuscular Diseases, Neurodegenerative Diseases, Pathogenesis, 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;
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 project is focused on improving our understanding of the mechanisms that play a role in neurodegenerative diseases that affect the nervous system in order to help develop new therapeutic strategies for these debilitating and often fatal disorders. There is currently no cure or disease modifying therapy for these disorders.

The project is particularly focused on disorders that affect the neuromuscular system including Motor Neuron Diseases, peripheral neuropathies and muscle disorders. However, recent clinical and genetic findings have revealed that some of these diseases are part of a disease spectrum that surprisingly, also includes forms of dementia, suggesting that the results of this project may have relevance for our understanding and possibly treatment of a wide range of neurological disorders. Although significant advances have been made in the past 10 years into our understanding of the genetic causes of many of these diseases, we still have a poor understanding of the underlying mechanisms that cause disease, and as a result, these diseases remain untreatable.

Therefore, this project has 2 key objectives: i) to advance our understanding of the mechanisms that play a role in these disorders, in order to ii) identify targets for therapeutic intervention, and to develop and test novel therapeutic strategies that will be effective in modifying disease progression in patients suffering from these diseases.

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

The results of this project will improve our understanding of the mechanisms that underlie several neuromuscular and neurodegenerative diseases, thereby advancing the current state of knowledge in these fields. A better understanding of the underlying pathological mechanisms of these diseases will help researchers to identify targets that can be treated with drugs or alternative therapeutic strategies. This project will also test the most promising of these novel therapeutic strategies in

models of neuromuscular and neurodegenerative diseases to establish their potential for therapeutic benefit in patients suffering from these diseases. This will include advancing the finding from this project through to human clinical trials.

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

This project will use mice and rats. Many of the mice that will be examined will be genetically modified to model aspects of the human diseases under study. We will use a maximum of 40,000 mice and 11,500 rats over the 5 year duration of this project.

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

The majority of procedures in the project will be mild or moderate and we expect there to be few adverse effects. this will be mainly limited to mild discomfort following surgery. In such cases, the animals will be given pain relief. As the animals will model neuromuscular and neurodegenerative diseases, deficits such as muscle weakness which are associated with neuromuscular diseases, usually restricted to hindlimbs, may be experienced. These deficits may include dragging of one paw, gait abnormalities of limb muscle weakness. In such cases, the animals will be provided with easy access to food and water, for example by providing a soggy diet within the home cage. The overall expected level of severity for the procedures described in the Project is likely to be Moderate. At the end of these experiments, all animals will be humanely culled.

Application of the 3Rs

Replacement

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

Replacement

Neuromuscular and neurodegenerative diseases involve changes in complex interactions between different types of neurons and several other cell types located within the central nervous system, in the brain and spinal cord. In addition, cells outside the central nervous system, in the periphery, such as nerves and muscles are also involved. It is impossible to fully model these hugely complex interactions in culture.

Furthermore, these diseases tend to manifest on a background of aging, appearing during middle to late stages of life, which is also difficult to model in culture.

Several models of neuromuscular and neurodegenerative diseases have been developed using non-protected species, including fruit flies and nematodes.

However, although these models may be of use for investigating some aspects of human disease, such as how do different genes interact, they have significant limitations for the objectives of this Project. For example, in fish models of ALS, motor neurons do not degenerate, making this a difficult model to use in the development of drugs that will prevent motor neuron death.

However, where possible, we will use cell culture models to model aspects of the complex interactions that are involved in neuromuscullar and neurodegenerative diseases, for example the interactions that occur between specific cell types (eg muscles and nerves). Indeed, as part of this project, a novel cell culture model of the neuromuscular junction will be developed, in a study funded by the Motor Neuron Disease Association. This will involve the use of stem cells -derived muscles, motor neurons and glial cells, which will model the key aspects of the neuromuscular junction.

Moreover, we are increasingly making use of human derived stem cell models of human disease, thereby avoiding the need for animal experiments. This area of our research is likely to become a major focus of our work over the coming years. Finally, as our overarching goal is to develop disease modifying therapies for neuromuscular and neurodegenerative diseases, we routinely compare our findings to available human data as well as our own studies on post-mortem tissue from affected patients.

Reduction

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

Reduction

We will design our experiments according to the NC3Rs ARRIVE Guidelines.

The experiments described in this Project have been designed in consultation with statistical advisors. In all aspects of this project measures will be undertaken to minimize the number of animals use wherever possible. For example, wherever possible, tissues from individual animals will be shared between group members as well as with other collaborative groups. In this way, different tissues from an individual animal can be used to support experiments undertaken by several researchers. This approach ensures that we keep our animal use to a minimum.

Depending on the experiment, control groups will include wildtype and/ or untreated littermates; for some experiments groups must be gender specific as disease progression will differ depending on gender

For most studies, in the first instance we will undertake small pilot studies which typically consist of experimental groups of smaller numbers than the definitive study, as it may be possible to obtain an indication of efficacy. However, due to the known

variability and gender effects of many transgenic models, the larger numbers indicated above are required to undertake statistically robust analysis to demonstrate efficacy.

Wherever possible, we will make use of stored tissue available in existing biobanks.

Refinement

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

Refinement

Most of the work in this project will be undertaken in mice. The mouse is unique in that it is relatively easy to create mutations in any gene of interest and therefore dissect the mechanisms involved in specific human diseases, and to generate models of disease in which to test new therapeutic approaches. In addition, since the ultimate aim of this research program is to relate the findings obtained in animals back to human disease; it is essential to work with a mammalian system.

Furthermore, we also have a comprehensive understanding of the mouse nervous system and most of our previous work has been gathered from mice. There is also a large body of background data on the normal functions and behaviour of the neuromuscular system in mice and rats, which therefore significantly reduces the total numbers of animals that need to be used in this project

Wherever possible we use in vitro models, eg-cultures of primary motor neurons and glia from genetically modified mice modelling disease to examine disease mechanisms and test potential therapeutics, prior to validation in animals. We also use models with as mild a disease as possible to test specific questions

We will minimise harms during surgery by undertaking the mildest injury required to meet the scientific objectives. In all cases, the harm will be the minimum and supportive therapy will be employed to minimise the impact of the intervention, eg provision of a soggy diet in the base of the home cage following surgery or when an animal's mobility becomes reduced as a result of the disease.

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	Mechanism of stem cell homeostasis, cancer and tissue regeneration
Key Words	Stem cells, cancer, 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):

The human body is made up of trillions of cells that together form various organs to carry out their unique functions. The growth and maintenance of each organ is supported by the stem cells in the resident tissues, which will respond to damage for tissue regeneration. This is achieved by delicate control of signals given to the stem cells for their fate decision (expand, mature, dormant or die). Dysfunction of these regulations can lead to uncontrolled expansion (cancer) or catastrophic loss (organ failure) of stem cells.

The purpose of this project is to understand how fate decision is controlled by specific genes and genetic pathways in both normal (healthy) and abnormal (trauma, inflammation, cancer and aging) situations. These genetic controls of stem cells are often similar in different organs/systems. The main system we use is the gut, which is one of the fastest regenerating tissues. The sheet of cells covering the entire gut lumen, which is approximately the size of a tennis court, continuously regenerate every 4-5 days. The high turnover rate and the unique architecture of the gut makes it an ideal model for stem cell study.

Stem cell fate decision, tissue repair and cancer are closely linked, while the underlying molecular control is not fully characterised. Our aim is to identify the key genes and their related signalling pathways involved in the stem cell control of normal and cancerous tissues, with the emphasis of finding new drug targets to improve cancer 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 project is likely to advance the basic understanding of how human body works by stem cell fate decision, to benefit diagnosis of genetic diseases, to inform clinical treatment, and ultimately to improve cancer treatment by providing options of targeted (personalised) therapy.

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

Around 33000 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 vast majority of our regulated experiments are of the mildest severity and concern the breeding and observation of genetically altered mice and/or minimally invasive procedures such as administration of substances by injection. Adverse effects are neither expected nor seen in all but a very few of these cases. In some cases, the animals will develop tumours, which can be associated with weight loss, signs of discomfort and slowing down of the normal activity. However, the procedures will never exceed the moderate severity level. Any animal approaching severity limits will be killed (Schedule 1), and all animals subject to a procedure will eventually be killed (Schedule 1) and tissues will be 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

Cell fate decisions in the animal or an organ take place within a complex environment, where events intrinsic to the cells are influenced by a variety of extrinsic signals. The latter can involve molecules that can act locally or over considerable distances (such as growth factors and hormones), and which may originate from neighbouring cells, or from anywhere within the body (or even be from the external environment such as microbes in the gut). Moreover, most tissues develop in a complex way in three dimensions over time in a carefully orchestrated manner, and require blood vessels and nerves to operate. Therefore, although some aspects of certain cell fate decisions can be studied in vitro, and we both use and develop such approaches, it is generally essential to study them in animals (as a minimum to judge the suitability of in vitro systems to give meaningful information). This is particularly true of the complex systems and processes we investigate.

Reduction

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

Reduction

Our experiments are designed to use the minimum number of animals required to give robust answers with the use of statistical methods.

We test methods and reagents in vitro whenever possible prior to their use in animals.

We always confirm the importance of the genes of interest in “organoid culture” (3D stem cell culture that consists of organ-specific cell type) in vitro before testing them in animals.

We use efficient method to generate and maintain genetically altered animals, and make use of sperm and embryo freezing to avoid keeping the strains as live animals when a particular study is finished.

We will make optimal use of several tissues, fluid and cell types per individual mouse and will provide the other affected tissues to appropriate scientists so that they do not have to breed mice specifically for their 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 have been selected for the majority of this work as it is an appropriate model for providing insights into human diseases and it is the species in which reliable transgenic and knockout technologies are most advanced.

We choose well-established protocols known to have minimal harmful effects whenever possible.

To minimise stress during breeding and maintenance, we will follow best practice guidelines and follow local refinements of husbandry.

Whenever practical, we prefer to make genetic alterations that are inducible, so that the animals do not show a phenotype until expression of the candidate gene or a deletion is induced.

For manipulations, we will adhere to local and national guidelines that aim to minimise suffering. If insufficient information is available, new manipulations will be pre-screened in small-scale pilot studies to obtain indications of the minimal dose and exposure time that is likely to be effectively, thereby minimising any potential suffering.

In all surgery, analgesia will be provided according to contemporary best practice and advice from the NVS/NACWO. Good aseptic surgical techniques, heat and fluid therapy will be provided as necessary.

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	Pathogenesis of influenza infections in murine models
Key Words	Influenza, Antiviral, Infection, 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;
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):

Influenza (flu) is a seasonal virus that affects everyone and can cause pandemics.

Our risk of getting sick from infectious disease is determined partly by our genes.

The aim of this project is to understand how our genes contribute to our susceptibility or resistance to infection.

Also, we want to develop new ways of treating viral infections that take advantage of the body's natural defense mechanisms.

We will use computer programs and lab work to identify new genes and new antivirals to help us in the fight against flu.

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

The benefits of this project will be the identification of new targets and treatments for influenza infections. This will provide us with new specific targets to generate novel antiviral molecules and so enable us to treat and prevent infection.

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

All the animals in this project will be mice, a total of approximately 3,500 mice are expected to be used over the 5-year period of this licence however there will be continued efforts to minimise these numbers

In the context of what you propose to do to 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 the animals used within these studies will be closely observed and monitored to ensure their health. Many animals will be anesthetised for viral administration reducing the stress/discomfort. Substances will be administered to

prevent or treat the symptoms of infection, thus testing the effectiveness of new antivirals. Some of the mice we use in this study will get very ill and we expect a high severity in these cases. We will closely monitor these animals and have set humane endpoints. Where possible animals will be removed from the study at the earliest time points where the science can still be achieved.

Application of the 3Rs

Replacement

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

Replacement

Although we can use computers and cells in the lab to investigate and test new antivirals and the effects of changing genes in the mice during infection, they cannot replace the complexity of the infection response in a mouse or a human which uses lots of different cells at the same time.

Reduction

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

Reduction

We have developed infection models that allow us to investigate multiple aspects of viral infection and the host response in a single animal. This greatly reduces the number of animals required to observe many changes at once.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 infection models permit the assessment of multiple aspects of infection in the same animal and so, we can look at the host/virus interaction in great detail. We have clearly defined end points, with experimental protocols that follow regulated standard operating procedures and are performed by trained staff. We are continually refining the experimental protocols we use.

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	Interactions between cancer drivers as determinants of tumour tropism, phenotype and response to therapy.
Key Words	Cancer, Breast, Treatment, Resistance
Expected duration of the project	5 year(s) 0 months

Purpose of 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 the project are to understand the fundamental reasons why different cancers arising in the breast appear different to each other and also why a single cancer will have regions of different appearance within it. We will also try and understand why different genetic mutations cause some type of cancer but not others e.g. why do people who inherit a mutation in the BRCA1 gene tend to get breast and ovarian cancer but not liver or skin cancer, as BRCA1 is a gene important for protecting all cells from damage. Finally, we will build on our previous studies to test treatments aimed at specifically killing the different types of cancer and understanding resistance to such treatments.

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

If we can understand the fundamental reasons for the differences between cancers, we can identify potential targets for treatment specific to different cancer types and begin to develop treatments for them. This could lead to ‘personalised cancer medicine’ for patients. We have already identified one such potential target previously and are beginning to develop possible therapies against it. These will need to be tested in cells in dishes and, if they prove effective, in animal studies. Understanding the reasons for differences in appearance within a tumour will enable us to understand how the different regions of a tumour may respond to treatment i.e. can some regions of tumour be killed by a treatment but others survive and cause a tumour to regrow. If we can understand why some regions of a tumour, but not others, are resistant, we may be able to identify ways to overcome that resistance. Understanding why different genetic mutations cause some type of cancer but not others will advance our fundamental understanding of the biology of cancer and also may lead to new therapeutic approaches if we can mimic the features that protect one tissue from cancer in another tissue that is sensitive.

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

We will use mice. We expect to use approximately 12,500 mice over 5 years. At least 60% of these will be used only for breeding.

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

Some mice will undergo surgery under general anaesthetic for implantation of cancer material. Others will develop cancers spontaneously. The adverse effects caused by the cancers will vary depending on the site of the cancer. For example, breast cancers, being located close to the body surface, have minimal health effects but if the get large or are close to the legs may affect walking. In contrast, gut cancers may cause digestive side effects and bleeding into the gut. This may be of varying severity. We will have a ‘welfare score’ system to assess the overall health of all animals carrying cancers as well as a number of absolute defined limits beyond which adverse effects will not be allowed to proceed. When the ‘welfare score’ reaches the prescribed limit the mouse will be humanely killed. The overall expected level of severity is ‘moderate’.

Application of the 3Rs

Replacement

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

Replacement

To eventually have benefit for patients, understanding what influences the development of cancer, its behaviour, interaction with the cells around it and response to treatment requires a mammalian whole organism system. Furthermore, development of new treatments requires proof that such treatments can work in animal studies.

Reduction

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

Reduction

We have calculated the minimum number of animals required for us to determine statistical differences in experiments.

Refinement

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

Refinement

Mice are the animals of choice for using genetics to study cancer and for the testing of new cancer therapies. Genetically modified mice are widely available, meaning that the biology of most genes can be studied in many organs in the body.

We will finish experiments at the first possible humane endpoint which enables the object of the experiments to be achieved with the least possible suffering.

We will increase our use of imaging techniques to enable non-invasive measurements to be taken of animals and allowing them to be humanely killed earlier.

Suitable anaesthesia and analgesia will be used under the guidance of a veterinary surgeon.

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	Evaluation of new treatments for polycystic kidney disease
Key Words	ADPKD, polycystic
Expected duration of the project	5 year(s) 0 months

Purpose of 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 the project is to identify novel therapeutic strategies to treat Polycystic kidney disease. Compounds are tested for their ability to reverse or slow down kidney cyst formation and improve kidney function and for adverse events to be minimal in order to provide significant improvement in the quality of life compared to current 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)?

Polycystic kidney disease is a debilitating disorder characterised by progressive kidney cyst formation leading to end stage renal failure. It affects about 70000 adults and children in the UK and accounts for 1 in 8 people in need for a kidney transplant. This work is expected to provide new information on mechanisms regulating kidney cyst formation, which will be used to identify new therapies with better efficacy and fewer side effects.

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

18000 mice 2000 rats both 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?

Kidney cyst growth and progressive related perturbations in function will be induced in rodents by subcutaneous or orthotopic implantation of cystic human cells or by genetic deletion of proteins known to cause the disease in humans. The majority of studies described in this license are well tolerated by rodents and those models are well described in the literature. It is not expected that serious adverse effects will occur but any side effects are likely to involve bodyweight loss and deterioration in clinical signs. Any animals exhibiting such signs will be removed humanely 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

Polycystic kidney animal models are required to assess the effect of a test compound on kidney cyst formation and global renal function (efficacy)

Cell and ex-vivo assays can give a good indication of the potential ability of a compound to modulate the formation of cysts but they cannot fully predict in vivo efficacy on global renal function or disease progression. In vivo models are therefore an absolute necessity to relate in vitro data to efficacy in order to predict a potential clinical benefit. In addition, the PK/PD relationship, driven by distribution, metabolism and elimination, cannot be accurately modelled in vitro.

Finally, proven in vivo efficacy data is a prerequisite of the regulatory bodies who have the authority to approve or reject a new drug application

Reduction

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

Reduction

Protocols covered by this project licence application are designed to use the minimum number of animals possible.
Tolerability studies are performed with small groups of animals in order to establish the maximum tolerated dose and suitability of dosing regimen prior to larger efficacy studies. Only then, can the more complex in vivo efficacy studies commence in the knowledge that the animals are likely to tolerate the compound.

Minimum group sizes for efficacy studies will be calculated using power analysis and will incorporate consultation with a statistician.

The use of non invasive techniques to repetitively record both cyst/kidney volume and glomerular filtration rate will avoid unnecessary sacrifice and enhance the amount of mechanistic data obtained in a single animal, therefore decreasing the number of animals necessary for each particular 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

It is well documented that deletion of either polycystin-1 or polycystin-2 proteins in mice leads to progressive development of kidney cysts and loss of kidney function, which reflect the pathology and biochemical changes associated with the human disease. These models are based on the gene mutations that have been shown to cause ADPKD in human patients, therefore improving the likelihood of translation from efficacy observed in these models to a clinical benefit. Studies in rodents deliver robust, reproducible data and so it is often unnecessary to evaluate efficacy of new compounds in higher species.

The project uses techniques that can also be used in patients during clinical trials such as total kidney volume and glomerular filtration rate. These techniques will provide efficacy data on the key symptoms of the disease and key information on the mechanism of action of the compound tested that will be directly translatable to the clinical situation.

In addition to tolerability studies, pilot studies may be conducted in a small number of animals in order to refine the parameters and methodology for ensuing studies. These are intended to define the risk/benefit ratio of each procedure to generate statistically significant data whilst causing the least adverse effects.

Finally, longitudinal non-invasive imaging and/or measure of specific urine markers will be used to assess disease progression and will be correlated to the general physical condition of the animal in order to define accurate, quantitative humane endpoints.

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	Understanding liver injury and disease
Key Words	Liver, Fibrosis, Myofibroblast
Expected duration of the project	5 year(s) 0 months

Purpose of 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 seeks to identify ways in which to treat chronic liver disease such as fibrosis, for which there are currently no clinical treatments available. The project also seeks to understand how the liver can become more susceptible to damage, and how we may be able to use stem cells to treat patients with liver failure.

We are doing this project because liver disease is the fifth most common cause of premature death in the western world and the only one in the top five predicted to increase over the next few decades. A common consequence of liver injury of any type is fibrosis, for which there is currently no treatment. This project seeks, in part. to find treatments for fibrosis. It also seeks to establish whether a reduction in fibrosis can reduce cancer in the liver, and enhance liver cell function.

An additional component of the project will examine the potential for dietary constituents such as alcohol to alter the absorption of chemicals like food additives and colourings, from the gut. This could result in liver damage and/or increased exposure for people and is something that is not taken into consideration by regulatory authorities when they evaluate safe levels of exposure of dietary additives. We therefore need to establish whether this may be the case and could be the explanation for liver diseases such as primary biliary cirrhosis, for which the cause is not known.

The general project plan will be to use established liver injury approaches to generate liver fibrosis in mice or rats. This may be induced by treating the animals with a selected number of toxins at a dose that specifically damages the liver without causing more than transient moderate adverse effects. Alternatively, the liver may be exposed to a transient loss of blood flow to part of the liver to model the effects that liver tissue undergoes prior to transplantation (as this can result in liver fibrosis in the recipient patient's new liver).

These models will be used to test potential drugs for their ability to reduce fibrosis.

In addition, the laboratory has developed an antibody-based strategy to target therapeutics to the cells that cause liver fibrosis. This strategy will be used to examine the role of myofibroblasts (and fibrosis) in a range of diseases such as cancer or stem cell engraftment in the liver and may demonstrate that an anti-fibrotic treatment may also help to reduce cancer growth in the liver or increase cell engraftment.

The project will also examine the potential for alcohol to modulate the uptake of chemicals in the diet and to increase the risk tf the chemical causing liver injury and fibrosis.

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

The benefits of this project include a greater understanding of the function of the cells which cause fibrosis; the identification of drugs with the potential to treat fibrosis in man; the potential for improved stem cell therapies and the identification of a mechanism by which the population may be exposed to harmful chemicals present in our diet.

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

Rats, mice and transgenic mice only will be used as these are established and effective models for human liver disease research. Approx 260 rats will be used. Approx 1080 mice will be used, 580 in experiments with approx 500 used for breeding transgenic mice, of which a proportion will be re-used in other experiments. In some cases, transgenic animals may be used if they enable a mechanism of action for a drug to be identified or confirmed.

In the context of what you propose to do to 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 include causing liver damage using chemicals or stopping the blood flow through a part of the liver for a period of time to initiate and sustain a fibrosis in the liver tissue. In some experiments, animals will be injected with tumour cells or stems cells to see if fibrosis affects them. No procedure exceeds moderate severity. In the absence of changes in body condition scoring and general clinical appearance, body weight losses of up to 25% of controls may occur.

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 panel of in vitro assays to identify potential anti-fibrogenics or potential liver toxins. In this way, we reduce our reliance on animal models as much as possible. However, liver injury and liver fibrosis is a multi-cellular and multi-tissue process which cannot be completely replicated in vitro. Accordingly, key observations such as the identification of an efficacious anti-fibrogenic, need to be tested in animal models before they could approach Pharma development and clinical trials.

Reduction

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

Reduction

Animal use is reduced in this project by using in vitro systems when possible. Our laboratory often employs a variety of cell culture systems using cell lines (which do not require animal donors) to investigate the effects of chemicals on cells. We also actively use human cells isolated from donors (e.g. from tissue removed during surgery or after a donor organ has not been used in transplantation) to perform much of our work. Only key questions are investigated in animals, when in vitro systems are not appropriate. This might be the case when we want to understand the effect of a chemical on an organ and when the response might be dependent on multiple cell types (some of which are localised outside the organ of interest). When animals are used, a variety of refinements are employed to provide some insight into any effects of treatments so that animals are not required to be put down. This has the additional benefit of reducing the overall number used. One refinement we have used is to use a transgenic mouse that can be live imaged to identify and quantify adverse effects. This not only reduces animal use because the animals can be used repeatedly, it also allows us to monitor severity and anticipate and avoid excessive severity. Another refinement we frequently employ is blood sampling. We can measure things in the blood that can also allow us to monitor severity and anticipate and avoid excessive severity. These refinements reduce the animals we have to use in the long term. Overall, we also try to use the least number of animals in any study in any case. We do this by looking at previous experiments and working out how many animals we need to use in each treatment group to see, or not see, a critical effect (so that we can have some statistical confidence in our results). This means we use the near minimum number of animals in each particular study but it also means we don’t need to go back and repeat 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

Transgenic mice may also be used if they enable live imaging experiments to be performed that relate to the degree of liver injury. This is because they provide more information about the injury and mean that we can use fewer animals. We have also replaced a severe model of liver injury(bile duct ligation) with a moderate replacement which markedly reduces mortality and provides a more controllable refined injury. These developments ensure that we will use the minimum number of animals necessary.

It is clear from animal studies and in man that significant liver injury can occur for many years without any symptoms because the liver regenerates (with the development of fibrosis). The liver injury protocols are designed to damage the liver sufficiently to cause fibrosis without causing more than transient moderate stress. So we can be reasonably confident that the animals used in these study protocols suffer the minimal degree of suffering required to undertake these studies

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	Breeding and maintenance of GA mice
Key Words	Breeding, Maintenance, Genetically-altered, Mice
Expected duration of the project	3 year(s) 6 months

Purpose of 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 aims and objectives of the project are to breed and maintain established colonies of Genetically Altered (GA) mice. These mice will be used for scientific research, which may require separate project licence authority. The project will allow us to acquire new lines of GA mice that are of interest to our researchers and it will allow us to cryopreserve embryo and sperm from GA mice when they are not being actively studied. It will also allow us to re-derive colonies to improve animal health and welfare.

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

This project is a continuation of a service licence to provide breeding and maintenance of genetically altered mice. A team of technicians with expertise and experience will care for these mice. Researchers will be provided with mice of a high quality that will allow for greater reproducibility in their results. The ability to cryopreserve embryos and sperm will lead to a reduction in the numbers of mice being bred. Rederivation will improve animal health and welfare and ensure that we provide high quality mice for our researchers. Results from studies undertaken on mice bred under this licence will be published in peer reviewed journals and lead to increase in the knowledge and understanding of the models being studied.

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

Mice (all developmental stages), 48,130 over 5 years (the current license is a continuation of a previous license; together the project is 5 years in duration).

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

There are no expected adverse effects from breeding of GA mice. Females of an appropriate size will be used and over vigorous males will be replaced. To identify the mice and to check the genetic make-up, a small tissue sample (normally from the ear) may be taken. This may cause transient pain, but trained and experienced people take the sample. Some female mice (less than 1% of the total animals requested) may be injected with small volumes hormones to increase the number of embryos they can produce, a procedure known as superovulation. The needle insertion may cause transient pain. Some mice (less than 1% of the total animals requested) may be anaesthetised for surgical procedures, this may by injection or by inhalation anaesthetic. This will be for relatively short periods of time. After being anaesthetised, one of two surgical procedures may be performed: either vasectomy in males or embryo transfer in females. For the vasectomy, a small cut is made in the scrotal wall and a small piece of the vas deferens cut away. The hole in the scrotal sac is then repaired. The mice are given analgesia before and after the surgery. For embryo transfer, a small cut is made on the back of the mouse, through the body wall. The uterus or oviduct is exposed and a small hole is made in the uterus or oviduct, embryos held in a very fine tipped pipette are then transferred into the uterus or oviduct. The incision is repaired and the mouse allowed to recover. The mice are given analgesia before and after the surgery. Animals are expected to make a rapid recovery after the anaesthetic. Mice with genetic alterations are affected in different ways depending on the gene/s affected, many look and behave as normal mice. In this project, some of the GA mice born may have balance problems making their walking unsteady. Other strains used in Alzheimer's research, may lose the structure or function of the cells in the brain, which may cause changes in their normal behaviour over time. Sometimes the mutation makes the mice smaller than their normal litter mates. All of these mice are monitored closely. To ensure that these animals can reach the food and water in their cage they will be provided with wet mash. The animals may be transferred to another authorised project, kept alive at the establishment or humanely culled. Trained and experienced personnel will carry out the procedures.

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 as although many research projects involve the use of in-vitro systems such as cell culture, human tissue assays and computer modelling, these cannot yet adequately model all aspects of the complex biological process involved.

The chosen species for this project is the mouse. Mice are biologically very similar to humans and mice can be genetically manipulated to mimic many human diseases.

Reduction

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

Reduction

The project licence holder and animal technicians have a good working knowledge of the colonies being bred and a good working relationship with the researchers. The breeding colonies will be managed efficiently to meet the research needs.

Each request for new GA mice will be reviewed by a committee and we will review the breeding colonies regularly and meet the research group to discuss their requirements.

Refinement

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

Refinement

Mice are the model of choice as their genetic, biological and behavioural characteristics closely resemble those of humans.

The mice will be cared for by animal technicians and veterinary staff who have experience of the husbandry and welfare relevant to their needs.

Experienced and competent personal licence holders will conduct the procedures and veterinary advice will be taken in respect to the use of anaesthesia, analgesia and aseptic technique.

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	Safety Testing of Medicinal Products Using Dogs and Minipigs
Key Words	Regulatory, Safety Assessment, Dogs, Minipigs
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):

This project licence authorises the conduct of studies in laboratory dogs and minipigs to evaluate the safety, quality and effectiveness of medicinal products for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening conditions in man, in terms of general toxicity and whole body system exposure.

The expansion of scientific and medical knowledge has led to the development of drugs which can treat or alleviate the symptoms of many illnesses but there is still a need to develop medicinal products to diagnose and treat many human conditions such as Heart Disease, Stroke, Obesity, COPD, Respiratory Infections, Cancer, Autoimmune diseases, Diabetes, Alzhiemer's and Schizophrenia, amongst others. When these needs are combined with the growing threat from antibiotic resistant bacteria the advancement of science and the development of new products are as important as ever.

The primary aims of this project are to support the development of these medicinal products through acquisition of data to;
1) Support selection of new candidate molecules for further evaluation and development.
2) Demonstrate the safety-hazard profile of a new medicinal product prior to the initiation of clinical trials involving humans.
3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of blood/tissues to support drug development and the validation of non-animal alternative methodology.

As a specially protected species, the dog is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species with relevance to man, and that there is no other acceptable candidate species of lower neurophysiological sensitivity/status.

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

People are exposed to medicinal products as patients, medical professionals, carers and during their manufacture. The products can be biological or non-biological materials and include diagnostic agents or substances associated with drug candidates e.g. metabolites, impurities and drug degradants. The principal benefit of this project is the provision of robust safety data to facilitate sound decisions by national and international Regulatory Agencies regarding human exposure to medicinal products. Without these studies, progression of new medicines to early human studies and to patients could not occur safely or in the current regulatory framework. Validation and refinement of test methods may be completed for specific techniques and may be published to the wider scientific community.

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

Over the 5 year life of this Project Licence, it is estimated that 4,800 dogs and 2,300 minipigs will be used. These numbers include a small proportion of re-use of the same animals.

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

The animals used under this licence will be given the test substances in a similar way to that in which they are expected to be given to humans. As most medicines at taken orally the majority of animals will receive the test material directly by insertion of a flexible rubber catheter into the stomach via the mouth. Most animals are treated in this way once per day daily, although studies may occasionally require two or three doses within 24 hours. For some test materials the oral route of administration may not be appropriate; for example, it may be broken down, not tolerated or not absorbed during the digestive process. In these situations, alternative administration routes such as sub-cutaneous or intravenous injection may be more appropriate. Animals may be manually restrained for “bolus” administrations lasting a few seconds or minutes, or they may be habituated to sit in specially designed slings for longer intravenous administrations. For test materials which will be administered to humans intravenously over extended period of time due to their inherent toxicity or fast clearance from the body it may be more appropriate to surgically implant a permanent catheter (with appropriate anaesthesia and analgesia) to allow the animal free movement whilst being dosed in their home enclosure. Test materials intended to treat a localised condition may be administered to that body part or area for example to the skin. Blood and urine samples may be taken to measure the level of test material or its metabolites within an animal’s circulatory system. These may also be analysed to detect any effects on body systems and organs for example liver or kidney function. Study animals are closely observed several times a day by highly trained technologists who monitor for any signs of discomfort. Other measures such

as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour such as reduced activity. Humane endpoints will be applied or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively fewer adverse effects. Many toxicological effects of the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia. Animals that do not need to be euthanased at the end of a study and have experienced no more than moderate effects and have met the requirements for keeping alive may be reused or rehomed under AWERB oversight, in line with ASPA/HO requirements. In the case of rehoming, animals will undergo a programme of training and socialisation to enable transition to a domestic environment. Potential adoptees will also be assessed for their suitability to provide for the animal's needs.

Application of the 3Rs

Replacement

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

Replacement

There are currently no scientific and legally acceptable evaluations of systemic toxicity that will satisfy regulatory requirements and provide sufficient safety data other than use of animals, though validated *in vitro* tests for specific organs are used wherever possible. As new *in vitro* methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace *in vivo* procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.

As a specially protected species, the dog is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species

with relevance to man, and that there is no other acceptable non-specially protected candidate species.

Reduction

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

Reduction

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

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

In general, toxicity studies are initiated in rodents before progressing into larger animals. This approach, combined with background literature searches and looking across at other study types, can lead to earlier decisions on whether or not to continue development of a particular test material, refinement of study designs and reduced use of dogs and minipigs.

In recent years, the general availability and use of minipigs has also increased, with an associated reduction in the proportional use of dogs.

Refinement

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

Refinement

Species choice and use of specific animal models is determined by the need to generate regulatory acceptable data. Where a choice of species is possible, care is taken to select the most biologically appropriate species, and the species which most closely relates to man.

Animal studies are only considered where there is a direct legislative or regulatory requirement and after review of all other available information to ensure that no alternative is feasible. A step-wise approach is taken, with higher risk studies, when little may be known about the test material, being performed early in a programme and using only small numbers of animals. As confidence in the data and the level of information grows, longer term studies in larger numbers of animals may become necessary and the design of these studies, whist adhering to regulatory requirements, can be refined and tailored to obtain the most relevant and valid

scientific information from the fewest animals and with the lowest level of adverse effects possible.

Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints.

Socially compatible species are routinely group housed with environmental enrichment which encourages species specific behaviours without adversely impacting study outcomes.

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

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	The physiological roles of phosphatidylinositol 3-kinases
Key Words	Therapeutics, immunology, Translational, cancer, 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):

We study signalling pathways, which is the processing of information within cells, tissues and animals. When these systems go wrong, this can lead to disease.

We have created mice with altered genes, called mutant mice, to study signalling pathways and assess if these mice develop diseases (for example malformed vessels) or are protected from some diseases (for example are protected from allergic stimuli). Ultimately, we wish to develop drugs that can alleviate human disease and to further our scientific understanding of the signalling pathways involved.

In order to achieve these goals mutant mice are initially created, followed by a full screening to find out if these mice have any abnormal pathology, behaviour or physiology. In some instances, we can challenge these mice, for example give them an allergic stimulus, to see if the inactivation of the gene protects. In this way, we can identify genes against which pharma can make drugs to treat human disease. In previous work, our studies have 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)?

The knowledge gained from this licence will not only contribute to the understanding of how signalling pathways operate, and when disrupted, cause disease, but also to develop therapeutics for these diseases. So far we have found that disrupting the pathways of the PI3K gene family may result in, or protect diseases of metabolism, immunology and cancer. Therefore therapeutics will be developed to help alleviate these disorders. As a result of our work and those of collaborators, drugs have been developed that are in phase II clinical trials and are helping to treat people with cancer.

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

Mice, 5000 per year Rats, 150 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?

Animals will be required for this project as many of the disease we study, such as allergy and cancer are very complex and cannot be replicated in cell or tissue systems. Also, for drug-based studies, the whole mouse is required to elucidate any side effects that may occur, which would not show up in cells or tissue systems. For these protocols, the likely severity bands will vary from mild to moderate. In the case of collecting organs or cells for tissue culture work, the severity reached would be sub-threshold for non-harmful mutant strains. Procedures will consist of humanely killing mutant mice, for cell-based tissues and blood for pathological analysis. Other experiments would consist of injection of cells that cause cancer or the injection of substances that cause allergies and to administer medicines to see if these diseases get better. Also, minor surgery, such as the removal of tumours may also be carried out. Adverse effects likely to be encountered would be weight loss or gain, subdued behaviour, and hunched posture. Adverse effects will be monitored for daily and mice will be culled before the humane endpoint is reached or before if sufficient scientific data has been collected. Protocols have been designed such that mice are subjected to minimal suffering. Also, pilot studies will be initially carried out on very small numbers, to give an indication of what scientific data would be generated and the degree of suffering imposed, such that subsequent experiments can be refined to minimise suffering. At the end of the experiment, animals will be killed and tissues used for in vitro studies or biochemical analysis.

Application of the 3Rs

Replacement

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

Replacement

Animals will be required for this project as many of the diseases we study, such as allergy and cancer are very complex and cannot be replicated in cell or tissue systems. Also, for drug-based studies, the whole mouse is required to elucidate any off target effects that may occur, which would not show up in cells or tissue systems. Mice are good models to study signalling pathways, as they resemble humans closely; anatomically, biochemically, physiologically etc.

Before animals are used, cells from tissue banks will be cultured in order to first characterise whether the signalling pathways we wish to study are affected in diseases such as allergy and cancer and whether drugs can restore these pathways to normal functioning.

Reduction

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

Reduction

We propose only to work with mice and rats. Statistical analysis will be used in order to keep the minimum number of mice required to make a valid scientific conclusion. We plan not to use more than 3000 mice per year for all our experiments and for collaborators specialised in other disciplines, such as behaviour and imaging. We are also developing tissue culture based systems such as making organoids - miniture organs in culture - which will help replace the need to do procedures in mice.

Refinement

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

Refinement

Protocols have been designed such that mice are subjected to minimal suffering. No mouse would be subjected to more than a moderate amount of suffering. Also, pilot studies will be initially carried out on very small numbers, to give an indication of what scientific data would be generated and the degree of suffering imposed, such that subsequent experiments can be refined to minimise suffering. The latest methodology for scientific procedures will be adhered to which causes the least amount of harm to the animals.

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	Molecular mechanisms of T cell mediated immune responses
Key Words	Immune response, autoimmunity, Egr, viral infection, tumour
Expected duration of the project	5 year(s) 0 months

Purpose of 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 the project is to understand how two molecules (Egr2 and 3) affect the functionality of T lymphocytes, and whether this can be optimized to treat virus infections and cancers, with the potential of developing a new therapeutic vaccine.

The immune system is responsible for protecting our body from invading pathogens. However, in autoimmune diseases, the immune system mistakes one's own tissues for pathogens, and hence the body begins to attack itself. In contrast, after contracting chronic infections such as hepatitis C, the immune system becomes too weak to attack the invading virus. It is unknown why these diseases cause the immune system to over-perform or under-perform.

However, we have recently discovered that the excess production of two proteins, Egr2 and 3, in T lymphocytes (a type of white blood cell), results in weaker anti-viral responses. Conversely, limiting the production of these proteins leads to the development of autoimmune diseases.

To correct the malfunction of T lymphocytes in these diseases, we must understand the mechanisms of Egr2 and 3.

By using unique mouse models, the objectives of this project are;

1. To discover the mechanisms of Egr2 and 3 in T cells at a molecular level.

2. To understand the function of T cells under conditions of virus infection, tumours and autoimmune diseases, in mice that are either genetically missing Egr2 or 3 or over producing Egr2 or 3.

3. To evaluate the effectiveness of regulating Egr2 and 3 production in T cells for treating autoimmune conditions.

4. Finally, to reconstitute immune activators into artificial cells (Nano-APC), and use these to correct the function of T cells in autoimmune diseases and chronic infection.

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

The outcome of these experiments will make an important contribution to the development of new methods for treating autoimmune diseases, as well as new vaccines for cancer, which are currently incurable.

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

Mice. Over the duration of the project, it is envisaged that approximately 4000 mice will be used, including those that are used only as breeding 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?

All of the proposed experiments are well established and used by many laboratories for more than 20 years. There are expected adverse effects from Experimental Autoimmune Encephalomyelitis (EAE), vaccinia virus infection and implanted tumour and irradiation to destroy bone marrow for bone marrow transplantation experiments. In EAE conditions, mice will have weak movement of tail and limbs.. Weight loss is the major sign of infection. There will be no signs of adverse effects in tumour bearing animals as long as tumours are less than 10mm3.. Irradiation has limited and short period of adverse effects such as less movement that may last for a few hours after irradiation. Once these signs are observed, mice will be humanely killed at the earliest sign possible.

Application of the 3Rs

Replacement

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

Replacement

Immune function is established in living organisms. In addition, the method for investigating the function of any given molecule is to genetically modify the molecule in animals. We cannot carry out life experiments in humans.

Reduction

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

Reduction

- We will assess some molecular mechanisms using cells after adding or removing Egr2 and 3 genes in the laboratory.

- To minimise the use of animals without affecting scientific results, we will design the each test with accurate number of animals which will be just enough to give us clear results. We will carry out, if possible, pre-experiments on cell lines to optimize the procedures in order to achieve 100% success of the animal 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 to use mice since the parallels between the mouse and the human immune systems are well understood and mouse models of the diseases well established. This means that reagents are readily available and alleviates the need to establish novel models which greatly reduces animal use.

- The newly established GFP-Egr2 knock-in model has proven to be generally normal without any health problems. It give us a great advantage for fulfilling the objectives under this new PPL, while greatly reducing the need of Egr2 KO model which showed some welfare issues with breeding difficulties, thus also minimising welfare costs for the animals.

- We apply well defined experimental techniques with minimal intervention to avoid distressing the animals, expert preparation of samples for investigation, strict adherence to protocols and keep the time that an animal is under experimentation as short as possible

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	Mechanistic basis of information processing in neural circuits
Key Words	Synapse, Neuron, Circuit, Behaviour, Learning
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Correctly interpreting sensory information (e.g. touch, visual scenes and sounds) and generating coordinated movements are essential for our survival and normal behaviour. Indeed, deficits in these functions, which are common hallmarks of neurological disorders (e.g. schizophrenia, ataxia and autism), have debilitating effects. However, surprisingly little is known about how neural circuits represent, distinguish and learn sensory sequences or how sensory information is disentangled from self-generated input arising from movements. The overall aim of this research is to discover how the synaptic and cellular properties of circuits enable the brain to perform these functions.

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

The main benefit of this research programme is to generate new scientific knowledge about how neural circuits in the brain represent, transform and learn sensory information and coordinate movement. Developing a better understanding of the physiological mechanisms underlying normal brain function will also provide a basis for understanding how genetic and disease-induced changes in proteins, synapses and neurons cause aberrant network behaviour during neurological disorders, which are currently poorly understood and impose a heavy burden on society. The data collected during this research programme will also be used to build, refine and test biologically accurate models of brain circuits. A lasting benefit of this animal research will be to provide computer models that can be used by other scientists in their future research. Such models consolidate quantitative knowledge, can generate new hypotheses through prediction and enable neuroscientists to develop a better understanding of complex neural systems. An additional benefit of such models is that they can lead to more informed/defined hypotheses, thereby enabling greater refinement in experimental design and reduced animal usage.

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

This 5-year program of scientific investigation into brain function will involve a team of 15 scientists. The breeding of multiple genetically modified mice strains accounts for the vast majority of animals used due to only a fraction of the progeny inheriting the transgene. These together with the mice that will be used for behavioural experiments and mice/rats for tissue preparation will total approximately 1200 p.a.

In the context of what you propose to do to 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 involve procedures of mild or moderate severity. Animals typically undergo short surgeries under general anaesthesia where we make very small windows in the skull so that we can image and record the activity of neurons. Potential adverse effects include postoperative stress and discomfort, but this will be minimised with analgesia. When unexpected clinical signs appear, we will immediately consult our designated welfare officers and vets. At the end of each procedure animals will either be euthanised according to schedule 1 or another humane method, under terminal anaesthesia and the tissue will be utilized for ex vivo experiments.

Application of the 3Rs

Replacement

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

Replacement

Experiments on live brain tissue and intact animals are essential for gaining new knowledge on the properties of synapses, neurons and networks and there is no equivalent or alternative to using animals. Cell culture is not suitable because synaptic, neuronal and network properties all change in the cell culture preparation. Moreover, investigation of how information about the body and surroundings is represented and processed requires that animals are intact. Wherever possible we use computer models to test possible scenarios before experiments are carried out, but it is essential to test model predictions experimentally for them to have scientific value.

Reduction

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

Reduction

The minimum number of animals will be used to achieve the aims of the experiments and ensure reliable and reproducible results. The data generated in this project will largely be differences in neuronal and circuit responses following a perturbation, sensory stimuli or a defined behavioural task. Each experiment is designed, as far as possible, to include its own control, reducing variability, increasing statistical sensitivity and thus minimizing the number of animals required to reach statistical significance. Variability across animals will be minimized by using well defined strains of mice, with little genetic variation. We also apply methods of experimental design which enable us to estimate the minimum number of animals required to detect an effect, where possible. We will be conducting our experiments to be able to publish according to the ARRIVE guidelines.

We use and develop methods and experimental techniques that maximise the data collection from the minimum number of animals. We will micromanage the breeding of mice to ensure that the number of animals bred matches as closely as possible the number required for experiments. Cryopreservation of transgenic lines is utilised whenever there is a sustained gap in experimental use in order to minimise breeding surplus. Lastly, for acute slices, tissue from an animal is shared between researchers whenever possible.

Refinement

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

Refinement

Mice are the least sentient spices that are appropriate for this type of study. The basic synaptic, neuronal and network properties found in mice are common to all mammals and makes them a good model system for studying human brain function. Moreover, the large range of genetically modified mice available are critical for labelling specific neuronal subtypes with fluorescent indicators that report neuronal activity and optogenetic transducers that can activate and silence neurons with light.

All surgery will be performed using appropriate anaesthetics/analgesic regimes to minimise pain. Surgery will only be performed by fully trained and competency assessed staff with procedures under regular review to identify further refinements to minimise animal suffering.

Mice will receive environmental enrichment and will be group housed wherever possible.

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	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. From our past experience characterising the kinetics of the immune response, 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 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	Translational Studies in Prostate Cancer
Key Words	Prostate cancer, 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):

In the UK, prostate cancer affects 1 out of 8 men. It kills more than 11,000 men each year, exceeding the number of breast cancer related deaths. Hormone treatment, which has been used for more than 50 years, controls prostate cancer in a palliative manner. We urgently need better and more specific treatment options. Better knowledge on how prostate cancer spreads (or metastasise) and survives (or resists) treatment (such as hormone, chemotherapy and radiation) will help researchers worldwide to formulate new ideas and approaches to defeat prostate cancer. During these studies, better tests to detect aggressive cancer and/or to predict how cancer will respond to treatment can be developed. Our project builds on our extensive expertise and resources, linking laboratory to clinical (surgical and oncological) practice, to study cancer metastases and treatment resistance. In addition, we are well placed to begin efforts to test the usefulness of novel treatment agents.

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

The likely benefit will come from knowledge on why cancer spreads and resists treatment. This information will shape ongoing and future efforts in drug development for patient benefit. Data from work carried out within this project will be tested using resources from clinical prostate cancer.

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

All studies use mouse, with ~50,000 over 5 years. Relatively high numbers of mice are required for the breeding protocols. One of the reasons is that only male animals will develop a prostate gland and are used as cohort animals. Also, there is a need for extensive breeding regimes, and as such, only ~25% of the mice bred will harbour the desired genetic alterations required for our studies. Hence, at least 50% of mice will not undergo scientific procedure but are used to generate the required

mice. We estimate that around 8000 mice will be studied as transgenic models and around 7000 mice (including immune deficient and immune intact but genetically compatible) from this project or other sources will be used in our transplantation models.

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

The majority of animals used (~70%) will not have any observable clinical signs. They are used for breeding programme only and will be humanely culled once their genetic statuses are known. Experimental (cohort) animals will carry patient-specific genetic events to recapitulate human cancer. Such animals will develop prostate cancer. When cancer develops in the prostate, animals may experience abdominal distension or develop urinary symptoms. We have refined the required surgical procedures (to administer treatment and/or facilitate imaging studies) described in this project, typically using a small lower abdominal incision, which is closed when the procedure is concluded and mouse recovery will be closely observed per protocol. Some of the study animals will be administered substances/therapeutic agents, or fed altered diet (e.g. high fat diet). We have refined the technique to produce prostate cancer in its natural environment by implanting cancer cells directly into the mouse prostate. This is a very useful method to test the growth behaviour of cancer cells with certain genetic contents. We will also use such technique to test the usefulness of new treatment agents in the project. By implanting cancer cells in the prostate gland or injecting into the mice (using different routes to mimic clinical metastasis to bone and other organs), we hope to study the reason for prostate cancer to spread and/or resist treatment. All animals on treatment or anaesthesia will be carefully monitored for discomfort, recovery or development of relevant clinical symptoms. Animals will be humanely killed at the end of the experiments and tissues collected at post-mortem to maximise data obtained.

Application of the 3Rs

Replacement

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

Replacement

Prostate cancer is closely controlled by male hormone function. Communication between the prostate and other organs/cell types in the body (including immune cells, adipose tissue and liver) are very important in determining the way cancer behaves. For some research objectives, there are currently no alternatives to the use of appropriate mouse models. However, our research group is active in trying out new ways of co-culture systems whereby prostate cancer and host cells (fat, immune and other cell types) can be studied in the laboratory, including the use of three

dimensional spheroid (or mini-organ) cultures. We also have extensive expertise to generate cell lines from our mouse models which will replace the use of the whole mouse for some exploratory experiments.

Reduction

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

Reduction

Statistical significance and power analysis are applied to design our studies to ensure our studies are informative using the least number of animals. We will also use our transplant models to reduce the use of transgenic mice and the overall study periods. We have extensive experience and support within our Institute to use the minimal number of mice to answer specific research questions. We have also pioneered the use of ultrasound scan and other non-invasive imaging methods such as magnetic resonance imaging and functional scans (e.g. positron emission tomography) to allow serial (multiple) monitoring which reduces numbers.

Refinement

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

Refinement

We have developed expertise in a range of mouse models that are selected to best answer individual research questions. For instance, if we were to test the usefulness of a new treatment, we can apply our implanted prostate tumour model which will minimise the need for breeding and substantially reduce the number of mice needed and duration of the entire experiment. We have developed expertise in non-invasive imaging using ultrasound scan to monitor tumour growth so animals can enter our studies at the optimal time to ensure robust comparison between mice and minimise suffering. Evaluation of new therapeutic agents will be tested using small number of mice (3-4) in the first instance. Analgesia will be applied to ensure the welfare of the animals.

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	Investigating cellular and molecular therapies for neural repair
Key Words	nerve, regeneration, spinal cord injury, brain 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):

The overall aim of the project is to find new ways to promote the repair of the damaged nerve cells in the brain and peripheral nervous system. After injury, most body tissues undergo repair that either totally or partially restores normal function. In contrast, damage to the nervous system invariably results in permanent loss of function with life changing consequences. The work conducted will assess the potential of genetic and cell based therapies to facilitate the repair and restoration of nerve function following damage incurred either as a result of traumatic injury or as a consequence of a neurodegenerative 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)?

It remains unclear why damaged nerve cells invariably fail to undergo effective repair. This project will advance our understanding of how the brain makes new neurons throughout life, why not all neurons can regenerate and why some are more vulnerable to injury than others. The findings of these studies will be of direct benefit to neuroscientists working in the field of nerve repair and will contribute to the discovery of new therapeutic approaches that bring about the repair and restoration of damaged nerve cells.

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

Rats and mice will be used because the anatomy and the physiology of their nervous systems is similar to that of humans. The study will last 5 years and is expected to use approximately 1500 mice and 400 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?

Some animals will undergo surgical procedures performed under deep general anaesthesia and the animals will be given pain killers throughout the post-operative

period. During the 1-2 hour surgeries, we will typically gain access to the nerves by making incision through muscle, or make small window in the spinal column to gain access to the spinal cord. We will use fine forceps to pinch a small and specific nerve and a fine needle to deliver our therapeutic intervention into the nerve; following which we will sew back the skin/muscles together and wake the animal with painkillers and post-operative care given just like people recovering in the hospital. In some surgical procedures, we will make very small windows in the skull to gain access to the brain, use a fine needle to inject a very small volume of chemical into a specific part of the brain and seal the skin over the skull and recover the animals as above. Some animals will experience weakness, or partial loss of function in one limb that does not impair their ability to move about, feed and drink, or cause them pain. Following recovery, we will use tasks to assess the animals' nerve function by watching how they move about, how they reach for objects, or how their memory functions recover. Some animals may have their diet restricted to motivate them to undertake tasks involving a food reward. The level of restriction will not impair their growth or activity level. Some animals will be kept for a period of time at which they might start to develop mild signs of age deterioration. However, this will not impair their ability to move about, feed and drink. All of the animals will be killed by a humane method at the end of the study and tissues taken for analysis after death.

Application of the 3Rs

Replacement

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

Replacement

Wherever possible cell culture will be used in preference to animal studies. However, the body's response to damage to the nervous system is highly complex and involves numerous interactions between many different cell types in a constantly changing biological environment, as yet it is not possible to replicate this in a meaningful manner in either a tissue culture dish or by using computer simulation.

Reduction

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

Reduction

In studies where we are evaluating the effectiveness of a treatment, the number of animals needed will be minimised by careful experimental design, the adoption of sensitive outcome measures with small variation (reliable measurements) and sampling at the most relevant time points. When possible longitudinal studies or within-animal controls 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

The mammalian nervous system of rodents shares many physiological and anatomical features with that of humans and are recognised, by the scientific community, as relevant models for the study of neural regeneration. Our studies will model the damage that occurs to nerve either as a result of traumatic injuries or neurodegenerative diseases. The models used to replicate traumatic injuries to nerves have been selected because they are the least severe needed to undertake the study and result in weakness or partial loss of function in one limb that does not impair the animal's ability to move around it cage, eat and drink or cause it any pain. The models used for assessing treatments to counter damage caused by neurodegenerative disease do not impair the animal's ability to move about, feed and drink, or cause it any pain. In order to address some scientific questions it will be necessary to use genetically altered mice, these are not expected to result in alterations that cause the animals any suffering. All surgical procedures will be performed under deep general anaesthesia and the animals will be given pain killers to prevent post-operative pain. The behavioural tests used to assessing functional recovery do not cause any pain or harm to the animals. The procedures used in the programme of work will be continuously reviewed and to refine to minimise their impact on the wellbeing of the animals.

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	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 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	Developmental and Reproduction Safety Testing of Chemicals, Plant Protection Products, Biocides and Substance added to Food or Feed Products Using Small Animal Species
Key Words	Regulatory, Safety Assessment, Developmental, Reproduction
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 drive for new and safer products in conjunction with human population expansion and developments in our habitat, drive the need for more effective solutions, for example, to develop “bee friendly” insecticides, environmentally acceptable weed killers, new disinfectants which counteract microbial resistance or safer (to humans) veterinary medicines, animal feed additives, food ingredients and preservatives.

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

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

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

During day to day life people are exposed to a wide range of substances at work, in their home, during leisure and other activities. If not properly assessed and controlled these substances can cause significant injury, health issues and/or lead to terminal illness or even death. Developmental and Reproductive Toxicology (DART)

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

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

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

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

Animals will be given the “test material” under investigation in a way which mimics possible human exposure. As the most likely route of exposure is orally the majority of animals will receive the test material either mixed in their food or directly by insertion of a flexible rubber catheter into the stomach, via the mouth. For some test materials the oral route of administration may not be appropriate for example the material is more likely to come in to contact with skin or other body membranes. Most animals are treated daily; occasionally studies may require several doses within 24 hours. The length of study is dependent on the tonnage of the test material produced each year as a higher tonnage increased the risk of repeated human exposure and ranges from a simple study to explore effects on reproduction with a small number of animals to a multigeneration study to explore effects of generational exposure to a compound. Blood and urine samples may be taken to measure the level of test material or its metabolites within an animal’s circulatory system. These may also be analysed to detect any changes in blood or urine chemistry, allowing in-vivo monitoring of body systems and organs for example liver or kidney function. Neurobehavioural assessments may be carried out to identify potential neurotoxicity by observing and describing behaviour. Many of the endpoints measured on reproduction studies do not adversely affect the life of the animals. For example, offspring may simply be observed for developmental milestones such as eye opening and the development of reflexes and as they grow they may be observed for evidence of sexual maturation, which may be precocious or delayed. Study animals are observed at least twice a day by highly trained technologists who monitor for any signs of discomfort. Other measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected

to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects. Effects on reproduction and fertility of a test material are not always evident during the in-life phase of a study and may not impact the animal's wellbeing (for example reduced numbers of maturing sperm and a reduced number of eggs). Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia.

Application of the 3Rs

Replacement

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

Replacement

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

Reduction

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

Reduction

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

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

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

Refinement

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

Refinement

Species choice and use of specific animal models is determined by the need to generate regulatory acceptable data. The rodent is the first choice for reproduction studies run using the OECD guidelines. Rabbit provides a second species for evaluation of teratology.

Animal studies are only considered where there is a direct legislative or regulatory requirement and after review of all other available information to ensure that no alternative is feasible. A step-wise approach is taken, with higher risk studies, when little may be known about the test material, being performed early in a programme and using only small numbers of animals. As confidence in the data and the level of information grows, longer term studies in larger numbers of animals may become necessary and the design of these studies, whist adhering to regulatory requirements, can be refined and tailored to obtain the most relevant and valid scientific information from the fewest animals and with the lowest level of adverse effects possible.

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

PROJECT 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	Regulatory and Investigative Toxicology
Key Words	Medicine, safety, toxicity, toxicology
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):

Understanding how safe a potential new medicine is before it is given to humans is an essential part of medicine development. Although some information on safety can be obtained without using animals, some tests must be carried out using animals to better understand how these medicines might affect the human body. The objectives of this project are as follows:

- Identify the right potential medicines for development which are safe to give to people and are most likely to be able to treat the target illness.
- Identify any possible safety concerns and understand how these might arise and whether they could cause harm to patients or human volunteers in clinical trials.

Where possible, improve and refine our tests using animals to provide more relevant information to humans whilst minimising the use and impact on 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)?

Achievement of the objectives will support development of safe, new medicines to improve health and quality of life of patients by generating high quality, regulatory acceptable data and will help to remove unsuitable candidates from the development pipeline at an early stage, thus minimising the use of animals and resources. The benefits gained by studies performed depends on the study purpose and type and include: Making decisions on whether potential new medicines are suitable for development as early as possible in the process to avoid wasting animals and money. We use the information generated during early studies to help to understand what we need to measure on future studies. To help us to decide the doses and endpoints to measure on early human studies to minimise the risk to human volunteers. To allow regulatory authorities to decide whether to allow the potential new medicine to be given to humans.

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

Over the 5 year period of the licence we expect to use approximately: 5500 rats 2000 mice 575 dogs 200 hamsters 200 rabbits 575 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?

Evaluation of safety to assess potential risk to humans requires the use high doses of a potential new medicine which can cause some adverse effects in animals. Adverse effects in animals are usually of mild or moderate severity. The most common effects will be loss of body weight or reduced weight gain, reduction in the amount of food the animals are eating and clinical signs such as reduced activity, postural changes, changes in faeces and in some species, vomiting. No animals will intentionally experience severe adverse effects but because early studies may be the first time that a potential new medicine is given to animals, effects may occasionally be more severe than expected. Animals are monitored closely and animals which show signs toward the limit of moderate severity are humanely killed. Most safety studies require examination of blood and tissues from animals to see whether the potential new medicine has caused any damage to organs or tissues, so the majority of animals are humanely killed at the end of a study and subjected to post mortem examination. Samples of tissues are then examined microscopically. On some early studies animals are not required to be killed and provided they have not shown adverse effects they may be used again in a subsequent study.

Application of the 3Rs

Replacement

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

Replacement

Whilst alternatives to in vivo animal models are being developed and are used where possible, there are currently no reliable models available for broad, primary toxicity screening and none that are acceptable to drug regulatory authorities, it is therefore necessary to screen for toxicity in animal models

Reduction

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

Reduction

For safety studies, guidelines require 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. We have a track record of
designing studies that provide us with the information we need to make decisions on the safety of our test substances (leading to continuing, or stopping, development).

For preliminary studies, small groups are acceptable because of the endpoints used give a clear answer. Where group sizes are sufficient data from definitive toxicity studies are analysed statistically. Statistical input is sought, where necessary, to strengthen the overall scientific quality and relevance of the studies to be performed, with sample size calculations performed for specific studies to determine the group size. Group sizes in dog and pig studies are usually insufficient for valid statistical analysis. However, because toxicity is the result of changes in multiple parameters, assessment is made by examination of data from each animal and by correlation of in-life and post mortem findings within an individual.

In order to minimise animal use, we will consider using animals on more than one stu dy when this can be justified on welfare and scientific grounds.

Refinement

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

Refinement

Regulatory guidelines state that toxicology studies in support of administration to man should be conducted in one rodent and one non-rodent species. Generally the rat is the rodent species of choice unless it is known to be an inappropriate model for man for the compound. The non-rodent species will be that likely to give the most satisfactory, reliable and regulatory acceptable results.

The pig will be used as the preferred non-rodent species in this licence, unless it is shown to be unsuitable based on scientific information available, when the dog will be used. Where evaluation of all information indicates that both the pig and dog are a suitable non rodent species, the pig will be chosen.

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	REDACTED 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) higher education or training for the acquisition, maintenance or improvement of vocational 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 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	Safety Testing of Medicinal Products Using Non-Human Primates
Key Words	Regulatory, Safety Assessment, Non-Human Primates
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):

This project licence authorises the conduct of studies in laboratory non-human primates to evaluate the safety, quality and effectiveness of medicinal products for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening conditions in man, in terms of general toxicity and whole body system exposure.

The expansion of scientific and medical knowledge has led to the development of drugs which can treat or alleviate the symptoms of many illnesses but there is still a need to develop medicinal products to diagnose and treat many human conditions such as Heart Disease, Stroke, Obesity, COPD, Respiratory Infections, Cancer, Autoimmune diseases, Diabetes, Alzhiemer's and Schizophrenia, amongst others. When these needs are combined with the growing threat from antibiotic resistant bacteria the advancement of science and the development of new products are as important as ever.

The primary aims of this project are to support the development of these medicinal products through acquisition of data to 1) Support selection of new candidate molecules for further evaluation and development. 2) Demonstrate the safety-hazard profile of a new medicinal product prior to the initiation of clinical trials involving humans 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of blood/tissues to support drug development and the validation of non-animal alternative methodology.

As a specially protected species, the non-human primate is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species, and that there is no other acceptable candidate species. This is usually based upon the test material's mechanism of action, target systems/receptor profile etc and an assessment of the appropriateness of the primate model in general. This is typically achieved via the availability of data (e.g. *in vitro* metabolism,

early pharmacokinetics, or other supporting information) demonstrating that the test material or metabolite is effective in primates, that primates are the most relevant model to man, and that the purpose of the programme of work cannot be achieved by the use of animals that are not primates. A record of the scientific rationale for the use of primates is always retained.

With respect to the high specificity of large molecule biotherapeutics (such as monoclonal antibodies and antibody-drugs conjugates) to the human target, non-human primates are often the only species exhibiting binding of the target and the desired pharmacological effect, and therefore, toxicology studies are most frequently performed in this single species.

Thus, the use of primates in carefully selected studies is an essential requirement in the successful development of new medicinal products.

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

People are exposed to medicinal products as patients, medical professionals, carers and during their manufacture. The products can be biological or non-biological materials and include diagnostic agents or substances associated with drug candidates e.g. metabolites, impurities and drug degradants. The principal benefit of this project is the provision of robust safety data to facilitate sound decisions by national and international Regulatory Agencies regarding human exposure to medicinal products. Without these studies, progression of new medicines to early human studies and to patients could not occur safely or in the current regulatory framework. With the increasing use of advanced drug technologies, targeting the immune system to combat life-threatening and debilitating illnesses like cancer and autoimmune diseases, the non-human primate is often the only suitable test species due to similarity of the immune system. Validation and refinement of test methods may be completed for specific techniques and may be published to the wider scientific community.

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

Over the 5 year life of this Project Licence, it is estimated that 4500 non-human primates will be used (900/year).

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

The animals used under this licence will be given the test substances in a similar way to that in which they are expected to be given to humans. As most medicines at taken orally the majority of animals will receive the test material directly by insertion of a flexible rubber catheter into the stomach via the mouth. Most animals are treated in this way once per day daily, although studies may occasionally require two or

three doses within 24 hours. For some test materials the oral route of administration may not be appropriate; for example, it may be broken down, not tolerated or not absorbed during the digestive process. In these situations, alternative administration routes such as sub-cutaneous or intravenous injection may be more appropriate. Animals may be manually restrained for “bolus” administrations lasting a few seconds or minutes, or they may be habituated to sit in specially designed chairs for longer intravenous administrations. For test materials which will be administered to humans intravenously over extended period of time due to their inherent toxicity or fast clearance from the body it may be more appropriate to surgically implant a permanent catheter (with appropriate anaesthesia and analgesia) to allow the animal free movement whilst being dosed in their home enclosure. Test materials intended to treat a localised condition may be administered to that body part or area for example to the skin. Blood and urine samples may be taken to measure the level of the test material or its metabolites to which the animal is exposed. These may also be analysed to detect any effects on body systems and organs for example liver or kidney function. Study animals are closely observed several times a day by highly trained technologists who monitor for any signs of discomfort. Other measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour such as reduced activity. Humane endpoints will be applied or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively fewer adverse effects. Many toxicological effects of the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia.

Application of the 3Rs

Replacement

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

Replacement

There are currently no scientific and legally acceptable evaluations of systemic toxicity which will satisfy regulatory requirements and provide sufficient safety data other than use of animals, though validated *in vitro* tests for specific organs are used wherever possible. As new *in vitro* methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace *in vivo* procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.

As a specially protected species, the non-human primate is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species with relevance to man, and that there is no other acceptable candidate species that is not a primate.

Reduction

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

Reduction

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

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

In general, toxicity studies are initiated in rodents before progressing into larger animals. This approach, combined with background literature searches and looking across at other study types, can lead to earlier decisions on whether or not to continue development of a particular test material, refinement of study designs and reduced use of primates.

The number of small molecule new chemical entity drugs (NCEs) developed using non-human primates has declined in recent years but is being offset by the proliferation of large molecule biotherapeutics (such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs)). The non-human primate is often the only relevant model for testing such materials due to the similarity with the human immune system but a reduction in the numbers of animals used per study is being achieved through knowledge gathering and refinement of study designs at an industry/regulatory level.

As most studies involve the post-mortem examination of tissues following treatment, opportunities for re-use are limited. Nevertheless, this licence does include the potential to re-use animals, in compliance with Home Office guidance and, where

possible, this is intended to help reduce the overall number of primates used at this facility.

Refinement

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

Refinement

Species choice and use of specific animal models is determined by the need to generate data that satisfies worldwide regulatory authorities. Where a choice of species is possible, care is taken to select the most biologically appropriate species, and the species which most closely relates to man.

Animal studies are only considered where there is a direct legislative or regulatory requirement and after review of all other available information to ensure that no alternative is feasible. A step-wise approach is taken, with higher risk studies, when little may be known about the test material, being performed early in a programme and using only small numbers of animals. As confidence in the data and the level of information grows, longer term studies in larger numbers of animals may become necessary and the design of these studies, whilst adhering to regulatory requirements, can be refined and tailored to obtain the most relevant and valid scientific information from the fewest animals and with the lowest level of adverse effects possible.

Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints.

Non-human primates are routinely group housed with environmental enrichment which encourages species specific behaviours without adversely impacting study outcomes.

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

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	Provision of Biologicals Materials
Key Words	Blood, tissues, service, biological materials
Expected duration of the project	5 year(s) 0 months

Purpose of 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 aim of this project is to provide blood and biological products (organs including brains, lungs and kidneys) from a range of animal species (mice, rats, rabbits, chickens, turkeys and dogs) to support in research, diagnostic and regulatory work. This can include ensuring new medicines are safe before release for use and checking the calibration of diagnostic devices used in treatment of both humans and animals.

To do this we produce fresh bloods, plasmas and serums after assessing individual customer requests looking at the purpose of the work to be carried out by the customer and the benefits it may provide.

By storing frozen plasma, serum and organs we can then ship them internationally to customers, giving a consistent timely service across different end users working on similar work. This allows researchers to purchase the specific product required as opposed to animals having to travel.

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

The benefits from conducting this work are dependent on the research projects of the customers ordering: a large proportion of products produced under the previous licence supported regulatory work, which is required under government guidelines and ensures the safety of drugs. Other products go to support calibration of assays and equipment to ensure results from work conducted are validated and that drugs produced are free of viral contamination. Regulations which guide the choice of species selected by customers to perform this testing include: Food and drug administration, world health organisation and the ICH (International council of harmonisation). The data from the assays performed will be used in regulatory submissions to the appropriate regulatory authorities or is used to help form a picture of the potential of putative new drugs to be more efficacious with a better side effect profile than existing therapies in a wide variety of human and animal health

indications. These data may not always be positive, and hence, some of these tests may prevent the further development of such entities, preventing the un-necessary use of animals in efficacy and regulatory testing prior to testing in human or animal clinical trials. The scientific benefits directly linked to this licence are dependent on the research projects of our customers; but under previous licences the tissues have contributed to the knowledge of disease processes in man, animals and food crops, understanding of the development of the immune system and its regulation, and extension of the knowledge of neurobiology and associated neurological disease. By offering the different products and species from one location we can give consistency across the samples, allowing direct comparisons in the end work performed, even if this is at different locations by different customers. We are able to reduce the movement of animals by shipping blood products to end users across Europe who would otherwise have to transport animals increased distances to produce products themselves. We also can take organs after the death of the animal (for example brains and lungs) and store these until needed. The customers we supply have a preference to outsource this work so they can benefit from the high levels of specific experience and knowledge we provide.

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

Over the 5 year term of the PPL we expect to use 160,000 mice, 60,000 rats, 6250 rabbits, 180 dogs and 1060 birds (chickens and turkeys). The majority of animals used will undergo non-recovery procedures (collection of blood or organs and tissues); i.e. carried out under terminal general anaesthesia. However, approximately 250 rabbits, 80 dogs and 160 birds will be used for the repetitive collection of small blood samples. Dogs, birds and a small proportion of the rabbits (5%) would have blood withdrawn from a superficial vein at approximately fortnightly intervals resulting in each animal having approximately 24 samples taken per year. Most rabbits would only have one blood sample taken.

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

The majority of the animals under this licence will only undergo procedures under non-recovery anaesthetic; these animals will only experience mild discomfort, due to being held still as the anaesthetic is introduced, such as experienced by human patients undergoing surgery. The only difference is that they will not awaken from the anaesthetic and will have death confirmed or be humanely killed at the end of the procedure. Anaesthetic will be introduced either by injection into the veins or by inhalation of gas. For rabbits and dogs sedation may be used before hand to reduce the need for longer periods of restraint. Chickens, Turkeys, Dogs and Rabbits will be kept as blood donors, and will have approximately 2 blood samples taken a month. These are small volumes that are under 10% of blood circulating volume and will be collected from superficial veins, similar to human blood donations. These animals will

only experience minimal restraint during the period of sampling and it is not expected to cause any adverse effects. Where appropriate topical local anaesthetic will be applied to the area before sampling.

Application of the 3Rs

Replacement

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

Replacement

The work performed, that uses products produced under this licence, is required under safety and regulatory guidelines; these include testing of drugs (both medical and veterinary) prior to their release to market, as well as ongoing calibration and quality checks of equipment and processes to ensure accuracy of the results that are published.

Currently there are no methods to generate animal specific blood products (cells, plasma, serum) without the use of animals.

Reduction

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

Reduction

By keeping donor colonies of dogs, rabbits and birds we are able to take small blood samples across a period of time from the same animals. This reduces the number of animals needed overall and provides a consistent product decreasing the need for retesting.

By collecting blood under non-recovery anaesthetic we are able to collect a higher volume of blood per animal compared to collection after the death of the animal. This reduces the numbers of animals used overall.

Our customer services department provides a central point to order blood and other biological products from, for a range of customers from small university groups to large contract research companies. This means we can collect different products (blood and organs, including brains, heart, liver and lungs) from the same animal and provide to multiple end users. This is frequently done with blood products from birds. All tissues are collected after death from all species.

Refinement

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

Refinement

The choice of animal is determined by the customers' and regulatory requirements, with species such as dogs only used where non-rodent species are required and it is the best model, due to either the research being related to dogs, or due to the similarities in systems that they share with humans.

The methods used for the collection of blood samples are based on guidelines of volume and frequency that will cause the least harm to the animals. The processing after collection is designed to get the highest quality and quantity of product so sample sizes can be kept as small as possible; we consider storage methods from across multiple fields including human transfusion services to ensure that we can maintain the quality of stored product.

Dogs and birds kept as donor animals are held in group living conditions, with dogs having access to both inside and outside areas as part of their housing; all donors are assessed individually and both their behavioural and physiological condition is monitored throughout the time they are a donor. Rabbits are only kept as repeat donors if the end use requires it, for example we work with a customer who uses fresh rabbit blood cells in human medical diagnostic work and before using the cells from any rabbit they have to validate it in line with ISO 15189 (International standards for medical laboratories). By keeping a donor rabbit they can complete the validation once and then only take small volumes thereafter.

For donor dogs, chickens and turkeys the jugular vein is used for collection of blood samples, this is a superficial, easily accessible, larger vein which means the time the animal is held for the procedure can be kept to a minimum and adverse effects, even for larger samples are rarely seen. For rabbits the marginal ear vein or artery will be used, with the vein mostly used as the samples taken are small and the vein has less chance of bruising. This is an accessible blood vessel that means the rabbit can be held in a natural position for the duration of the sample. For all the animals used as blood donors the sample time and experience of feeling is similar to a human blood donation or blood test performed medically.

Dogs will only be used when the product is required for work that cannot be done without using dog specific materials, currently there is a requirement under EU legislation that drugs are tested in a non-rodent species before release into the medical and veterinary markets. Dogs are used in cases where they have similarities with humans in how they deal with the drugs at a cellular level.

PROJECT 91.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 Toxic Hazards to Fish
Key Words	Ecotoxicology, Freshwater, Marine, Fish
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):

To generate quality-assured data on ecotoxicological properties of anthropogenic substances for clients as part of the evidence they present to regulatory authorities for assessment of the risks to the environment posed by those substances when they are produced, transported or used. The data required will normally include studies on effects on representatives of the major trophic levels in the environment including bacteria, plants and/or algae, invertebrates and fish.

These data, in addition to data for environmental fate and exposure, will be used to assess the level of environmental risk associated with those substances.

The studies with fish, which is the purpose of this licence, constitute an important and mandatory part of the risk assessment process.

A number of study types or protocols involving fish may be required depending on the level of production and fate and potential risk of the substance.

These include:

Acute toxicity to fish (96-hour LC50 test or limit test) e.g OECD203

Prolonged toxicity to fish over 14 days e.g. OECD204

Fish growth study over 28 days e.g. OECD215

Bioconcentration in fish e.g. OECD305

Effects on fish early life stages e.g. OECD210

What 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 assure environmental safety of substances by assessing the intrinsic ecotoxicological hazards of chemicals, products or effluents. The environmental risk will be assessed by a regulatory authority and where unacceptable risk to the

environment is identified the production and use of the substance will be restricted or banned. Appropriate labelling and risk phrases on packaging for products on general sale will be applied according to the results of environmental fate and ecotoxicology studies. This will allow appropriate precautions to be taken when using and disposing of those chemicals to minimize risk to the environment.

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

The species used will be one of the following:Rainbow trout (Oncorhynchus mykiss) Carp (Cyprinus carpio)Zebra fish (Brachydanio rerio)Turbot (Scophthalmus maximus) Sheepshead minnow (Cyprinodon variegates)

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

Mortality and other sublethal effects such as loss of equilibrium, abnormal swimming or unresponsive to external stimuli.Levels of severity will be moderate or severe.Fish will be killed by a Schedule 1 method at termination of a test (AC).

Application of the 3Rs

Replacement

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

Replacement

There is limited scope for replacement as the acute fish test is a regulatory requirement under many regulatory submissions

Reduction

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

Reduction

Reduction of numbers of fish used is achieved in three ways; 1) by always using the minimum number of fish allowed by the guideline to generate statistically valid data, 2) by carrying out limit tests (tests using a single concentration) using toxicity results previously generated from algae and invertebrate tests to define the test concentration (e.g. the use of the OECD Threshold Approach), 3) by using toxicity results previously generated e.g. using the results from a freshwater test for registration of an offshore chemical or the use of data generated from older studies that were not GLP-compliant.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 is achieved by performing careful visual inspections of the fish under test in order to minimise their suffering. For example moribund fish (fish showing no response to external stimuli and /or little respiratory activity) will be removed and humanely killed.

PROJECT 92.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	Bioelectronic Medicines
Key Words	Electrophysiology, Implantable Devices
Expected duration of the project	5 year(s) 0 months

Purpose of 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 purpose of this project is to understand how implantable devices and electrical signals can be used to regulate the nervous system to treat disease and organ dysfunction.

To do this we must first gain a better understanding of the anatomy and function of the nervous system, and how it exerts control of organ function. Secondly we must ascertain whether electrical regulation of the nervous system can be accomplished safely and effectively.

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

Currently medicines treat a wide range of ailments in billions of people. However, there are a multitude of side effects and treatment resistant populations. Although in general successful, current treatments are expensive, socially limiting, and in most cases only a treatment and not cure. The potential for Bioelectronic medicine is broad, as all organs are controlled by the nervous system. Through implantation of devices that regulate the nervous system, and in turn organs, one can potentially reverse organ dysfunction and disease states completely.

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

Pig (650 over 5 years) and Sheep (300 over 5 years)

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

The project has 3 stages. The first two phases will involve anaesthetised animals that are euthanised at the end of the study before they recover from the anaesthetic. Beyond the induction of anaesthesia, these animals will not experience any pain or suffering. In addition, these animals will give us the information we need to more

effectively and safely move to the next step of investigating treatment in animals with disease. There are no expected adverse effects with implantation and treatment as this is a terminal procedure. The final phase will investigate the safety and efficacy of Bioelectronic medicines and therapies in conscious and freely moving animals. Animals will undergo surgical implantation of the devices. The stability/reliability of the device in a conscious animal can then be investigated. This will be achieved using imaging technology e.g. MRI or CT scanning, to see how the resting body responds to the device, then the biological response following activation of the device will be studied. Looking both at the normal resting response and when the body is exposed to a minor inflammatory insult. At the end of the study, all animals will be killed by a schedule 1 method or will be perfused to allow tissue to be analysed.

Application of the 3Rs

Replacement

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

Replacement

A limited amount of testing has been done without using animals to give confidence nerve stimulation may treat disease. The science cannot be advanced further without using animals. Only a whole body system biology approach will give conclusive evidence and understanding that manipulation of the nervous system can be an effective treatment of disease.

A computer model does not yet exist to test nerve stimulation as a treatment of disease.

Reduction

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

Reduction

Pilot studies in small numbers of animals will be used to develop optimal methods**,** assess feasibility and outcome measures, and will define go/no go criteria for further studies. Statistical advice will be sought for study design to ensure adequate animal numbers are used. The number of animals used in the studies will not exceed the study size required by statistics to ensure reliable significance and result confidence.

In many cases chronic (recovery) animals will provide their own internal controls (e.g. stim on versus stim off), and multiple repeated doses (of mediators and stimulations) reduces group sizes further.

Refinement

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

Refinement

Pigs and sheep will be used for all experiments because they are the most appropriate species to determine efficacy and safety with respect to the devices tested. Their neuroanatomy and physiology is very similar to that in humans.

We will work with manufacturers and academic experts to ensure a continued refinement approach is adopted for all implantable devices, electrodes and leads. We will work toward fully implantable devices as advancement to external wires and head caps.

The systemic inflammation model is well-characterised and used experimentally in clinical and non-clinical studies, to determine the efficacy of medical treatments. Many diseases have an inflammatory component to them. Developing a chronic low-level inflammatory model allows investigation of a range of diseases and the potential benefits of Bioelectronic medicines and therapies in these diseases.

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	Mechanisms of neurodegeneration.
Key Words	Motor Neurone Disease, Aging
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Motor Neurone Disease can be caused by genetic mutations – mistakes in the internal code that shapes all living things. The aim of this project is to test if one particular mutation found in humans can cause a similar disease in rats. If so, it may be possible to use these animals in experiments to better understand the basic process of Motor Neurone Disease and determine if there are mechanisms in common with other debilitating progressive motor disorders. For instance, it is not understood why the disease mainly affects just the ability to use your muscles; nor why it occurs more in older people.

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

There are no effective treatments for this insidious disease, and it is invariably fatal. If we understood all the changes that took place in the body before the disease took hold and as it progressed, it might be possible to help develop new treatments or cures. This information is difficult or impossible to get from human patients. The short-term benefits of this work is new scientific information on the pathological mechanisms of neurodegeneration. These results will benefit researchers in fields such as neuroscience, cell biology, physiology and gerontology. In the mid-term, a detailed molecular characterisation of the pathological mechanisms of ALS may identify prognostic biomarkers that could form the basis of new diagnostic reagents and also highlight new potential drug targets. These may be exploited by the pharmaceutical industry, to produce new diagnostic kits and initiating new drug screens. These will have mid-term benefits to clinicians and patients by improving disease diagnosis and individual prognosis. In the longer term, the results from this work will stimulate rational drug design and provide a screening platform to quantify therapeutic potential. There are no treatments for ALS/MND or other progressive motor disorders so this would be a major benefit to patients, clinicians and the NHS. If the, as hoped, there are mechanistic links between ALS and other

neurodegenerative conditions then this longer term benefit could be even more significant and could influence public health policy developments.

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

Approximately 1500 rats and 500 mice across a range of ages will be used in the study, which will last 5 years.

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

The human diseases we wish to study are relatively slowly progressing and starts in latter life. Human patients do not appear to suffer any pain from the disease. As the experimental animals age, we anticipate mild to moderate effects of the mutation where they find simple tasks such as walking and gripping objects more difficult. Similarly, as they age, the general physical fitness of the animals will diminish. Animals would be humanely killed before their ability to eat or drink was significantly impaired. During the study animals will be tested on behavioural tests such as their ability to walk and balance. As the animals will be accustomed to being handled these tests should not result in significant adverse effects. Sampling of blood will require a hypodermic needle, which represents a mild stress for the animal. Sampling of CSF will require the animals to be sedated for approximately 5 mins, and a small incision to be made in the skin at the back of neck, just bellow the head. The incision will be closed and drugs will be provided post operatively to alleviate the discomfort from this procedure.

Application of the 3Rs

Replacement

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

Replacement

Humans, like all animals are made from many different tissues such as muscle, fat skin and bone. These tissues themselves are made from many more different types of building blocks or cells. The cells affected in ALS control the voluntary muscles in the body. The way these control cells, or neurons, interact with muscles is quite different between animals with a central nervous system compared to other animals. We know very little about how this system collapses in ALS and other motor disorders, so at this point it is necessary to study a vertebrate rather than the related but distinctly different systems working in non-protected animal alternative.

Reduction

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

Reduction

Rats are strong and large enough that it is possible to monitor how a disease may start and progress in an individual animal that you can monitor closely over the course of its life. This makes it possible to design more reliable experiments that use less animals to learn reliable information as does using previously characterised mouse models with known timelines of disease progression.

Refinement

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

Refinement

To maintain a healthy body different tissues communicate with each other by sending small signals such as hormones through the blood and other biological fluids. These signals change in response to disease, and knowing the details of these changes can help to understand how the disease is causing its effect. Changes in the constituents of the fluid surrounding the brain have been found in motor neuron disease patients. Rats are the smallest experimental animals from which it is possible to sample this fluid without killing the animal. This allows multiple samples to be collected at times before and after the disease has taken hold. Making it much easier to determine which changes are important for the disease. Motor neuron disease is a progressive fatal condition. For the purpose of this study is it is not necessary for the animals to suffer the full extent of the condition leading to respiratory failure. Once clear effects are evident animals may be humanely killed. In regards to the mouse models being used in this project they possess quantifiable features and yet the disease does not affect the general cage life of the mouse. Excellent care for the animals will be provided, specialised equipment used for all the behaviour and surgical procedures and advice from vets sought immediately for any welfare issue.

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	Regulatory and Investigative Toxicology
Key Words	Medicine, safety, toxicity, toxicology
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):

Understanding how safe a potential new medicine is before it is given to humans is an essential part of medicine development. Although some information on safety can be obtained without using animals, some tests must be carried out using animals to better understand how these medicines might affect the human body. The objectives of this project are as follows:

- Identify the right potential medicines for development which are safe to give to people and are most likely to be able to treat the target illness.
- Identify any possible safety concerns and understand how these might arise and whether they could cause harm to patients or human volunteers in clinical trials.

Where possible, improve and refine our tests using animals to provide more relevant information to humans whilst minimising the use and impact on 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)?

Achievement of the objectives will support development of safe, new medicines to improve health and quality of life of patients by generating high quality, regulatory acceptable data and will help to remove unsuitable candidates from the development pipeline at an early stage, thus minimising the use of animals and resources. The benefits gained by studies performed depends on the study purpose and type and include: Making decisions on whether potential new medicines are suitable for development as early as possible in the process to avoid wasting animals and money. We use the information generated during early studies to help to understand what we need to measure on future studies. To help us to decide the doses and endpoints to measure on early human studies to minimise the risk to human

volunteers. To allow regulatory authorities to decide whether to allow the potential new medicine to be given to humans.

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

Over the 5 year period of the licence we expect to use approximately: 5500 rats 2000 mice 575 dogs 200 hamsters 200 rabbits 575 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?

Evaluation of safety to assess potential risk to humans requires the use high doses of a potential new medicine which can cause some adverse effects in animals. Adverse effects in animals are usually of mild or moderate severity. The most common effects will be loss of body weight or reduced weight gain, reduction in the amount of food the animals are eating and clinical signs such as reduced activity, postural changes, changes in faeces and in some species, vomiting. No animals will intentionally experience severe adverse effects but because early studies may be the first time that a potential new medicine is given to animals, effects may occasionally be more severe than expected. Animals are monitored closely and animals which show signs toward the limit of moderate severity are humanely killed. Most safety studies require examination of blood and tissues from animals to see whether the potential new medicine has caused any damage to organs or tissues, so the majority of animals are humanely killed at the end of a study and subjected to post mortem examination. Samples of tissues are then examined microscopically. On some early studies animals are not required to be killed and provided they have not shown adverse effects they may be used again in a subsequent study.

Application of the 3Rs

Replacement

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

Replacement

Whilst alternatives to in vivo animal models are being developed and are used where possible, there are currently no reliable models available for broad, primary toxicity screening and none that are acceptable to drug regulatory authorities, it is therefore necessary to screen for toxicity in animal models

Reduction

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

Reduction

For safety studies, guidelines require 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. We have a track record of designing studies that provide us with the information we need to make decisions on the safety of our test substances (leading to continuing, or stopping, development).

For preliminary studies, small groups are acceptable because of the endpoints used give a clear answer. Where group sizes are sufficient data from definitive toxicity studies are analysed statistically. Statistical input is sought, where necessary, to strengthen the overall scientific quality and relevance of the studies to be performed, with sample size calculations performed for specific studies to determine the group size. Group sizes in dog and pig studies are usually insufficient for valid statistical analysis. However, because toxicity is the result of changes in multiple parameters, assessment is made by examination of data from each animal and by correlation of in-life and post mortem findings within an individual.

In order to minimise animal use, we will consider using animals on more than one stu dy when this can be justified on welfare and scientific grounds.

Refinement

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

Refinement

Regulatory guidelines state that toxicology studies in support of administration to man should be conducted in one rodent and one non-rodent species. Generally the rat is the rodent species of choice unless it is known to be an inappropriate model for man for the compound. The non-rodent species will be that likely to give the most satisfactory, reliable and regulatory acceptable results.

The pig will be used as the preferred non-rodent species in this licence, unless it is shown to be unsuitable based on scientific information available, when the dog will be used. Where evaluation of all information indicates that both the pig and dog are a suitable non rodent species, the pig will be chosen.

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	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 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	Neuromuscular and neurodegenerative disorders: pathogenesis and therapy
Key Words	Neuromuscular Diseases, Neurodegenerative Diseases, Pathogenesis, 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;
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 project is focused on improving our understanding of the mechanisms that play a role in neurodegenerative diseases that affect the nervous system in order to help develop new therapeutic strategies for these debilitating and often fatal disorders. There is currently no cure or disease modifying therapy for these disorders.

The project is particularly focused on disorders that affect the neuromuscular system including Motor Neuron Diseases, peripheral neuropathies and muscle disorders. However, recent clinical and genetic findings have revealed that some of these diseases are part of a disease spectrum that surprisingly, also includes forms of dementia, suggesting that the results of this project may have relevance for our understanding and possibly treatment of a wide range of neurological disorders. Although significant advances have been made in the past 10 years into our understanding of the genetic causes of many of these diseases, we still have a poor understanding of the underlying mechanisms that cause disease, and as a result, these diseases remain untreatable.

Therefore, this project has 2 key objectives: i) to advance our understanding of the mechanisms that play a role in these disorders, in order to ii) identify targets for therapeutic intervention, and to develop and test novel therapeutic strategies that will be effective in modifying disease progression in patients suffering from these diseases.

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

The results of this project will improve our understanding of the mechanisms that underlie several neuromuscular and neurodegenerative diseases, thereby advancing the current state of knowledge in these fields. A better understanding of the underlying pathological mechanisms of these diseases will help researchers to identify targets that can be treated with drugs or alternative therapeutic strategies. This project will also test the most promising of these novel therapeutic strategies in

models of neuromuscular and neurodegenerative diseases to establish their potential for therapeutic benefit in patients suffering from these diseases. This will include advancing the finding from this project through to human clinical trials.

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

This project will use mice and rats. Many of the mice that will be examined will be genetically modified to model aspects of the human diseases under study. We will use a maximum of 40,000 mice and 11,500 rats over the 5 year duration of this project.

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

The majority of procedures in the project will be mild or moderate and we expect there to be few adverse effects. this will be mainly limited to mild discomfort following surgery. In such cases, the animals will be given pain relief. As the animals will model neuromuscular and neurodegenerative diseases, deficits such as muscle weakness which are associated with neuromuscular diseases, usually restricted to hindlimbs, may be experienced. These deficits may include dragging of one paw, gait abnormalities of limb muscle weakness. In such cases, the animals will be provided with easy access to food and water, for example by providing a soggy diet within the home cage. The overall expected level of severity for the procedures described in the Project is likely to be Moderate. At the end of these experiments, all animals will be humanely culled.

Application of the 3Rs

Replacement

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

Replacement

Neuromuscular and neurodegenerative diseases involve changes in complex interactions between different types of neurons and several other cell types located within the central nervous system, in the brain and spinal cord. In addition, cells outside the central nervous system, in the periphery, such as nerves and muscles are also involved. It is impossible to fully model these hugely complex interactions in culture.

Furthermore, these diseases tend to manifest on a background of aging, appearing during middle to late stages of life, which is also difficult to model in culture.

Several models of neuromuscular and neurodegenerative diseases have been developed using non-protected species, including fruit flies and nematodes.

However, although these models may be of use for investigating some aspects of human disease, such as how do different genes interact, they have significant limitations for the objectives of this Project. For example, in fish models of ALS, motor neurons do not degenerate, making this a difficult model to use in the development of drugs that will prevent motor neuron death.

However, where possible, we will use cell culture models to model aspects of the complex interactions that are involved in neuromuscullar and neurodegenerative diseases, for example the interactions that occur between specific cell types (eg muscles and nerves). Indeed, as part of this project, a novel cell culture model of the neuromuscular junction will be developed, in a study funded by the Motor Neuron Disease Association. This will involve the use of stem cells -derived muscles, motor neurons and glial cells, which will model the key aspects of the neuromuscular junction.

Moreover, we are increasingly making use of human derived stem cell models of human disease, thereby avoiding the need for animal experiments. This area of our research is likely to become a major focus of our work over the coming years. Finally, as our overarching goal is to develop disease modifying therapies for neuromuscular and neurodegenerative diseases, we routinely compare our findings to available human data as well as our own studies on post-mortem tissue from affected patients.

Reduction

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

Reduction

We will design our experiments according to the NC3Rs ARRIVE Guidelines.

The experiments described in this Project have been designed in consultation with statistical advisors. In all aspects of this project measures will be undertaken to minimize the number of animals use wherever possible. For example, wherever possible, tissues from individual animals will be shared between group members as well as with other collaborative groups. In this way, different tissues from an individual animal can be used to support experiments undertaken by several researchers. This approach ensures that we keep our animal use to a minimum.

Depending on the experiment, control groups will include wildtype and/ or untreated littermates; for some experiments groups must be gender specific as disease progression will differ depending on gender

For most studies, in the first instance we will undertake small pilot studies which typically consist of experimental groups of smaller numbers than the definitive study, as it may be possible to obtain an indication of efficacy. However, due to the known

variability and gender effects of many transgenic models, the larger numbers indicated above are required to undertake statistically robust analysis to demonstrate efficacy.

Wherever possible, we will make use of stored tissue available in existing biobanks.

Refinement

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

Refinement

Most of the work in this project will be undertaken in mice. The mouse is unique in that it is relatively easy to create mutations in any gene of interest and therefore dissect the mechanisms involved in specific human diseases, and to generate models of disease in which to test new therapeutic approaches. In addition, since the ultimate aim of this research program is to relate the findings obtained in animals back to human disease; it is essential to work with a mammalian system.

Furthermore, we also have a comprehensive understanding of the mouse nervous system and most of our previous work has been gathered from mice. There is also a large body of background data on the normal functions and behaviour of the neuromuscular system in mice and rats, which therefore significantly reduces the total numbers of animals that need to be used in this project

Wherever possible we use in vitro models, eg-cultures of primary motor neurons and glia from genetically modified mice modelling disease to examine disease mechanisms and test potential therapeutics, prior to validation in animals. We also use models with as mild a disease as possible to test specific questions

We will minimise harms during surgery by undertaking the mildest injury required to meet the scientific objectives. In all cases, the harm will be the minimum and supportive therapy will be employed to minimise the impact of the intervention, eg provision of a soggy diet in the base of the home cage following surgery or when an animal's mobility becomes reduced as a result of the disease.

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	Effects of chronic intermittent hypoxia on carotid body and cardiac function and the exacerbating effect of poor glucose control and obesity
Key Words	Breathing, Heart, Blood glucose, Hypoxia
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

To investigate the effects of repeated low oxygen levels seen in obstructive sleep apnoea (OSA) patients on oxygen and carbon dioxide sensors in the neck and heart function and how this affects control of blood glucose levels.

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

OSA is a disease in which patients periodically stop breathing during sleep. It is linked to obesity and type 2 diabetes with multiple adverse health outcomes in common. High blood pressure (hypertension) is strongly associated and might be caused by changes in the function of the carotid bodies (sensors that respond to changes in oxygen and carbon dioxide levels in the blood as well as other substances). Chronic changes in carotid body function may cause higher background sympathetic nerve activity resulting in the development of heart disease. Additionally, poor control of glucose (seen in diabetes) is linked to damaging oxidative stress that also may contribute to the altered function of the heart or carotid bodies. This licence will help unravel the changes seen with repeated periods of low oxygen (when breathing stops in OSA) on the control and function of the cardiovascular and respiratory systems.

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

1000 Mice/rats will be used over the course of the next 5 years

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

Exposure to repeated short periods of low oxygen (intermittent hypoxia) induces long-term changes to the cardiovascular and respiratory systems, however, we have never seen any observable adverse effects and the animals exhibit normal behaviour. Measurements made on awake animals are short-lasting and generally

non-invasive (eg nothing greater than a needle prick). Tissue collection for in vitro experiments or measurements made on anaesthetised animals are all carried out under terminal anaesthesia and so the animal will not suffer any pain or distress.

Application of the 3Rs

Replacement

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

Replacement

To determine how the cardiovascular and respiratory systems work together and the nature of reflex responses requires a whole animal that has sensory inputs (carotid bodies) and relevant outputs (heart function or blood glucose levels) to understand control mechanisms.

We will continue to review the published literature so that we will be aware of any developments in this area of research where *in vitro* techniques could replace animal use. We will use the

NC3R's systematic review tool to help with searches

www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf

Reduction

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

Reduction

Use of isolated tissues will be made where possible to investigate mechanistic questions and this reductionist approach will generally allow smaller numbers of animals due to less biological variability. In whole animal experiments, collection of as much data as possible from each animal will allow greater interpretation of the data and reduce the need to carry out multiple separate experiments to answer the scientific question. We will use the NC3R's EDA to help design our experiments and we will publish in peer reviewed journals that support the ARRIVE guidelines to share out findings with the wider scientific community.

Refinement

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

Refinement

The integrative physiological nature of the research in this licence requires the use of whole animals, however, rodents have been selected as the lowest order of animals displaying the complexity required to interpret the results in the context of human physiology. Where the scientific questions can be answered without using live animals (eg in isolated tissue) this will be done. Where whole animals are used, these will usually be done under terminal anaesthesia to remove any animal suffering whilst allowing the maximal amount of data to be generated. In experiments where conscious animals are used (eg measuring ECG or breathing), steps are taken to minimise the stress on the animal. These include familiarising the animal to the chambers prior to carrying out experiments, transplanting some home cage bedding to the chambers to increase the familiar scent of the chambers, keeping the measurement duration as short as possible, keeping animals in group housing except when experiments require short periods of single housing for measurements. All procedures are kept under review and new refinements published will be incorporated whenever possible.

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	DNA repair in development and tissue homeostasis
Key Words	stem cells, ageing, neurodegeneration, DNA 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;
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 has 3 aims:

1) to understand the DNA repair mechanism underlying Fanconi Anaemia (FA), a human genetic illness (currently incurable); 2) to uncover sources of natural factors that we produce, that damage our DNA; 3) to understand how these molecules alter our genomes in fundamental ways.

FA patients stop producing blood and also have a very high predisposition to cancers and a cure is yet to be discovered. Over the last ten years, our research with cells and animals (mouse) has led to the discovery of a natural source of DNA damage that could explain why FA patients stop producing blood and develop cancer. These natural substances are known as aldehydes which are produced in our body and they can damage our DNA. First, cells are equipped with factors that repair this damaged DNA, in order to keep the genetic information intact in our cells. Fanconi Anemia patients are unable to repair some forms of DNA damage. It is the accumulation of damaged DNA that ultimately leads to the loss of blood and the onset of cancer. Second, other proteins are tasked with the “mopping up” of aldehydes. It is the joint action of DNA repair and aldehyde detoxification that allows our bodies to remain cancer-free and produce blood for several decades.

We have shown that the blood stem cells (that reside in the bone marrow cells and that produce blood throughout life) are easily damaged by these aldehydes. Thus, blood stem cells need both efficient DNA repair and aldehyde clearance to remain healthy and continue to make fresh blood throughout life. We want to know if other organs also protect their DNA in the same way: the skin and the brain are two major tissues for which we have preliminary data supporting this hypothesis.

We think that, as we get older, aldehydes (or other chemicals) produced in our bodies accumulate and might be a contributing factor to ageing. It is also important

to mention that aldehydes present in our environment (alcohol, food, air pollution etc) could play a role. We will develop mouse models to study this phenomenon.

Finally, we will investigate how aldehydes can impact the immune system (the cells that help combat infection by killing germs and producing antibodies). Indeed, a mutation in the detoxifying gene *ALDH2* is very common among Japanese and South East Asian individuals (over 400 million people). Paradoxically, preliminary work suggest that having a mutation in *ALDH2* helps to fight an infection. We will test this hypothesis in mice by mimicking human infections.

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

It is clear that being able to fix damaged DNA is crucial: patients of FA develop anaemia and have a 1000-fold risk of cancer. By understanding how DNA is repaired by the FA proteins in a small mammal like the mouse, we will be in a better position to understand cancer and loss of blood production. This will benefit research focussing on other genetic diseases where DNA repair is also faulty. Lastly, the identification of new sources of DNA damaging agents (environmental, dietary or produced in our bodies) could have public health implications.

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

We use genetically altered mice in our research. We anticipate that we will need ~100,000 animals over the next five years. This estimate is based on our usage in the past decade.

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

All the mice generated throughout this programme of work are ultimately killed. Because we breed mice to obtain mutants, a large number of mice do not carry the mutation and are of no use (so-called wrong genotype). These animals are killed as early as possible. The mice that are useful to us are used in experiments and are killed at the end of the experiments. We anticipate that some of our mice may suffer developmental defects, loss of blood production (anaemia) and cancer. This will happen while breeding animals to produce mutants, and by letting these mice age. In the last ten years we have developed accurate methods to measure signs of disease in mice and from these signs to predict outcome. This allows us to kill mice before their welfare is compromised, allowing us to gain maximum information from every mutant mouse we generate. A first example of experiments we perform is bone marrow transplant, similar to what is done in humans. Here, “donor” mice are killed and their bone marrow is harvested. “Recipient” mice are exposed to radiation to destroy their bone marrow. Cells from the donors are injected in the recipients to allow them to continue making the blood and survive the irradiation. The blood of

recipients is analysed every 4 weeks for 4 months to assess the capacity of donor bone marrow to produce blood. After 4 months, the recipients are killed. Adverse effects: irradiation often leads to transient weight loss. If the donor bone marrow is not rejected, the recipient makes a full recovery from the irradiation. Rarely, the transplant is not successful and 10-14 days after the irradiation the mice show signs of radiation sickness and are killed promptly to avoid suffering. A second example of experiments involves giving alcohol to the mice. This is because inside the body, the alcohol is converted into an aldehyde that will cause DNA damage. By treating mice with alcohol, we can study how DNA is protected and repaired. Usually, the alcohol is given by replacing water with a mixture of alcohol and fruit juice to make it palatable for the mice. Another way to administer the alcohol is to inject a dose into the abdomen, using a syringe and a very fine needle. In some instances, we will inject alcohol in pregnant female mice. This is to study the effect of alcohol and aldehyde during pregnancy. In this type of experiment, the mice are injected at a time equivalent to the first trimester of human pregnancy. The pregnancies are terminated a week later or in some cases, they might be carried to term. Pups that are not healthy will be humanely killed. Adverse effects: the injection procedure results in mild and transient discomfort. When treated with alcohol, the mice are unsteady on their feet, hunched and their fur bristles. These symptoms cause mild and transient discomfort and within 30 minutes to 1 hour, the animals have made a full recovery. Finally, we want to understand how the immune system might be a source of aldehydes. To this end, we will perform experiments where mice will be given infections agents to trigger an immune response. Since we do not know which pathogenic agent(s) might lead to the production of aldehydes, we will need to test models of viral and bacterial infections. Besides infection, inflammation is also a possible source of aldehydes. We will therefore perform experiments where mice will be given drugs that can induce an inflammatory reaction. Adverse effects: the effects of infection and inflammation are variable and depend on the nature of the infectious/inflammatory agent. Generally, weight loss and subdued behaviour are to be expected. Mice will be closely monitored and humanely killed if the severity limit is to be reached, as defined by the humane endpoints for each model of infection or inflammation.

Application of the 3Rs

Replacement

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

Replacement

The main reason for the use of this animal model is that we intend to investigate how such DNA repair pathways enable normal development, help mammals to deal with

common toxins present in our environment and diet, preserve stem cells and finally protect against DNA changes that lead to cancer.

It is really only possible to study the development of embryos/fetuses (pregnancy) in the context of a whole animal. Furthermore, to study stem cell biology and cancer in a way that can be compared to the human situation, it is necessary to use animal models that are mammals like us humans. For these reasons, the mouse is the best model at our disposal.

Reduction

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

Reduction

When we plan an experiment, we make sure we use the least number of mice that allows us to make a valid observation (confirmed by statistical calculations).

We carry out small pilot studies to refine our experiments.

All of our Fanconi mice are sterile and born at low ratios compared to what is expected. This necessitates large breeding programmes with many mice with the incorrect mutation being generated (wrong genotype). Over the past 3 years we have devised strategies that allow us to greatly reduce the number of mice that we have to breed to get animals with the useful genetic modifications.

Finally, we will cryopreserve our strains when they are not needed so that we can thaw out embryos and produce new mice when needed. This technique means that we do not need to maintain many mice alive.

Refinement

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

Refinement

We use the mouse as a model because it is a mammal and its genome (DNA sequence present in each mouse cell) has been decoded and is very similar to the human genome.

The development of a mouse and the function of their stem cells is also comparable to that of humans.

Our mouse models are the best available models to study the effects of DNA damage caused by aldehydes. Furthermore, these are the only mouse models that recapitulate the key features of Fanconi Anaemia as seen in humans, which makes

them relevant models. For the first time these models enable us to study the physiological role of the Fanconi DNA repair pathway.

As mentioned above we have invested in genetic modifications that allow us to control where/when the mutation has an effect. This allows us to further refine our models.

In these mice, the DNA repair pathway can be “switched off” in response to an inducing agent (e.g. tamoxifen). This allows us to generate mutant mice when we need them, further reducing the possibility that the mice will develop disease when not in an experiment.

As outlined above, our mutant mice that may develop signs of disease will be identified very early (14 – 21 days old). These mice will be monitored carefully by daily inspection for signs of disease and also through weekly weighing. Mice that develop signs of disease will be killed and analysed promptly to avoid unnecessary suffering.

PROJECT 99.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 Cardiovascular Remodelling
Key Words	Cardiovascular disease, Lesion Formation, Aortic Stenosis, Angiogenesis, Risk Factors
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Diseases involving remodelling of the heart and blood vessels (such as heart attack and stroke) are the biggest single cause of death in developed (and increasingly in developing) countries. The purpose of this project is to improve our understanding of the processes that regulate remodelling of the heart and blood vessels, to identify new therapeutic targets, and test promising new treatments.

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

This project will clarify the processes that block arteries, preventing blood reaching sensitive organs (e.g. heart, brain, limbs). It will also investigate control of new blood vessel formation, which is important for supplying oxygen and nutrients, during tissue growth and repair. Identification of key signalling factors, will highlight new targets for medical. These factors will be modified using existing and novel medicines, and new methods of administration, to assess their potential for treatment of clinically-important conditions (heart attack, stroke, heart failure, gangrene, cancers, respiratory failure).

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

A 5 year programme will predominantly use mice, as relevant models of the conditions under investigation that can be investigated using genetic modification and the latest imaging techniques. Rats are more appropriate for some investigations provide and, in limited cases, rabbits may provide improved representation of disease/ response to treatment in humans. Specific investigations will use zebrafish, which provide relevant and accessible models of inflammation and angiogenesis. The predicted numbers are: Mice: 13,750, Rats: 3250.

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

Regulation of cardiovascular remodelling will be assessed in established rodent and fish models of injury and repair; making use of genetic modification to identify mechanisms contributing to remodelling. High fat diets and/ or surgical intervention will induce changes in cardiovascular structure and function. Surgical procedures involve directly damaging an artery, removing/ blocking an artery to reduce oxygen supply to tissues (including the heart), or implantation of devices/ compounds to stimulate growth of new blood vessels. Adverse effects will vary for different protocols. Dietary manipulation is expected to produce few overt effects (eg. weight gain, mild metabolic dysregulation). Surgical manipulations may be associated with temporary pain following surgery, mild weight loss, and transient irritation at the site of skin incisions. There is a small possibility of infection but this is reduced by aseptic technique. Surgical induction of lesions can cause temporary lameness but is otherwise well tolerated and not associated with death or disability. Similar effects (lameness, impaired exercise tolerance) can occur with hindlimb ischaemia (femoral artery removal). Gangrene and autoamputation reported in some strains will be avoided by analysing the response to lower levels of ischemia in pilot studies. Pulmonary arterial hypertension is well tolerated in rodents. Coronary artery ligation and aortic stenosis are associated with peri-operative and post-operative (occasionally sudden) death in up to 20% of animals but this approach is necessary for investigating causes and treatment of heart attack. The impact of these techniques will be managed by good surgical technique and post-operative care. Appropriate use of anaesthetic and analgesics (pain killers) is important in reducing the impact on animals. Potential adverse effects of procedures that do have a high impact on animals are well understood and will be monitored and treated accordingly to reduce suffering. Such procedures will only be used for translational proof-of-concept experiments.

Application of the 3Rs

Replacement

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

Replacement

Cardiovascular remodelling in humans is a slow (decades long), symptomless process. This makes it difficult to detect onset of disease, and to predict (often life-threatening) cardiovascular events, and to determine the role and significance of initiating factors.

Animals provide essential models of components of the remodelling process, with changes developing over much shorter timescales. They also provide tissue samples at planned intervals during disease progression. Mice will be the main species used, primarily because this enables investigation of pathways of interest using genetic modification. Transgenic manipulation will be used to refine experiments (e.g. by

deleting a gene of interest) or by producing animals which more closely mimic aspects of human disease.

Replacement will be achieved by the use of *ex vivo* and *in vitro* techniques, often using immortalised cells lines. In many cases, these experiments use human cells and tissue (e.g. arterial ring) culture instead of animals. Furthermore, collaboration with colleagues in physics has advanced mathematical modelling of blood flow shear stresses, to improve understanding of the influence of haemodynamics on lesion formation and vascular network development. Generation of complex, scaffold-free bioartificial blood vessels *ex vivo*, using cultured human cells, will help clarify the mechanisms that regulate arterial formation, stabilisation and lesion development.

The translational potential of this work is strengthened by comparing results with data obtained from cellular, molecular and functional analyses of tissues samples (and cultured cells) from patients and healthy controls. In addition, many of the imaging techniques (eg. magnetic resonance, ultrasound) used for *in vivo* analysis are directly comparable to modalities used in patients.

Reduction

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

Reduction

Non-invasive *in vivo* imaging techniques are used to reduce the number of animals required. Furthermore, *in vivo* analyses are extended by the use of complementary techniques (histology/ immunohistochemistry, molecular biology, cell isolation and culture, tissue culture, and functional analysis) for analysing tissue samples *post mortem*. Similarly, relevant *ex vivo* models (e.g. cell culture) are used to model processes that occur *in vivo*. The advances in trascriptomics and bioinformatics have greatly increased the information obtained from experiments and allow powerful, detailed analyses of signalling pathways, improving target identification and assessment of new interventions.

Refinement

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

Refinement

The welfare of animals used in this work is extremely important. They are kept in state-of-the art facilities using best husbandry practices, with regular checks by suitably qualified staff and recourse to veterinary advice. Animals are provided with environmental enrichment (nests, nesting materials, tubes, chew sticks), as

appropriate. Surgery is performed by well-trained staff using good aseptic technique and appropriate anaesthesia and pain relief, to minimise distress and discomfort. Animals are individually monitored to assess the actual severity of the procedures they experience; they are killed humanely at the point of the experiment that allows the most meaningful analysis of outcomes. Any adverse effects of procedures that exceed expected limits will be referred to the named veterinary surgeon and, if necessary, affected animals will be euthanized.

PROJECT 100. 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 welfare implications of low atmospheric pressure stunning in pigs
Key Words	Pig, Welfare, Slaughter, Stunning, Decompression
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Approximately 167,000 pigs are slaughtered for food every week in the UK. Meeting this demand requires a humane method that can cope with high throughput. The law requires that animals are stunned (rendered unconscious) before slaughter. Pigs are commonly stunned using carbon dioxide gas as it is reliable, allows pigs to be killed in groups and enables high-throughput. However, this practice gives rise to important welfare concerns because it induces breathlessness and above certain concentrations, pain. Achieving unconsciousness with lack of oxygen (hypoxia) is more humane, but achieving this by exposure to other specific gases has been explored and is technically problematic and too expensive for commercial use. Low Atmospheric Pressure Stunning (LAPS) is a possible alternative, whereby animals lose consciousness and die by being placed in a chamber where gradually reducing air pressure reduces oxygen availability. This is called hypobaric hypoxia and is equivalent to rapidly ascending to high altitude, which is reported as not unpleasant or painful to humans experiencing similar rates of decompression.The aim of this project is to systematically evaluate, for the first time, the potential of hypobaric hypoxia (low atmospheric pressure stunning (LAPS)) as a humane method of commercial stunning for pigs. LAPS has already been validated as a humane method to kill chickens and therefore there is already robust equipment commercially available.

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

There is an urgent need to find alternative humane methods of slaughter for pigs and LAPS is the most promising option. This project will generate detailed data on the behavioural, physiological and pathological responses of pigs to LAPS, including negative effects on welfare. We will also conduct a parallel detailed evaluation of CO2 stunning to determine whether LAPS is more humane than current methods. The results of the research will directly inform policy makers in the UK government; if LAPS is found to be a welfare friendly approach to stunning for pigs then an

application will be made to the EU commission to add it to the permitted approaches in Regulation (EC) no. 1099/2009 On the Protection of Animals at the Time of Killing. If successful, commercial application of LAPS has the potential to improve the welfare of 8.6 million pigs in the UK each year, and many millions more globally

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

We expect to use 300 pigs over the five year course of the project.

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

The project has moderate severity because (1) we will implant pigs with electrodes to measure their brain activity (essential to accurately assess time to loss of consciousness) and (2) we will expose pigs to carbon dioxide gas which is known to be extremely unpleasant (despite being routinely used commercially). Given that the purpose of the work is to assess the welfare impact of non-recovery stunning methods, the animals will not survive the procedures. To understand the welfare impact of LAPS, pigs will be exposed to LAPS and carbon dioxide and their behavioural and physiological responses compared. We will use measurements of brain activity (EEG) to determine when the pigs become unconscious, so that we can determine what welfare harms occur up to that point. Some pigs will just be placed in the LAPS chamber for the same amount of time as a LAPS cycle then removed, after which they will be culled by one of the humane methods used for research animals. This is to determine the effect of being placed in a novel chamber. We will train pigs to indicate that they want to leave a situation, and then measure these responses during LAPS or exposure to carbon dioxide.

Application of the 3Rs

Replacement

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

Replacement

There are no alternatives to the use of pigs for this work because intact animals are required for the study of the specific welfare effects of each method of killing on this species. We have fully considered alternatives but the nature of animal welfare assessment is that many body systems contribute to the animal's conscious experience which cannot be adequately reproduced by other methods.

Reduction

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

Reduction

We have carefully calculated the minimum meaningful numbers of animals for each experiment, based on previous studies. We will employ a factorial design to maximise statistical power and allow identification of interactions between our measures and causal factors, minimising animal numbers. We will randomly assign animals to experimental groups and where possible, the same pigs will be used for behavioural, physiological, pathological and/or meat quality assessments, which will further strengthen the data we gather in terms of commercial relevance.

Refinement

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

Refinement

Our use of pigs is essential as we are investigating a welfare issue specific to this species. Measurement of brain signals requires the surgical placement of very small electrodes through the skull. We will refine this by using anaesthetic techniques that have proven efficacy and suitability in pigs and we will provide post-operative pain relief. After surgery, the pigs must be individually housed to prevent damage to the electrodes, but we will minimise the duration of individual housing and will ensure pigs can see, hear and smell neighbouring pigs which reduces stress. For surgical work, we will use smaller pigs as they have thinner skull bones and this will make the surgery less traumatic. We will train the pigs so that they are accustomed to handling and all testing and stunning equipment before each trial. In trials where we need to train pigs to complete an action when they are in the chambers, pigs will be selected according to their competence in the task (e.g. pushing a button with their snout and receiving a food reward) and this will occur at their home farm. Emergency methods to euthanasise pigs will be in place in case of unexpected events. As the work proceeds we may find that LAPS is not a suitable method for stunning pigs because of unexpected and/or unavoidable welfare costs or from technical or application difficulties such as failure to achieve a timely death similar to commercial standards. As such, we have built into our project key decision points, where we will carefully consider our findings to determine if the work should continue.

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	Mitochondrial Dysfunction and Therapy in Atherosclerosis
Key Words	Atherosclerosis, Vascular 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 project's objective is to better understand the implications of how and why blood vessels become blocked with fatty and cell material called atherosclerotic plaque and then find a treatment.

We aim to understand how the cells of the plaque respond to accelerated vessel ageing by removing genes required for DNA repair, essential for plaque cell health.

The project aims to develop the best possible model of atherosclerosis in which only the cells involved in the disease are modified and this is performed in the most elegant way possible through adding a low dose drug to the diet to remove genes predicted to be important in the disease.

If successful we propose a test a treatment in which other useful drugs are delivered directly to the plaque to improve the life span of the plaque cells. The hope is that by stabilising the plaque cap cells we can prevent plaque rupture heart attacks and strokes

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

Atherosclerosis is a human disease that accounts for 31% of all deaths, around 17.5 million per annum. The majority of these are caused by heart attacks and strokes caused by arteries blocking with fatty material called plaque. When the fatty plaque ruptures they also cause a blood clot that deprives the heart or brain of oxygen which then dies. Existing strategies rely on life style modification such as diet and exercise which can't always be maintained or relied upon. Surgical intervention to bypass the blocked arteries or stenting them open with a metal cage in a process called angioplasty, has significant costs and limitations and complications from the procedure itself. The plaque structure ruptures because a thin layer of cells called the "cap" ages more quickly than the rest of the vessel, most likely because it has to

continually repair itself and this is finite biological process called cell division. Cell division is inhibited by the accumulated toxic plaque components including dietary fats and chemicals called free radicals that damage the DNA. The DNA is essential not only for replication as it provides the blue print of all cells components, but is required for generating the chemical energy for life. This project proposes to better understand the molecular processes of how the cell copes with damage and then extend the life of the plaque cap cells by switching the way their cells generate their energy for cell division. We have tested drugs in vitro that suggest it may be possible to reprogram the way cells generate energy and then target these drug in ultra low doses to just the faulty plaque cells. This would be a significant refinement in current drug and drug delivery technologies and have global impact to cardiovascular patients with atherosclerosis. This project provides the opportunity to test healthier, less dangerous, less toxic treatments, with fewer side effects that could offer patients longer and healthier lives.

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

Mice: 1000 over 5 years

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

Specifically, mice will be bred and at 6 weeks of age the mice will be weaned and a tissue biopsy made to identify the wildtype from transgenic animals. This involves the smallest piece of tissue possible (~ 2-4 mm2) being removed from the ear under full anaesthesia which heals rapidly and offers mild discomfort. Mice are then weaned onto a high fat “western diet” that causes the arteries to develop fatty plaques also called atherosclerosis. The diet contains a low dose of drug (tamoxifen) that will remove activity of a gene important in generating energy for the cells of the arteries. This step will only affect the arteries and leaves all other body cells unaffected. Pilot data already shows this is well tolerated and a significant improvement over using injections and offers mild discomfort. At the end of the study the mice will be humanely culled and their blood and blood vessels collected. All mice in these groups will develop atherosclerosis and the effect of removing the important gene will be compared between control and experimental groups. Some of the mice will have their blood pressure, blood glucose and blood lipid levels measured on up to 3 occasions, usually just before the Western diet is started for baseline measurements, half way through the study at 7 weeks and again at the study end at 14 weeks (20 weeks of age). Some of the mice may have genetic alterations that affect the way their food is metabolised for energy. Therefore an insulin and/or glucose tolerance (GTT/ITT) test may be made to determine changes in this important pathway that can affect atherosclerosis. In a similar way to the way the test is performed in humans at risk of diabetes the mice will be given a single dose of glucose or insulin. The smallest possible volume of blood samples (often a single small drop ~15ul) to

achieve a test result will be taken at regular intervals and used to check how long the glucose can stay in their bloodstream before being removed from the blood by insulin these procedures are also mild. Drugs already approved for use in humans can often be found to treat other conditions. These medicines used for humans improves the lifespan of heart cells. If these drugs could be targeted to atherosclerotic plaques they may also improve the survival of the cells that cover plaques. Thicker stronger plaque cap cells are less likely to rupture and thereby prevent heart attacks and strokes in humans, responsible for 1 in 3 of all human deaths. We propose to test this idea in which these drugs are encapsulated inside small vesicles. These are delivered in to the blood stream and designed to preferentially bind the plaque and have minimal effects on other body cells. While this procedure is expected to be mild as the carrier is a naturally derived lipid substance and the drug is already used in humans. However, as this approach is new it has been categorised as moderate due to the potential of an unknown effects such as the accumulation of the carrier or drug in the liver or immune cells that may cause a very low chance of anaphylactic like response. To address this a staged and stepped escalation processes will be used to protect the very first recipients. If successful, patients of the future could have a targeted drug therapy that would avoid hospitals and lead to longer healthier lives.

Application of the 3Rs

Replacement

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

Replacement

Atherosclerosis is specifically a human specific disease that can not bemodelled in non mammalian systems. As such, the ApoE transgenic mouse provides the best cost to benefit ratio and most realistic model to obtain the best quality data to date.

Reduction

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

Reduction

We have conducted pilot studies that predict the minimum number of animals requir ed to find an statsitcal difference.

These data inform on the minimum numbers required for these studies without wasti ng resources or underpowering the study.

Refinement

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

Refinement

Transgenic mouse models of atherosclerosis are the simplest mammalian system that permits these molecular manipulations and yet retain the degree of complexity required to translate to human models of disease. The protocols have been refined where possible to use diet rather than the previous standard of using intravenous injections to induce the genetic rearrangement in the flox'd mice. This is a significant refinement and reduction in undue suffering.

We can now confirm the use of this approach as we have successfully been able to prove removal of our gene from the vessel of treated animals.

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	Brain mechanisms underlying cognition and emotion
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):

The overarching aim of this project is to identify the neural, genetic and neurochemical circuitry in the brain that underlies the cognitive and emotional impairments that are important symptoms of psychiatric disorders such as depression, anxiety and schizophrenia. This includes identifying how genes and physiological stressors impact upon the development and subsequent functioning of this circuitry, how this affects cognitive and emotional processes, and how current therapies (ie antidepressant drugs) interact with this circuitry to treat these symptoms. These are important questions because over 40% of patients suffering from neuropsychiatric disorders are not helped by current therapies for reasons that are unknown, and when the therapies are effective, we don't understand why and thus can't predict which patients will do well on which treatments. This severely limits treatment options and treatment development. It is recognised that this is because we have very little understanding of the different brain mechanisms that can cause these symptoms, and until we understand how the neural, genetic and neurochemical circuitry within the brain contributes to the normal and symptomatic cognitive and emotional processing we will not be able to improve treatment strategies for the sufferers of psychiatric disorders.

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

This project will provide a basic understanding of how some frontal brain areas contribute to a variety of cognitive and emotional behavioural impairments (for example, compulsivity, anxiety and loss of sensitivity to rewards) that are common in patients with neuropsychiatric and neurodegenerative disorders. It will provide an understanding of how damage to different brain mechanisms contributes to the different cognitive and emotional processes that cause these impairments, such as problems in switching attention away from negative stimuli or problems in predicting when negative events may occur. By identifying the underlying psychological, neural, genetic and neurochemical causes this will not only help stratify patients but also

improve their chances of getting personalised therapy. For example, if you are anxious because you find it difficult to switch attention away from negative things due to dysfunction in one region of prefrontal cortex, this will require different treatment than if you are anxious because you can't predict when negative things will happen due to dysfunction in a different part of the prefrontal cortex. It is this basic knowledge that is currently lacking. Thus, understanding the different brain circuits that mediate different aspects of such psychiatric symptoms, and combining it with information about how particular therapies interact with such circuits will help us to identify particular symptoms and eventually target existing therapies more effectively.

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

We expect to use approximately 340 marmosets over 5 years

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

All animals are housed in either stable male/female pairs or live in their family groups in cages that exceed the UK and EU guidelines and contain an extensive array of environmental enrichment aids. Animals may occasionally be housed without a partner in the event of an argument with their cage mate or if their cage mate is humanely euthanased upon study completion (e.g., in cases where timing of brain assessment is critical). A new partner will be provided at the earliest possible opportunity, depending, e.g., on availability of opposite-sex partners, experimental status, etc. A typical study lasts between 18 months to 2 years. During that time marmosets are likely to receive behavioural testing 5 days a week on a range of cognitive and emotional tests that either last 15 minutes or 40 minutes. The rest of the time they are in their home cage with their partner or family. Over that 18 month to 2 year period they are likely to have between 3-5 general anaesthetics, 2-3 involving a surgical procedure such as brain surgery and implantation of a measuring device, the remaining for restraint purposes only in order to e.g. perform brain scans. Normally, the animals recover well from their surgery or general anaesthesia and are back in their home cage within 2 hours of coming round from the anaesthetic. With all surgical procedures, animals will be fully recovered from one surgical procedure before undergoing another, with a minimum of 2-3 weeks between procedures. At the end of a study the animals are euthanased. In such a study an animal will undergo behavioural testing either in the home cage or in a specialised apparatus. The latter is a purpose built test box including a computer and touchscreen. It allows animals to be presented with positive stimuli (e.g. food rewards and visual and auditory stimuli predictive of food rewards) and mildly negative stimuli (e.g. mildly aversive loud noise (0.3-0.7sec) or darkness and visual and auditory stimuli predictive of these negative stimuli) to study learning, attention and emotion. Animals learn to voluntarily enter a transport box for transfer to the testing apparatus, to which they have been gradually acclimatised to minimise stress. Testing away from

the home cage is limited to 40 min, typically once, but very occasionally twice a day, and is halted if the animal exhibits signs of distress. No adverse effects are associated with behavioural testing, and even when mildly aversive stimuli, such as brief loud noises, are used, animals enter the transport box for testing. Animals undergoing restricted access to water during more intellectually demanding experiments utilising a liquid reward receive 2 hours of unrestricted water 5 days a week in addition to rewards received during testing. Water restriction does not affect the weight of the animals, who often ignore the water when it is returned to their cage, suggesting that they are not very thirsty. To study the brain mechanisms underlying behaviour and cognition, selective surgical procedures may be carried out under anaesthesia. Animals are gently caught from their home cage by an experienced handler and carried to the surgical suite. Premedication with a sedative is achieved via an injection into the muscle which causes only mild, momentary discomfort. A gas anaesthetic is used thereafter to ensure no pain is experienced during the surgical procedure (typically lasting 3-6 hours depending upon the procedure). Through small holes made in the skull, we can infuse substances that permanently or temporarily alter brain function in a discrete region or insert an implant that allows the later injection of substances to the implanted region. The latter is fixed in place using screws attached to the skull and dental adhesives. We may also temporarily implant devices to measure local brain function. Animals are monitored closely throughout the procedure and during recovery, and are usually fully recovered and back in their home cage eating, drinking and behaving normally within 2-3 hours. Long-lasting pain relief is given prior to surgery via an injection under the skin, and for several days after as an oral treatment delivered in marshmallow to minimise the need to catch them. Extra care is taken during the first week after surgery to observe any changes in normal behaviour or appearance. Long term implant sites are cleaned regularly throughout the life of the animal to prevent infection. Surgical procedures (lasting 90-120 mins) are also performed in some animals to implant a small radio transmitter into the abdomen to record physiological measures of emotion (heart rate and blood pressure) in animals that move freely during behavioural testing. Brain imaging (typically lasting 90 mins) may be carried out using anaesthesia to keep the animal still so as to ensure good quality images. Animals receiving certain brain scans may have an intravenous access device implanted under the skin to allow the injection of a radioactive substance without the stress of injecting directly into a vein. Following these surgeries animals typically return to the home cage within two hours.

Application of the 3Rs

Replacement

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

Replacement

The aim of the proposed work is to investigate how neural circuits in the brain control cognition and emotion. To do this, functional brain circuits are required. Furthermore to be able to determine the contribution of a particular brain region or circuit to the expression of a certain behaviour it is essential to be able to alter its function. As such interventional experiments cannot be done in humans for ethical reasons, and cell cultures are unable to contribute to a functional, behaving circuit, animal models are indispensable for this work.

Reduction

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

Reduction

We are very aware of the need to minimise the number of animals that we use in a year while optimising the validity of our scientific results. For this reason, and to keep numbers to approximately 70 per year, we screen all our animals for their suitability for studying particular behaviours and for their genetic background to optimise which animals go into which study. We also use brain scanning to ensure the precise targeting of the location within the brain which are of interest, and plan to investigate the use of imaging as a way of measuring brain structure, connectivity, chemistry, and function, allowing individual animals to act as their own control rather than requiring both control and experimental animals. All new surgical techniques are piloted in rodents first where possible, and any new techniques are tested first in one or two animals to ensure the experiment is optimised. We repair surgical implants, when possible and when there is no risk to the animal, rather than implanting additional experimental animals. We regularly consult with local statisticians to ensure that we are using the optimal group size for the results that we see, to ensure that we use the minimal number of animals while optimising the mathematical power of our analyses.

Refinement

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

Refinement

Primates are used specifically because their brains, in particular, those brain regions most important in regulating our cognitive abilities and emotions, such as the prefrontal cortex, are far more similar in structure and function to that of humans than lower species, e.g. rodents. To illustrate this, the cerebral cortex, that region of the brain with the most sophisticated processing abilities, makes up 80% of the brain

mass in humans and 60-70% in primates, compared to just 26% of the brain mass in rodents. Marmosets are a particularly valuable species to use for the proposed work as their relatively small primate brain makes it possible to target cortical and subcortical structures and to make regionally selective neurochemical interventions with relative ease, with little risk to the animal. Often, the same approaches cannot be used in larger primates, such as the macaque, because the surgical procedure involves too many brain entries, which increases the risk of collateral problems such as damage to major internal blood vessels. Having a breeding colony in the same establishment as the experimental program affords us considerable experimental control over the entire lifetime of the marmoset. This is an important factor, particularly when studying negative emotion and its regulation, since it is known that stress and early life experiences can have an enormous impact on the cognitive and emotional regulatory processes under study. The on site breeding colony means that animals do not have to experience the stress of transport to the laboratory and allows us to separate some of the environmental and genetic influences on behaviour. We constantly review all of our behavioural and surgical techniques to ensure that we refine procedures in order to minimize potential animal suffering.

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 the immune system in tissue damage
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):

We want to understand how and why we develop an abnormal immune response to organs like the skin and kidney. Understanding this abnormal response is important because it causes damage. Our experiments aim to identify how to stop the damage in a safe manner.

The immune system comprises a large network of cells and molecules that can detect and eliminate harmful substances e.g. toxins or disease-causing microorganisms. Induction of an immune response in this setting is appropriate and necessary to maintain health. However, if the immune response is uncontrolled or induced inappropriately (i.e. in the absence of any harmful substances) it can cause damage to organs like the skin and kidney through a process termed inflammation. Inappropriate immune responses are seen in many human illnesses including cancer and kidney diseases. The reason for our animal experiments is to work out the fundamental mechanisms driving abnormal immune responses. By doing this we will be able to identify where to develop drugs to prevent unwanted damage and to develop drugs to promote repair. There is a need to do this since although some of our available treatments are very effective in certain conditions, the side-effects are significant. Only by a detailed understanding of pathways that cause injury will we be able to develop more selective treatments that will work more effectively and have little or no side-effects.

In this application we aim to generate and analyse genetically modified mice in which we have removed one or more immune genes. Studying how their immune response to an insult differs from wild-type mice is a powerful way of revealing the function of these genes in the development of organ damage

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

Our research will provide valuable insights into the mechanisms leading to the development of human diseases whose underlying cause are abnormalities in the

immune system (e.g. cancer, autoimmunity and renal disease). Our results will advance the knowledge that clinical researchers and biomedical companies use to develop new treatments for these conditions

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

We estimate that we will use approximately 33000 adult mice, including breeding, during the course of the 5 year project

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

None of the experiments planned under this project involve procedures that are expected to cause the mice severe distress or discomfort. The majority of the animals will only be subjected to mild/moderate procedures. For example, the administration of a substance or bleeding which have only minor adverse effects, such as transient pain after the injection. A small proportion of animals (approximately 20%) will undergo moderate surgical procedure (e.g. skin punch biopsy), which will be performed under general anaesthesia with pain-relief peri- and post-operatively. Some animals (approximately 15%) will be exposed to a tumour challenge. These animals will be monitored daily to check for signs of adverse effects (e.g. signs of lethargy and of deteriorating body condition) and humane end points have been defined to avoid unnecessary suffering. In all cases animals will be killed before they develop signs of ill health. Some animals (less than 15%) may develop renal impairment spontaneously or as a result of the experimental procedures. By measuring the presence of protein and blood in the urine (leaking through a damaged kidney) we can readily identify this and mice will be culled at the onset of clinical signs of renal disease. All procedures have been designed to be terminated as soon as the animals appear to be suffering.

Application of the 3Rs

Replacement

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

Replacement

The immune response is a very complex and dynamic process that involves a number of different cells and molecules. Experiments using intact animals are essential to study the immune response since *in vitro* cell culture systems or *in silico* modelling are at best only able to recapitulate partial interactions. Despite these limitations, every *in vivo* study will be preceded and followed by *in vitro* work with the aim of developing new experimental approaches that can replace animal work. For example we are currently working on 3-dimensional skin culture systems. However,

presently the tissue changes present in skin cancer or kidney damage cannot be studied using isolated cells alone and whole animal studies are still required. For these reasons the use of genetically-modified mice remains the most scientifically robust experimental approach to achieve the objectives in our application

Reduction

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

Reduction

To ensure that we always minimise our use of animals we: (i) use strategies that tell us which are the most important experiments to perform to answer our objectives; (ii) ensure that all animal experiments are designed so that we use the minimum number of animals to answer the experimental question; (iii) maximise the information that we obtain from each animal experiment.

To reduce the number of animals in each experiment we perform statistical calculations that tell us the minimum number of mice needed so that we can be scientifically confident of the experimental results. These calculations are termed power calculations and from our previous work typically result in us using 5-6 animals in each experimental group. These calculations are continuously updated as we generate new data and this means that in some cases we can be perform future experiments using fewer animals per group.

We maximise information from each experiment by:

- examining multiple organs and tissues simultaneously.
- breeding together mice in which both copies of the genes are changed to reduce the generation of mice without the required alteration to the gene.
- using inbred mouse strains to reduce experimental variability
- applying, whenever possible, longitudinal imaging studies to maximise the data output from live animals.
- utilising strategies to minimize bias such as blinding.

Refinement

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

Refinement

Our animal research uses only mice. Mice develop immune disorders that are functionally comparable to human disease. We can also reliably measure changes in markers of organ damage (e.g. protein leaking into the urine in kidney disease) that are comparable to those measured in clinical practice.

Mice have proved invaluable in experiments studying the immune response because of the availability of genetically-modified strains lacking specific immune genes. The alternative to this would be to administer to healthy mice compounds that specifically inhibit gene function. This is technically challenging, not widely available, and often toxic to the animals.

To minimise animal suffering the experimental protocols have been refined so that they are all mild or moderate. In our program the majority of experiments are typically mild in practice. In all cases these models have been chosen because they accurately mimic features of the human disease without causing systemic distress to the mice. To our knowledge, there is no alternative less severe *in vivo* protocol. However, should such a protocol arise during our research we would adopt this to refine our existing protocols. However, we have not seen any significant or unexpected complications with our current models. In all experiments we design the protocols to keep the experimental duration as short as is necessary to achieve the scientific objectives. Animals will be housed in groups where possible, with appropriate environmental enrichment and fed according to institutional recommendations.

Procedures will be performed under local, general or terminal anaesthesia as appropriate and with pain-relief wherever necessary. In all experiments, animals will be inspected daily to ensure general well-being and any animal showing signs of illness will be humanely killed by schedule one or another approved method at the end of each study.

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	Preclinical evaluation of cancer therapeutics
Key Words	cancer, chemotherapy, immuno-oncology,
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):

- Identify tolerated dose levels of test drugs and side effects not predicted by cell culture based model systems.
- Study the effects of the drug on the body, and also the effects of the body on the drug.
- Demonstrate that anticancer activity can be shown at specified doses and with dosing schedules that are tolerated.
- Identify the best tumour models to use pre-clinically that correspond with a specific therapeutic target.

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

The aim of the data generated in these studies is to provide pre-clinical supporting information for clinical trial applications. A drug requiring evaluation will be supplied to Epistem along with summary evidence supporting the rationale for testing the agent. By having a much more thorough investigation into the efficacy and mechanism of action of a drug they will be able to make a more informed decisions on whether to proceed into clinical trials, reducing the risk of later stage failures. This will speed up the clinical trials and make them less expensive. The benefit is therefore a reduced number of unproductive human volunteer studies (and a reduced risk of adverse effects) and most importantly the development of improved and more effective therapeutics. The benefit to patients will be the identification of new anti cancer drugs. These studies will help identify the best potential drugs early in the drug development process.

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

We would expect to run 150 studies on behalf of sponsors using approximately 7,500 mice and 2,150 rats over the 5 year duration of this project licence.

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

Side effects of tumour treatment can include lethargy, anaemia, loss of appetite, diarrhoea, dysuria, bruising, bleeding or peripheral neuropathy. Animals exhibiting these signs will be humanely killed. This is likely to manifest as weight loss. A general dose limiting sign will be a 15% loss in bodyweight, and animals showing this will be considered unwell. Any mouse reaching a 20% bodyweight loss, or any rat reaching a 25% bodyweight loss along with other signs of distress will be humanely killed (schedule 1 method). Subcutaneous tumours may grow to a size that could cause discomfort or interfere with the animals' ability to satisfy thirst or hunger. Also, tumours could ulcerate through the skin dependent on the cancer type, or if intra-tumoural therapy is administered. Animals will be killed if their ulcers do not heal within 48 hours or if their tumour reaches more than 15mm in any direction. Injection at the tumour site may cause temporary bleeding which should stop within a few hours of injection. In the unlikely event that bleeding does not cease, and if the animal shows signs of discomfort, it will be humanely killed. Orthotopic (implanted at the natural tumour site, eg a breast cancer cell line injected into the mammary fat pad) tumours may have site specific adverse effects and elicit metastatic disease. Metastatic disease will be monitored by imaging of the whole body wherever possible. However if such techniques cannot be employed using a specific model, any deviations in physiology or behaviour will be treated as indicative of metastatic disease, and animals will be humanely killed when there is loss of condition consistent with the severity limit as defined by the Home Office regulation. For leukaemias, animals may gradually become weak, lethargic and lose body weight. Infiltration of the spleen or liver can lead to enlargement of these organs which may be palpable. Any animals showing signs of distress or symptoms at the limit of moderate severity will be humanely killed. Immunocompromised mice will be maintained in Individually Ventilated Cages in a barrier environment to avoid unwanted infections. If animals develop unwanted infections or surgical wound complications, they will be given antibiotic treatment after advice is sought or will be humanely killed. Animals will receive analgesia following surgical procedures such as bone marrow aspiration or orthotopic tumour implantation. Prolonged periods of anaesthesia can lead to animals losing body temperature. To counteract this, animals will be warmed throughout the procedure, either by the use of heating mats, warm air blowers or temperature regulated stages. All work will comply with the UKCCCR (United Kingdom Co-ordinating committee on Cancer Research) guidelines for the welfare of animals in experimental procedures.

Application of the 3Rs

Replacement

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

Replacement

The programme requires that the models used are ones which closely mirror human disease. All compounds to be tested would have previously been screened in relevant *in vitro* models to determine those candidates suitable for *in vivo* testing. Rodents (rats and predominantly mice) are suitable for these studies as the work cannot be conducted in lower vertebrates, invertebrates or cell lines due to the poor resemblance of these options to the clinical setting. Animal models address issues which current in vitro tests cannot accurately determine.

Reduction

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

Reduction

Animal models will be restricted to the minimum number of animals needed for a statistically valid result. The number of animals used will be the minimum safely necessary to allow meaningful statistical analysis of the data generated.

The most important aspect of the proposed programme of work that will reduce the number of animals used is careful selection of drugs, on the basis of preclinical data. Only those potential drugs that offer a realistic prospect of therapeutic exploitation will be investigated.

The investment by the team in the purchase of small animal imaging technology also reduces animal numbers in these experiments. The development of disease can be followed in each animal over time, abrogating the need to humanely kill satellite groups to examine disease progress, and thereby reducing total animal numbers.

Refinement

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

Refinement

Rodents provide a cost and time effective platform in general for most pre-clinical testing. For the purposes of oncology testing, the use of higher species is not required because there is a wealth of knowledge on different types of cancer in rodents, as well as decades of in-house expertise with such models. Internal expertise, and more recent technological advances, such as the use of whole body imaging, allows for a more refined study design that will minimise the number of animals. These techniques will maximise the output and will provide a more thorough assessment. They will also help in selecting the best models.

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	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 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 behind T-cell function and regulation
Key Words	T cells, Immune regulation, 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):

T cells are an important population of white blood cells that protect us from infections and cancer. T cells sense germs or damaged cancer cells through a specialised structure, called the T cell receptor (TCR). Each TCR is unique, enabling T cells to respond to any potential threat to the body. As there exists both a large number of T cells and many unique TCRs in the body, this presents a great challenge to study. Scientific approaches in the past have used techniques to label T cells with green proteins when their TCRs sensed a threat. However, these green proteins can remain in a cell for up to a week, many days after a T cell has responded. We have developed a new Tool, which labels T cells temporarily blue (within hours of TCR sensing a threat), meaning we can follow T cell behaviour with much greater precision. Using this system, we aim to reveal how T cells behave under normal healthy conditions, as well as in diseases such as allergy, infection and autoimmunity. In addition, we will look to understand how drugs may alter the function of T cells in autoimmune disease.

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

This project will advance scientists' understanding of how T cells respond during allergy, infection and autoimmunity. In addition, the breeding protocol will create new tools for use by others within the scientific community to take forward their own research. The project has the potential to identify new targets on T cells that we could design drugs to alter how T cells respond to threats in the body. This could help the development of future immunotherapies – which are drugs designed to alter how the immune system works. In addition, a key objective of the proposal is to investigate how a particular type of immunotherapy – called peptide therapy – works through altering the behaviour of T cells. This could better inform strategies for treatment of autoimmune diseases, such as multiple sclerosis, diabetes and arthritis.

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

The project will use both normal and genetically modified mice (which express the blue colour when T cells are activated). Over a period of five years, it is anticipated that a total of 3000 mice will be bred in order to address the aims and objectives of the project. In addition, of these mice, 2000 are anticipated to undergo direct procedures.

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

To study allergy, a refined model is used which results in a slight reddening and thickening of ear skin 24 hours after applying a substance to the skin. In addition, to test T cell responses during infection, we will use a well-established model that does not result in clinical symptoms for mice. Infection will be performed via infection with droplets in the nose, with the mouse anaesthetised for a short period of time to minimise suffering. Multiple rounds of anaesthesia will be avoided to reduce the cumulative harm to mice. For studying T cell responses during autoimmunity, it is necessary to use a mouse model of multiple sclerosis (mMS). These models require the injection of proteins immersed in oil containing heat killed bacteria under the skin of mice. The majority of these studies will look at the early stages of mMS, before more severe symptoms set in. These experiments will be restricted to no longer than 2 weeks duration to assess T cell responses during the initiation of mMS disease. However, to test drugs to potentially prevent mMS, a small group of mice may experience moderate symptoms (such as paralysis of tail and back legs). This is unfortunately necessary in order to reveal whether drugs are effective at preventing or improving disease. In some experiments, mice will receive substances in the diet, or be given orally, that can alter genes within T cells. All animals will be carefully monitored by trained staff throughout experiments, and all mice will be humanely killed following the end of experiments.

Application of the 3Rs

Replacement

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

Replacement

T cells are part of a very changeable and complex biological system, which involves numerous cells that migrate around the body and interact with each other. Whilst simple test tube models exist for investigating their behaviour in the lab, these do not always predict how T cells may behave in the actual body.

In order to study T cell responses that are relevant to human disease, we must use a species which shares all the major parts of the immune system. Amongst species that share the main components of the immune system with humans, mice are the least sentient option. Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use.

Reduction

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

Reduction

We will use a very refined model of MS, where each T cell has a TCR that makes them activate when they sense nervous system tissue. This model (called Tg4 MS model) is very reliable, and can induce disease in 100% of mice, which will mean experiments can be performed using only 3 mice as disease controls (compared to 5-8 using other MS mouse models). In addition, T cells from these modified MS model mice can be cultured in a dish in the lab and tested for how they response to nervous system tissues, reducing the number of mice that have to undergo experimentation.

Refinement

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

Refinement

REDACTED, we can capture more information during the responses of T cells to infection, allergy and during autoimmunity. This will require fewer mice to be used. The Tg4 mMS model is very reliable, and protocols have been designed to induce moderate disease levels in the greatest number of mice. In order to make sure mice suffering is minimised, we have a dedicated scoring system for disease, which grades from 1 (mild) to 5 (most severe). Any mouse that develops grade 1 or 2 disease will be checked on by trained lab members twice a day. Mice that develop grade 3 disease will be humanely killed. Protocols have been refined to generate no greater than grade 3 disease. However, a balance is required that minimises disease whilst also maintaining a level and incidence of disease that is sufficient to enable whether a given treatment is effective.

Following potentially painful injections, mice will receive Sudocrem® to reduce irritation and prevent ulceration

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	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 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	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 which will be reduced through anaesthetic. A high fat diet used to develop heart disease in this study is well tolerated by the animals and causes no adverse effects. This study will use only enough animals to answer the experimental questions. All animals will be humanely killed at the end of the study to allow detailed investigation of the heart and surrounding blood vessels in relation to heart disease; something that cannot be otherwise achieved in live animals.

Application of the 3Rs

Replacement

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

Replacement

The use of animals in the proposed study has been carefully considered and we are not aware of any alternative which does not use animals that would allow the experimental questions to be answered. Mice are mammals and develop heart disease in a similar way to humans providing a good model for studying details of this disease. While we will use isolated cells relevant to the development of heart disease grown in culture to complement the work, such experiments do not allow for the influence of the whole-body and systems within it to be fully considered and therefore cell experiments cannot be used as a replacement of the animal study.

Reduction

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

Reduction

Every attempt has been made to reduce the number of animals used through careful experimental design in consultation with statisticians familiar with animal studies. Throughout the project animals will be regularly monitored and experiments altered as necessary to ensure only the correct number of tests are performed to answer the specific questions of the study. These ongoing decisions will again be made with input from statisticians.

Refinement

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

Refinement

The use of mice for the study of atherogenesis is advantageous due to the similarities in the development of the disease to human. The specific mouse used in this project develops heart disease at an accelerated rate promoting disease in the arteries similar to that found in humans without the need for long experimental times and prolonged discomfort.

Any negative impact on the animals will be reduced through providing the highest levels of skilled care by trained and competent personnel. Discomfort of the experimental animals will be minimised through the use of appropriate anaesthetics during any procedures where it is considered necessary.

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	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 our society costing over £100 billion per year. Presently, we don't exactly know how stress acts on the brain and how it can cause disease, particularly after repeated exposure. As long as this remains unknown we can't develop new and improved medication to help these patients. It should be mentioned that most medicines used at the moment to treat patients were discovered by chance and actually we don't completely understand why they help some (but not all) patients. It is of great importance therefore to understand the impact of stress on the brain.

Stressful events result in the secretion of stress hormones ('glucocorticoid hormones') from the adrenal glands, which are hormone-producing glands located close to the kidneys. These hormones can act on nerve cells in our brains and influence their functioning. Importantly, research so far indicates that glucocorticoid hormones play an important role in the development of stress-related mental diseases. Presently, however, we do not know how these hormones act on nerve cells. For proper functioning of nerve cells (so the whole brain and thus the person can function normally), many substances ('molecules') are interacting in these cells. Many of these molecules are the result of the expression of genes located within the DNA in the nerve cell nucleus and may be affected by stress hormones. Thus, our aims are:

1. To determine which genes are affected by stress-induced glucocorticoid hormones

2. To investigate how these hormones are changing the affected genes after stress and the consequences for nerve cell function and behaviour

3. To study whether, in addition to these hormones, other molecules are involved in these effects

4. To study how repeated exposure to stress disrupts the normal effects of glucocorticoid hormones on genes, nerve cells and behaviour.

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

This project will deliver the following direct benefits: 1. A vast increase in, publicly available, knowledge about how stress and stress hormones impact on the brain, which includes: - The identity of the genes affected by stress/stress hormones - How this results in changes in nerve cell function and behaviour - Through the comparison of juvenile and adult rats, we will learn why adolescents are more vulnerable to developing a stress-related mental disorder than adults - How repeated stress disrupts the normal action of glucocorticoid hormones in the brain 2. The scientific results will help the development of computer models, which can reduce the need for animal experimentation in the future. In the medium-term, the results of this project will help to identify the genes that are critically affected by stress hormones. These genes should then be further investigated as potential targets for novel drugs for the treatment of stress-related mental diseases. Identification of the genes will also help to screen for stress-sensitive individuals 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 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.

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

Word limit; 1000 words

Project Title	Examining the control of female meiosis and early development
Key Words	Oocyte, Aneuploidy, Women's Health
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:
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 want to understand how a healthy egg is produced. This has a number of facets but the most prevalent to go wrong is having the right number of chromosomes. Eggs that are made with the wrong number of chromosomes are described as being aneuploid. Such aneuploid eggs go on to form aneuploid embryos, and these are mostly non-viable and die on or before the time of implantation. It is estimated that up to 60% of ovulated eggs from women are aneuploid. This leads to infertility, early pregnancy loss and birth defects – because a few aneuploidies can result in live births. The most prominent type of aneuploidy is trisomy 21 (Down Syndrome).

Currently we do not know why eggs should end up being aneuploid at such a high a rate. So we need to investigate this phenomenon if we are to move forward and to either develop ways of reducing aneuploidy or screening for it more effectively. Especially intriguing is how the rate of aneuploidy increases with maternal age. There is an increasing trend to have children later in life, hence the relevance of aneuploidy to human fertility is on the rise.

Mice also show a high rise in aneuploidy as they age and are therefore an appropriate tractable system to study this area- without the ethical issues of using human oocytes. Put simply there are also too few human oocytes available for research to produce much scientific progress. Therefore in order to understand why aneuploidy happens and how a healthy egg is produced this project will investigate the process in mice

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

Women and couples: the main benefactors in the long term are going to be women who will have an increasing control on their reproductive health. Knowledge from this project will help underpin future strategies to develop ways of improving egg health during the 40 years of reproductive life women have. Especially relevant is finding ways of reducing the effects of ageing on aneuploidy. However any strategy to do

this has to be based on scientific knowledge of how aneuploidy comes about. Such knowledge will then aid in developing appropriate methods to circumvent or reverse the deleterious effects of ageing.

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

Numbers are reduced during the entirety of the project (3Rs) by using only hormonally treated animals, a process that increases the numbers recovered. I request animal numbers on this 5-year Project licence of 3500. This equates to 14 mice per week (50 week year), 700 pa, 3500 over the 5 year window. This oocyte number is the average we can use in the experiments we perform following oocyte collection (on average just under 3 mice a day).This is based on the average week using (2,3,3,3,3) mice. Some experiments use more (e.g. Western blotting), while some (e.g. pilot experiments) use less. It is based on a historical average for the same sets of experiments that we have performed in the laboratory under previous grants and licences, which use the same mix of experiments.

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

Female mice are used as a source of eggs. The protocol is very mild, and involves either 1 or two intraperitoneal injections of hormones. These hormones mimic endogenous hormones, and help follicle growth and ovulation. This simple and quick procedure ensures that we can reduce animal numbers by obtaining the most useable numbers of oocytes per mouse. The only feasible adverse effect is infection from injection, however this is minimised by only using sterile equipment and solutions.

Application of the 3Rs

Replacement

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

Replacement

Replacement: the long term goal would be to replace the use of mice. However, there are no in silico models or cell cultures that can be used. The only available source of oocytes is from the ovary.

Reduction

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

Reduction

Reduction: we only use mice that have been hormonally treated, so reducing the numbers we need to a minimum. Hormonally treated mice will produce more eggs than non-treated.

Refinement

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

Refinement

Refinement: we are using a priming and superovulation hormonal treatment that has been refined over the past 40 years. One of the great advantages of using mice is that this procedure is so common, it has been refined over decades to be used with the utmost effect.

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	Development, function and regulation of the immune system
Key Words	Lymphocytes, immunology, cancer, vaccines, signals
Expected duration of the project	5 year(s) 0 months

Purpose of 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 must be precisely regulated so that it will fight off infections and tumour cells effectively, while not damaging the normal organism. T lymphocytes (“T cells”) are cells found throughout the body and which have key roles both in attacking “foreign” material but also regulating the activity of the immune system as a whole. Our research objective is to systematically map and identify the key signalling pathways and molecules that control lymphocyte function. One focus is how lipid and protein kinase pathways integrate information from antigens, cytokines and nutrients to control metabolism, inflammatory cytokine production and T cell migration/trafficking and hence determine cell fate choices in lymphocytes.

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

When T cells fail to function correctly the immune system fails, often resulting in death. The present project will characterise molecules that act as messengers inside T cells to regulate their function. The control of T cells is critical for immune responses. Understanding the signalling pathways that control T cell activation is essential to identify targets relevant for the treatment of autoimmune and inflammatory diseases. The laboratory has an integrated research program to explore signalling pathways inside lymphocytes. These studies will generate new information about mechanisms that control immune responses and identify new targets for therapeutic intervention in the immune system that can be used for vaccination, to fight bacteria and to treat cancer.

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

This application is to support a group of 8 scientists for a period of 5 years and proposes to use up to 20,000 mice including wild type and genetically modified mice.

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

Based on previous experience, more than 95% of the mice to be used in this programme will exhibit only mild outward signs or none at all. The great majority will be killed humanely for the collection of tissues and cells to be studied in detail in the laboratory. Some animals (up to 5%) will be challenged with microorganisms or tumour cells. Infections are likely to cause deviation from normal welfare in some of these mice, but all will be killed humanely at as early a scientific endpoint as possible. Similarly, tumour studies will be conducted (in up to 5% of animals) in a way that will ensure that only minimal changes in welfare are caused. Some animals (2-3%) will be irradiated, to permit the introduction of immune cells derived from other mice (akin to bone marrow transplants as carried out in humans). We will use a standard procedure for this and mice will receive welfare support to ensure that the effects of the irradiation are minimised and resolve completely.

Application of the 3Rs

Replacement

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

Replacement

Lymphocytes isolated from peripheral blood from normal human donors can be used to study some aspects of lymphocyte behaviour but immune responses are very complicated and require that lymphocytes traffic between the blood lymphoid organs and peripheral tissue. To identify important modifiers of immune responses, it is necessary to carry out immune function tests in live animals.

Reduction

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

Reduction

We work in partnership with skilled animal experimentalists and use well validated mouse models. We ensure through constant discussions and consultation with statisticians that the number of mice we use for experiments is appropriate. We constantly seek to reduce our animal usage through improvement of in vitro models. The minimum number of mice required to show scientifically and statistically significant data are used.

Refinement

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

Refinement

This project will use mice because mice are an appropriate, well established model for studies of the mammalian immune system and are a good model for the human immune system. We will adopt early scientific endpoints for all experimental interventions.

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.

This summary will be published (examples of other 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: 2,050 Over a period of 5 years

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

Potential harm results from hydrocephalus, which will be created under general anaesthesia. In the vast 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 113. 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	Nutritional programming of metabolic disease
Key Words	Suboptimal nutrition, pregnancy, early life, programming, adult 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):

Our overall purpose is to identify the mechanisms leading to adult disease, secondary to suboptimal maternal nutrition during pregnancy and/or lactation, and to investigate the efficacy of interventions that are potentially translatable to humans.

The key questions are:

1. **How does suboptimal gestational and/or lactational nutrition affect maternal and offspring health and what are the underlying mechanisms?**

- What are the key maternal factors (e.g. hyperinsulinemia, hypertension) in an obese mother that program insulin sensitivity, cardiac dysfunction, energy balance in offspring?
- As proof of principle, can we mimic these programming effects (e.g. insulin) by exogenous introduction of these maternal factors directly into the fetuses?
- Does altered maternal diet lead to altered vascular function and/or blood flow between the mother, placenta and the fetus?
- Does altered maternal diet lead to defects in cellular proliferation, cellular apoptosis, hypoxia or oxidative stress in maternal, placental, fetal or adult offspring tissues?

1. **What are the effects of pharmacological and/or exercise intervention to the mother, and the metabolic health of her offspring?**

- Does perinatal maternal exercise in an obese mother improve her insulin sensitivity and cardiovascular tone and that of her offspring?
- Does perinatal pharmacological treatment improve her insulin sensitivity cardiovascular tone and that of her offspring?
- Do the interventions affect cellular proliferation, cellular apoptosis, hypoxia or oxidative stress in maternal, placental, fetal or adult tissues?

1. **What are the effects of dietary/pharmacological intervention in the offspring on the programmed effects on metabolic health?**

- Does dietary/pharmacological treatment to the programmed offspring improve insulin sensitivity and cardiovascular tone?
- Do the interventions affect cellular proliferation, cellular apoptosis, hypoxia or oxidative stress in neonatal/adult offspring

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

Lifestyle (increased physical exercise) intervention to an obese mother around the time of pregnancy is likely to improve her metabolic fitness and the long-term health of her offspring. This is an important translational message.

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

Over the 5-year period of this project, we expect to use no more than 6,900 adult and 7,500 neonate Rats; 11,600 adult and 10,400 neonate Mice.

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

The large proportion of animals to be used in this licence (81.9% of the adult rats and 87.5% of the adult mice) will experience no adverse effects. Female animals fed differing diets, with or without exercise or pharmacological intervention, will then be paired with a male. Both mothers and offspring health will be monitored throughout adult life using longitudinal assessments of a) non-invasive cardiovascular imaging techniques, sometimes with a general anaesthetic to negate stress b) metabolic testing involving All animals will be killed humanely at the end of the experiment. 15.2% of the adult rats and 10.8% of the adult mice will undergo recovery surgery under appropriate general anaesthesia. Following the surgery, they will experience minor discomfort with itching around the wound stitches. This will be managed with appropriate analgesia and antibiotics. Animals will be killed humanely at the end of the experiment. In addition to this, 2.9% of the adult rats and 1.7% of the adult mice will undergo surgery (without recovery) under general anaesthetic throughout the experiment and be killed by an anaesthetic overdose at the end of the experiment.

Application of the 3Rs

Replacement

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

Replacement

Although experiments in cultured cells can provide a lot of useful information on specific workings at a cellular level, we need to study how different cell populations behave and interact as part of a complex environment in a living animal. Each tissue type e.g. brain, fat tissue, heart, working muscle or liver, are themselves made up of

different cell populations (including stem cells which go on to divide and mature into fully functioning adult cells). The different tissue types send out and respond differently to the signals present in the peripheral blood system such as occurs in the whole living animal. This level of complexity cannot be attained in cell culture based experiments.

Our previous work has used the strategy of identifying specific mechanisms due to suboptimal maternal environment in animals which lead to cardio-metabolic disease in the offspring, and then using this information to guide parallel human studies (e.g. in Danish low birth-weight men). This successful strategy clearly underlines the value and justification for our work using animals, which has significant parallels with the human pathophysiology. We will where possible, use a similar approach to translate our observations in animal models into humans by working on human serum and biopsy material and we will continue to use a forward and back translation approach.

Reduction

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

Reduction

It is important to note that as the environmental stimulus is made to the mother, it is the mother which becomes the statistical unit for all our studies, and this is therefore reflected in our animal numbers as we are constrained to using only 1 offspring in each litter for any given outcome. We will collect all tissues and organs at post mortem-even ones that are not required at the time for any particular study. These include brain, heart, aorta, lungs, liver, pancreas, fore-gut, hind-gut, kidneys, intraperitoneal fat, retroperitoneal fat, gonadal fat, vastus lateralis & biceps femoris muscle, brown fat, bones and testes or ovaries. This extensive bank of tissues allows us to follow up several lines of disease pathology, so that we and other groups through internal and international collaboration, are able to facilitate later studies without the need for additional numbers of animals, thus significantly reducing the need to use more animals.

Where possible we will follow up observational studies in the animal with non-animal cell systems to gain mechanistic insight into specific cell signaling pathways. These studies will help define the specific pathways involved and thus inform specific intervention, resulting in a reduction in the number of animals and a high degree of refinement to the proposed intervention models. For example, we observed that maternal obesity “programmed” a loss of IRS1 (an insulin signalling molecule) in the fat tissue of the offspring of obese mouse dams. In the same tissues, we also observed a gain in the expression of a small RNA molecule (microRNA) known to negatively regulate IRS1 protein levels. In order to investigate if these programmed changes could be replicated in a cell system, we obtained precursor cells from the fat tissue of these mice and grew them into mature adipocytes in vitro. This

experiment showed us that despite being grown and outside the animal, these precursor cells carried the information encoding the programmed phenotype observed in the mature fat cells of the animal. This strategy will allow us to **reduce** animal numbers further by adopting a cell system widely used in studies of regulation of fat metabolism for our more complex studies. We will also couple the use of this cell system with a global approach to identify other proteins regulated by the overexpression of any microRNA in a non-biased manner and without *a priori* bioinformatics prediction.

We will use non-invasive echocardiography with recovery anaesthesia to monitor cardiac function longitudinally, in the same animal that **reduces** the number of animals required. Isoflourane is very well tolerated in every animal as we maintain anaesthesia for no longer than 20 minutes and recovery is quick (under 1 minute). This provides substantial gain in power and data quality and robustness thus reducing animal numbers.

We are introducing some new techniques: 1) Labelling with a marker for impaired oxygenation of fetal tissues and placenta and 2) Ultrasound imaging of the pregnant dam at critical stages of fetal development. These 2 techniques will inform on the effects of maternal condition to her offspring in early life and during organ development, even before the pups are born. In the first approach, a chemical which binds to tissues affected by reduced oxygenation, will be injected into the pregnant animal shortly before killing. The label will identify both placenta regions/specific cells and developing fetal structures affected by maternal condition/treatment using histology with antibodies. In the second approach, we will monitor maternal heart function and blood flow in major vessels connecting the mother to the fetus as well as fetal heart function using ultrasound. Additionally, by administering various drugs that modulate contraction or dilation of the heart and blood vessels during the ultrasound, we can gain more specific information on the types of loss of function and the mechanisms involved. Similar information will be gained by using this ultrasound technique in the adult offspring.

We currently use a technique for labelling fetal tissues that are actively growing during gestation by injecting a dye into the mother which is trackable. We now wish to extend this approach to measure the expansion of cell populations during early postnatal life.

Refinement

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

Refinement

Females from our breeding colony will be randomised (by animal technicians blinded to the study) to receive experimental diets and or interventions. At sexual maturity, they will be mated with male studs that are refreshed every 6 months to optimise breeding and minimise ageing paternal effects on the offspring. Offspring are again randomised (by technicians blinded to the study) to be weaned onto control or obesogenic diets with or without pharmacological intervention. Where possible, investigators carrying out cardiometabolic measurements will be blinded to the experimental groups.

In our exercise intervention studies, we apply the knowledge that rodents are nocturnal and therefore they respond better to exercise training at the beginning of their wake cycle (which is after 6pm in the evening when the lights are out). Our researchers therefore go in to train the animals in the dark with a red headlamp to minimise disturbances to their circadian rhythm.

We use non-invasive TDNMR that does not require anaesthesia to measure body composition of mothers and offspring. As this allows longitudinal body composition to be measured in the same animal, it also reduces the number of animals required. We combine this data with other repeated longitudinal measures such as non-invasive tail cuff blood pressure and Echocardiography. Finally, at post-mortem, we take blood to measure metabolites and tissues for molecular studies. All the data can be correlated and then also compared to aged controls to identify if there is an advanced aging phenotype. From this, we can identify markers at the cellular level indicative of ageing, which enables us to assess the effects of early nutrition on life span without the need for maintaining mice and rats for their full lifespan. During the next 5 years, we hope to acquire access to more advanced imaging equipment that would allow in-utero measurements and measurement of ECG in conscious animals.

Offspring of obese or undernourished mothers may be affected by a lack of oxygenation during development in the womb. Introducing a chemical that directly labels tissues and cells so affected is a refinement to various indirect techniques currently in use (e.g. manual techniques using whole blood, which are prone to errors as once a fetus is removed from the amniotic sac, it is exposed to ambient oxygen levels). Ultrasound imaging of both the mothers' and offspring heart and vessels whilst still in the womb greatly advances our understanding of blood flow and therefore the availability of nutrients to the developing embryo.

PROJECT 114. 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	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. We are primed to move rapidly into early phase clinical trials through the NIHR Biomedical Research Unit with the partnership of the pharmaceutical industry. Our pioneering studies have already illustrated common mechanistic regulators of disease in several organs and extension of these studies has the potential to not only identify new therapeutic targets but also to extend the licensed use of pre-existing therapeutics. Thus our data is thus likely to be used by basic scientists and clinical scientists to inform the design and outputs of their own experiments. As required by our funding partners, data originating from these studies will be published in high impact scientific journals confirming with the ARRIVE guidelines provided by NC3Rs, and presented at national and international symposia and conferences. Thus benefits from our work include transfer of knowledge, training opportunities for future clinicians and academic scientists as well as improvements in treatment for UK patients and the healthcare industry.

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

We will use mice for our experiments and expect to use up to 800 over the five year term of the licence.

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

This model is classed as severe because it is necessary to induce liver injury and inflammation in our mice. We would expect all of the mice in the untreated injury groups to exhibit some degree of weightloss (<15%) and deterioration in condition (ruffled coat and reduced mobility) for a transitory period after alcohol administration. All animals are humanely killed at the end of the experiment.

Application of the 3Rs

Replacement

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

Replacement

The complex disease pathways we are interested in involve the interaction of several cell types and regulatory signals that are hard to recreate *in vitro*. We also do not have access to samples from humans in all stages of alcohol-induced injury. Mice share the main components of their immune systems with humans, and established alcohol injury models recreate the patterns of disease seen in humans. A wide range of genetically manipulated strains and therapeutic reagents are available for mice and thus they are the best model for us.

Reduction

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

Reduction

We have prior experience using the model, which will inform the design of experiments in this study. Importantly we have noted inter-individual variation in response and so our experiments are powered to take this into account and in conjunction with our local facility we have devised a flexible dosing approach based on clinical scoring to maximise our outputs and minimise animal loss. We have built in checks in our workflow to ensure that experiments do not progress if statistically significant results are not evident upon an intervention. Similarly experiments run serially with outcomes from initial animal groups informing the design of subsequent experiments. For all experiments the scientific team meet regularly to discuss data and seek advice from local statisticians and clinical staff. Importantly our experimental design strategy is informed by use of the NC3R's experimental design assistant (EDA : http://www.nc3rs.org.uk/experimental-design-assistant-eda)and in conjunction with adherence to the ARRIVE guidelines, to ensure the minimal numbers of animals are utilised in order to gain valid experimental outputs.

Many of the molecular pathways we investigate operate in more than one organ. Therefore to maximise the useful information we can collect from each animal, we will collect blood, liver and other solid organs. These samples can later be used to investigate the wider significance of our pathway or therapeutic intervention. We work closely with collaborators at other institutes and have a policy to ensure tissue is shared with our colleagues so that maximal use is gained of each individual animal and that new knowledge generation is facilitated.

Refinement

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

Refinement

Although many alcohol injury models are used worldwide, few both recreate the histological picture seen in humans AND meet the strict welfare conditions we adhere to in the UK. We have chosen a model that is quick to perform, recreates human alcoholic hepatitis, and has been refined by our past experiments. This means we individually tailor our monitoring and alcohol exposure to ensure weightloss and loss of condition are minimised.

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	Models advancing knowledge and treatment of paediatric brain cancer
Key Words	Paediatric, brain, Cancer, Treatment, Biology
Expected duration of the project	2 year(s) 9 months

Purpose of 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 main objective is to understand the development of and find new treatments for four types of paediatric brain tumours.

Ependymoma (EPY), Medulloblastoma (MB), High-Grade Glioma (HGG), and Choroid Plexus Carcinoma (CPC) are the most common solid cancers to affect children. Each presents a unique set of clinical challenges, and all require new treatments. With few exceptions, childhood brain tumours remain one of the biggest killers from disease in children and require aggressive surgery, radiation and chemotherapy that have changed little in several decades. Radiation is especially damaging to the developing brain and results in devastating long-term cognitive side effects for survivors. Fewer than 70% of all patients are cured following initial therapy and once these tumours recur they have a dismal prognosis. Importantly, Ependymoma and CPC are relatively insensitive to chemotherapy and there is therefore a great need for effective new treatment strategies. By investigating the development of these detrimental diseases, we will advance the understanding of the underlying biology of all four tumour types. By exploring new and innovative treatment strategies we will hopefully be able to translate new treatments into the clinic.

HGG is a particularly lethal form of childhood brain tumour, the origins of which are only recently beginning to be understood. In order to direct the design of new treatments further study is needed to understand how the tumours initiate and how they progress. The recent development of mouse models for this disease type is a critical step in that process. The research proposed in this project will use these models to fill gaps in knowledge about the disease and be crucial in understanding the mechanisms of acquired resistance to treatments.

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

Our project holds great promise to make fundamental and much needed progress in advancing understanding of the origins, biology and treatment of paediatric brain tumours. The benefits of this project are numerous and include but are not restricted to: (i) advancing the knowledge of four paediatric brain tumours (ii) Provide brand new and repurposed drugs for clinical testing.

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

We will be working with mice (including genetically engineered mouse models). We expect to use around 20,665 mice over the licence period of 5 years.

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

A number of the animals required in this project will be used to breed subjects for further study. Animals used purely for breeding and that do not undergo any procedures except for ear notching will be humanely killed when they are no longer required. Some of the animals will develop adverse effects, including cancer as a result of their genetic makeup or because tumour cells have been implanted and allowed to grow. This may require administration of an inducing agent or a virus that will switch on/off particular genes. This administration will only cause momentary discomfort, but all animals will be monitored closely for clinical signs related to tumour growth, including loss of 15% body weight, limited normal behaviour, loss of movement on one side. Animals in distress will be humanely killed. Tumour development may be monitored using imaging techniques, such as MRI scans. These methods may require anaesthesia and/or administration of imaging agents, which will not result in any harm to the animals. Some of the animals that develop tumours will be treated with surgical resection and/or irradiation, in order to mimic the clinical standard. Many of these will go on to receive treatments with anti-cancer drugs, including potential new therapies. All animals on treatment studies will be closely monitored and may be blood sampled to follow uptake of the drugs which should only cause only mild momentary discomfort. Any animal that displays clinical symptoms such as those listed earlier, will be humanely killed. At the end of the study all animals will be humanely killed, and tissues collected post-mortem to gather as much information from the study as possible.

Application of the 3Rs

Replacement

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

Replacement

Because our approach requires the use of specific cancer-susceptible cell types at specific points in development, this is currently only possible by using live animals that fully recapitulate the complexities and cell populations present in development.

Regulatory and research bodies require preclinical assessment of potential therapies in animal models prior to their translation to the clinic. Therefore, our translation of optimal new therapies to the children's cancer clinic requires the animal studies proposed here. Nonetheless we will continue to use *in vitro*drug sensitivity studies, including radiation/chemotherapy combination studies*in vitro*to optimise the selection of agents and thereby minimise the use of animal models in exploratory studies.

Reduction

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

Reduction

Our use of *in vitro*methods limits the number of animals required for the *in vivo*investigation stage. Furthermore, each of our *in vivo*mouse model experiments has a careful statistical design that is aimed at minimising the use of animals while ensuring robust and meaningful statistical end points. These animal numbers are selected in collaboration with our statistical colleagues and our extensive experience with brain tumour mouse models. In addition, we have optimised the use of material from each mouse, often harvesting fresh cells for culture, frozen tumour for RNA and DNA studies and fixed material for histology from the same animal.

Refinement

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

Refinement

Mice are the only species employed in our protocols. They are the least sentient species that best fit our criteria for the following reasons: Their lifespan (approx. 2 years) allows for "development to humane endpoint" studies; the scientific community has a range of techniques to manipulate the mouse genome, allowing us access to many transgenic/knock-in/knock-out mice with which to answer specific key questions; mouse gestation is less than three weeks, and embryogenesis in this species is extremely well documented in the literature, allowing us to look at the effect of genes on normal brain development. Non-animal models cannot recapitulate the complex context existing in developing tissues in which cancers actually form and are treated. The advancement of knowledge and development of concepts to improve human and animal health and well-being requires the use of

living animals. Exhaustive literature searches in brain tumorigenesis show that our tumour systems are the most accurate models for the study of these diseases.

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	Developing an ocular melanoma model for drug discovery
Key Words	ocular melanoma, uveal melanoma, Patient derived xenograft, MEK
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):

Uveal melanoma is a rare form of cancer that arises from the eye. Unfortunately it often spreads to the liver and treatments are mostly ineffective. In this project we hope to find out how and why this cancer is resistant to a type of drug called selumetinib. We anticipate that the results of the study will help us design new combinations of drugs (including selumetinib or relted drugs) that will overcome this resistance.

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

Uveal (also known as ocular) melanoma is a rare cancer arising in the eye. Unfortunately it spreads to the liver in about half of all cases and is invariably fatal. At present there are no established therapies for uveal melanoma, and resistance to drug therapy is very common. We intend to decipher the main mechanisms by which this occurs thus developing new combinations, which may help control the disease and prolong survival.

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

We will use approximately 300 mice over 3 years.

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

The severity limit of the experiments is moderate, and most animals will have limited adverse events. All animals will have tumour cells implanted under the skin and these will be allowed to grow up to about the size of a pea. Some animals will have drugs given to them which may cause some adverse effects, however in all cases the side effects will have been established previously and a dose used to minimise these. At the end of the experiment the animals will be killed humanely and tissue extracted for experiments.

Application of the 3Rs

Replacement

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

Replacement

Wherever possible we will perform experiments in cell lines or directly on human samples. However, these experiments cannot model many of the effects of growing tumours inside humans such as the presence of other cells, growth of blood vessels into the tumour and varying concentrations of oxygen and nutrients in differing parts of the tumour.

Reduction

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

Reduction

We will only use enough animals to establish our model systems and for the purposes of our assays. Where we perform experiments to contrast different treatments, we will perform calculations to identify the minimum number of mice needed to show a meaningful result.

Refinement

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

Refinement

While simpler organisms may be used to perform experiments on the basic biology of cells, the experiments we will be performing need us to be able to grow tumours derived from humans. Mice are the simplest model system, which will allow us to do so and then treat with drugs. We will monitor the condition of mice daily and weigh at least weekly. Where animals appear sick we will observe more closely, and if they are not recovering, the animals will be euthanized. Tumours will not be allowed to grow beyond a specific limit which has been established in previous experiments. Where drugs are given, these will be used at a dose that has been shown to be tolerated well by animals without significant adverse effects.

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	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 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	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 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	Strategies for Brain Repair
Key Words	Brain repair, transplantation, neuroscience
Expected duration of the project	0 year(s) 6 months

Purpose of 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 develop novel strategies for treatment of brain damage, whether caused by injury or disease, with a particular focus on the development of novel cell and gene therapies for Parkinson's disease (PD), Huntington's disease (HD) and stroke

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

This work underpins clinical trials of fetal tissue transplant¬ation in HD and PD taking place now, and provides the biological foundations for the next generation of major new applications using more efficient sources of cells, including pluripotent stem cells.

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

Rats and mice. The project will use approx. 300 rats and 500 mice over 6 months.

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

The project involves surgical, anatomical, physiological and behavioural procedures of mild, or at most, moderate severity, including breeding genetically modified animals, that express modest impairments of motor and cognitive disability, that are the targets for structural repair and functional amelioration. The experimental procedures are reliable, and serious adverse effects are rare and not expected, but procedures are in place for rapid alleviation of distress in the case of unexpected adverse events being detected. All animals are killed at the end of each experiment by the most humane methods appropriate to the species.

Application of the 3Rs

Replacement

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

Replacement

Motor and cognitive behaviours are complex features of the living sentient animal, dependent upon the intact functioning of a complex living nervous system, and impaired in human neurodegenerative diseases. The survival, growth and connectivity of cells in this complex environment cannot be adequately modelled in vitro or in simulation. Thus, in order to develop effective new cell-based therapies for devastating human conditions, the experimental use of live animals is the only way to model the disease processes, to determine the survival integration growth and connectivity of cell repair processes, to test the effectiveness of alternative cell therapy procedures, to develop the transplantation technology and to test protocols for safety and efficacy prior to human application.

Reduction

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

Reduction

All protocols are designed for maximum sensitivity, and experiments are designed to maximise power to detect significant results with the smallest numbers of animals achievable. Non-animal alternatives e.g., tissue culture are used to optimise all cell preparation protocols prior to assessment in animals, but ultimately the in vivo situation cannot be avoided if the goals for human health are to be achieved.

Refinement

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

Refinement

The organisation of motor and cognitive functions and of the brain systems that underpin them are relatively consistent among mammalian species but differ progressively from non mammalian brains. Rats and mice are used as the least sentient mammals to model the relevant systems and functions disturbed in human neurodegenerative disease. These species tolerate well living in the laboratory environment, and provide the most extensively validated models for addressing the physiological, anatomical and behavioural functions under investigation. All animals are housed in licenced facilities and cared for by professionally trained staff following procedures designed to optimise health and welfare, operating under a rigid

inspection system to ensure compliance with full and continuous attention to welfare regulation and best practice.

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	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 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	Bioelectronic Medicines
Key Words	Electrophysiology, Implantable Devices
Expected duration of the project	5 year(s) 0 months

Purpose of 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 understand how implantable devices and electrical signals can be used to regulate the nervous system to treat disease and organ dysfunction.

To do this we must first gain a better understanding of the anatomy (**Protocol 1**) and function (**Protocol 2**) of the nervous system, and how it exerts control of organ function. Secondly we must ascertain whether electrical regulation of the nervous system can be accomplished safely and effectively over time (**Protocol 3**). Thirdly we wish to apply this regulation treatment in animal models of disease, such as diabetes (**Protocol 4**), infertility (**Protocol 5**), and arthritis (Protocol 6), to understand the magnitude of treatment effectiveness.

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

Currently molecular medicine treats a wide range of ailments in billions of people. However, there are a multitude of side effects and treatment resistant populations. Additionally continual reliance on molecular medicine is expensive, socially limiting, and in most cases only a treatment and not a cure. The potential for Bioelectronic medicine is huge. Through implantation of devices that regulate the nervous system, and in turn the target organs, one can reverse organ dysfunction and disease states completely. We have chosen to focus initially on 3 disparate diseases that we have clear reason to believe that bioelectronic medicine could be a success, therefore ultimately improving people’s quality of life. Type 2 Diabetes is wide spread, and will continue to devastate people’s lives in the developed and developing world. Reliance on molecular medicine is debilitating. Polycystic Ovarian Syndrome affects millions of women and currently has no clinical treatment options. Rheumatoid Arthritis is a chronic progressive inflammatory condition affecting millions.

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

Mouse (550 over 5 years) and Rat (2750 over 5 years).

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

Work will initially involve non-recovery models for assessment of anatomy and physiology, and by definition no side effects will be encountered. Recovery studies will only be initiated once correct surgical approach and treatment refinement has occurred in non-recovery models. The expected adverse effects are associated with the 3 disease models, and they will manifest as hyperglycemia and weight increase (T2D), loss of ovulation (PCOS), and joint swelling in the limbs. This will not lead to any behavioural effects in these animals. Implantable devices will be thoroughly refined and miniaturised for use in rodents. The only expected side effects are related to post-surgical complications, such as infections, broken sutures, local inflammation and swelling. Levels of severity will not exceed moderate. All animals will be killed by a schedule 1 or perfusion-fixation methods.

Application of the 3Rs

Replacement

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

Replacement

A limited amount of testing has been done without using animals to give confidence nerve stimulation may treat disease. The science cannot be advanced further without using animals. Only a whole body system biology approach will give conclusive evidence and understanding that manipulation of the nervous system can be an effective treatment of disease.

A computer model does not yet exist to test nerve stimulation as a treatment of disease.

Reduction

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

Reduction

Pilot studies in small numbers of animals (e.g. 1 to 3) will be used to develop optimal methods**,** assess feasibility and outcome measures, and to estimate required group sizes for larger studies. Statistical advice will be sought on adequate animal numbers for each recovery study. Imaging techniques will be used to monitor evolutions within the same animal over time.

Refinement

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

Refinement

Rodents will be used for all experiments because they are the lowest order species with a nervous system that is anatomically and functionally similar to that of humans.

Aseptic surgical techniques, anaesthetics and pain preventing medicines will be employed to minimise potential of post operative infection and pain. Veterinary care will be provided throughout.

We will work with manufacturers, to ensure a continued refinement approach is adopted for all implantable devices, electrodes and leads. We will work toward fully implantable devices as advancement to percutaneous leads or head caps. We will always use the least minimally invasive option for each study.

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.

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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 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	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 3500 mice will be required to perform the planned experiments over the five year time period.

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

Animals used in these experimental procedures will be given tumour cells under the skin resulting in the formation of a local tumour at this site. These tumours do not spread so discomfort to the animal is minimal. This will cause local discomfort at the site of the tumour and inflammation at this location. Some mice will receive carcinogens on or under the skin to induce local tumour formation. This approach will irritate the local skin and cause discomfort. The mice will then experience a local tumour at this site with the discomfort associated with this. In some experiments mice will undergo minor surgery including exposure of the kidney to graft tissue under the kidney capsule. Mice will only undergo one form of surgery. Mice will be handled frequently (ranging initially from approximately three times per week, increasing to daily towards the end of each study). During these times animals will be injected and / or monitored for tumour burden. Through good handling techniques, distress caused to the animal from being restrained will be minimised in terms of time and discomfort (a single animal will typically be restrained for less than 30 seconds). Mice will be monitored for tumour burden frequently and tumours will be scored for size, position and ulceration. Should tumours limit mobility, appear ulcerated or reach a maximum permissible size, animals will be humanely killed by a schedule 1 method. Mice will be humanely killed by a schedule 1 method when the tumour reaches a certain size (1.2 cm mean diameter).

Application of the 3Rs

Replacement

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

Replacement

This project requires animals as we seek to understand the movement of cells into and out of tumours to inform treatment of cancer in humans. Such interactions

cannot be modelled in vitro due to the many complex parameters and multiple three-dimensional environments involved. Thus we require an in vivo approach to recapitulate the complex situation present in patients. We can use existing mouse models to accurately assess cellular migration in several different tumour models. These have been selected as they are currently used to assess therapeutic treatments that have been demonstrated to work very well in some patients.

We regularly review the literature to keep informed of any new developments in experimental approaches that might enable the replacement of animal experiments with in vitro work.

The use of the animal models described means that our data can rapidly inform current treatments, since the models used are currently those that inform clinical work. The insight our work can provide into the action of checkpoint inhibitors is of a very broad applicability to cancer treatment and is fundamental information that can improve our understanding of how these therapies work and how they can be improved.

Reduction

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

Reduction

Small scale pilot experiments will be used to establish models building on expert advice from collaborators using these approaches. Experiments will be designed following NC3Rs EDA guidelines, using power calculations and previous advice from in-house statisticians. We reassess group sizes as our experience with models develops and we will continuously look to use the minimal number of mice that provide robust experimental data. I have been publishing the results of my in vivo analyses of immune responses for over 12 years in high impact journals reflecting the expertise we have in the appropriate design of this type of experimental work.Publication of this work requires peer-review and this process ensures robust assessment of our experimental work and dictates that our experiments are well performed. We will continue to do this. Should further assistance be required we will reach out to local statisticians and/or the local NC3Rs advisor.

Refinement

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

Refinement

Choice of Species:

The mouse provides an excellent model in which to study the relationship between the immune system and tumour growth, since mice are well characterised immunologically, and their immune systems closely resemble those of humans. In addition, several genetically modified mice lacking various immune molecules/cells have already been generated and provide an ideal opportunity to perform detailed analyses of immunological function. Fundamental to this study is the use of specialised mice in which violet light can be used to label cells at a specific site to allow direct analysis of cellular movement. This in turn enables the dynamic changes in the cells to be assessed and factors affecting this movement to be precisely tested.

Choice of Models:

Tumour models that are proven to work in the mice have been selected based on the suitability for use in our mouse models and the use of these tumour models by many labs to inform treatments of human patients.

Minimising Animal Suffering:

In all procedures animal suffering will be minimised through good animal handling techniques and strict adherence to monitoring procedures outlined in detail in Section E. These monitoring procedures ensure that any potential adverse effect of tumour growth is spotted before pain or distress is caused to an individual animal. In the case of the tumour models described herein, particular attention is paid to ulceration of tumours and to effects on mobility as well as to the general well-being of individual animals.

Review:

We critically review experimental approaches at the end of each experiment and look constantly look to refine our work as it progresses.

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	Pig models of poisoning & drug toxicity
Key Words	poisoning, antidotes, treatment, mechanisms
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Poisoning is a major global health problem causing hundreds of thousands of deaths each year. Self-poisoning with medicines (‘attempted suicide’) is responsible for 10% of all medical presentations to hospital in the UK. Medicines such as diltiazem and paracetamol are responsible for several hundred deaths each year in the UK.

Self-poisoning is an even greater problem in rural Asia. Here pesticide self-poisoning is a major public health problem and one of the three most important means of suicide worldwide, killing more than 150,000 people each year. Many of these suicides occur from organophosphorus (OP) insecticide poisoning, but other types such as paraquat and aluminium phosphide can be devastating.

The study of poisoning in humans (clinical toxicology) is a neglected area of medicine, with little active research. Few animal models exist with which to study what happens after poisons enter the body - information that is essential to find novel treatments. Few effective and affordable antidotes exist for severe poisoning.

This project will use pigs to identify effective antidotes for poisoning and to better understand what poisoning does to the body. This will be done by giving poisons to anaesthetised animals and studying the effect of poison and treatment. Lessons learnt from these animal models will be rapidly considered for studies and trials in humans

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

This project will increase our understanding of how poisons affect the body, in particular how OP insecticides cause our muscles and nerves to stop working and the lungs to become damaged. It may also find new treatments (or antidotes) for cyanide poisoning that are better at saving lives than our current treatment options

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

We will use up to 150 pigs over 5 years. Previous work shows that detailed studies in a small number of pigs are able to provide scientifically powerful data that will guide human treatment

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

All animals will be anaesthetised at the beginning of the study so that they are unaware of any study procedures. They will then have minor surgical procedures to place monitoring and blood sampling tubes into an artery and veins so that blood samples can be taken for tests and the condition of the heart carefully watched. The wounds will be stitched up after insertion of the tubes. Poisons and/or treatments will also be administered via these tubes or by a tube placed into the stomach. All animals will be cared for by veterinarians who will closely monitor for adverse effects. They will be watched for the effects of the poison and how this is controlled (or not) by the antidote. At the end of the study, the animals will be killed by a humane method and tissues taken for analysis after death. There are no severe protocols on the license and no animal suffering except that associated with routine administration of sedative or other drugs before anaesthesia.

Application of the 3Rs

Replacement

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

Replacement

It is not possible to set up models of poisoning in humans or to test new antidotes that have not previously been tested in animals. Studies done in test tubes or on computers are unable to determine the efficacy of antidotes or therapeutic interventions for poisoning in living humans because they cannot reproduce the complex multiorgan effects of the poisons against which the antidotes must work. Animal studies are therefore required.

Human patients presenting to hospital with self-poisoning are very variable. They have ingested differing amounts of different poisons, at different times, and have received different treatments before coming to hospital. Furthermore, the dose ingested is rarely known and the actual compound ingested may well not be known for several days, if at all. This marked variation between human patients makes clinical research difficult.

Large controlled studies in hospitals allow the variation to be balanced out but such trials are expensive, difficult, and only to be attempted when there is good evidence from both animal studies and early human studies that there is a reasonable likelihood of effectiveness.

Animal studies can be more controlled, with a specific dose of a particular poison administered at a specific time point, thus allowing much smaller numbers of participants.

We have shown that pig models of poisoning provide a large amount of relevant information on what poisons do in the body and whether treatments work - all information that can be rapidly translated into human studies

Reduction

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

Reduction

We work with experienced statisticians to ensure that the minimum number of animals are used for each study, while maintaining scientific quality

Refinement

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

Refinement

We have chosen pigs (either the Gottingen minipig which has been bred to be small or outbred farm pigs) for our studies because pigs are much closer to humans than rodents. Due to similarities with humans in how pigs handle and break down medicines, they have become an increasingly important model species for understanding the benefits and harms of new drugs.

The large size of the species has several further advantages including: a longer, and more clinically relevant, time course of study for most diseases; ability to repeatedly sample blood and tissues; and the use of readily available hospital equipment for humans to record changes and to image the animals.

Unfortunately, previous animal models of poisoning using rodents have not been closely related to the human situation and their data could not be extrapolated to clinical practice. For example, most studies of OP insecticide poisoning and its antidotes have involved measuring how many animals survive to 24hrs with or without certain treatments. However, these studies do not mirror what happens in people. The OP pesticide has been given in the wrong form and by the wrong route.

The treatment has been started: at the wrong time; with treatment doses that differ from doses used for humans; without the typical intensive care support available to humans; and without the intention of giving the animal comprehensive treatment. Our pig models address all these limitations.

All studies on this license will involve anaesthesia before poisoning. There are no severe protocols on the license and no animal suffering except that associated with administration of sedative or other drugs before anaesthesia.

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	Understanding the regulation of brain monoamine neurotransmission in health and disease
Key Words	Neurotransmission, Dopamine, Basal ganglia, Parkinson's disease, Acetylcholine
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Overall objective:

Brain cells, or neurons, that use the transmitter dopamine, carry out key functions in our everyday motivated actions as well as out learned habits. We think these neurons tell us about things in our environment that have some motivational value that help us to detect them, and then respond optimally to benefit from them. These neurons die in the neurodegenerative disease Parkinson's disease. There is therefore a need to understand the workings of these cells better so that we can not only advance biological knowledge, but also improve our understanding and treatment of Parkinson's and related diseases.

The work we propose will promote our understanding of how dopamine regulates our everyday behaviours, and it will also allow us to explore at a subcellular level how these neurons communicate from synapses.

We will work towards these goals through a program of work that will identify how neurotransmission by dopamine (and related transmitters) is regulated by neural circuits with other neurotransmitters, neurotransmitter receptors, cellular signalling pathways, regulatory genes, and related mechanisms. We will also examine how dopamine release governs behaviour.

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The work we propose will promote our understanding of how dopamine regulates our everyday behaviours, and it will also allow us to explore at a subcellular level how these neurons communicate from synapses.

We will work towards these goals through a program of work that will identify how neurotransmission by dopamine (and related transmitters) is regulated by neural circuits with other neurotransmitters, neurotransmitter receptors, cellular signalling pathways, regulatory genes, and related mechanisms. We will also examine how dopamine release governs behaviour.

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

This work should therefore advance basic biological knowledge and understanding of many brain functions relevant to our everyday motivations and actions. It shed also light on mechanisms relevant to key brain disorders. In turn, we hope to gain insight into potential future therapies for these disorders for which there are currently still very few effective treatments.

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

Over 5 years, we estimate that we may use up to 2,600 mice in procedures other than simply breeding and maintenance. We may breed and/or maintain up to 12,000 mice, some of which will be the same ones used in the additional procedures. Mice will be the species used because they are the lowest vertebrates in the phylogenetic tree for which brain dopamine systems are suitably well characterised and comparable to that of humans, as well as there being models for neurodegenerative disease. The mouse is also currently the most tractable mammal for use in genetic studies.

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

We will raise genetically altered mice that allow us to explore the functions of key molecules in these mechanisms. In some animals we will insert genes into the brain during general anaesthesia which allow animals to express proteins that can be targeted with flashes of light or designer drug tools to activate the neural circuit we want to explore. Some animals might instead receive a toxin or will be genetically altered to make the animals begin to develop a Parkinsonian condition so that we can understand the disease better, and explore some options that might treat it. Some animals might be given drugs regularly when awake over a few weeks to enable us to understand better the processes which become disturbed, or how we might treat them. And a small number group of animals will have small microelectrodes implanted in their brains and then be allowed to roam freely so that we can understand how neural circuits are important to behaviour. The adverse

effects that some animals might experience might include the effects of brain surgery under general anaesthesia which might include transient pain and bleeding, some disturbances to normal movement particularly some slowness or other slight difficulties in initiating movement, which might compromise their ability or a failure to thrive. Animals will be well supported during these times. At the end of the experiments, the animals will be humanely culled.

Application of the 3Rs

Replacement

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

Replacement

This work is an exploration of fundamental mechanisms of operation of the brain and also studies the adaptive mechanisms and/or the impact of drugs in neurodegenerative disease. Use of live animals and real brains is therefore needed to provide tissue with synaptic circuitry that resembles the in vivo scenario. We are not aware of any alternative which does not use animals that would allow progress to be made towards the objectives. No cell or culture alternative can adequately provide this. We will use virtual neuron computer models in the limited experiments for which this is appropriate.

Reduction

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

Reduction

We will keep animal numbers to a minimum by using power calculations and pilot studies where appropriate. We will also use experimental designs and powerful techniques that are high yield, by allowing multiple refined measurements per sample, or per brain or per animal.

Refinement

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

Refinement

We have chosen mice because their dopamine systems are very similar to those of humans and because they are highly suited to using genetic manipulations that will help us understand how the dopamine system works in health and disease. The

genetic tools we can use allow experiments and manipulations to be highly targeted to the cells we are studying, and therefore very refined.

We will select for each experiment the most refined mouse brain preparation. Sometimes brain slices are ideal because they have a good balance between containing substantial normal circuitry, unlike isolated cell preparations, whilst also allowing good access to the neurons we want to visualize and study. Sometimes, we need to use whole animals, when they are the only means to understand brain cell function in relation to behaviour.

The mouse models of disease we will use are the best available, and each one has been chosen because it closely mimics key aspects of the disease and with minimal suffering, and so is very refined.

We will use the lowest severity models applicable to each of our aims. Our general measures to minimise suffering in interventional experiments include appropriate use of anaesthesia, aseptic surgeries, close post-operative care, analgesia, and support. In all cases where an intervention is applied *in vivo*, monitoring systems and humane endpoints will be in place.

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	Mechanisms of Acute and Chronic Inflammation
Key Words	Inflammation, Lung Diseases
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Inflammation is a normal biological process that the body uses to protect itself against diseases and for restoring damaged tissues back to normal function. However, when inflammatory processes are poorly controlled or directed against normal bodily functions they are harmful to the affected individual and result in diseases such as Asthma and Chronic obstructive pulmonary disease. The inflammatory response involves directing specific white blood cells to sites of disease where they interact with other cells to secret substances which allow the cells to divide and fight the disease. However, how the cells are directed to and function at sites of disease and damage are not well understood. The overall objective of our investigations is to identify molecules that start and propagate the inflammatory response.

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

Understanding the mechanisms involved in the inflammatory response is fundamental to analysing the processes of infectious disease control on the one hand and inflammatory diseases on the other. This understanding is critical for the design of new drugs for common inflammatory diseases for which there are currently few effective treatments.

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

We expect to use only mice (approximately 30000) over 5 years, amongst 20 researchers.

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

The following procedures are expected to cause some moderated discomfort, that animals will fully recover from; bone marrow ablation and reconstitution (tiredness

and reduced appetite), splenectomy (pain) and pathogen exposure (weight loss). Other procedures, such as administration of substances, assessment of lung function and taking of blood samples cause mild transient discomfort and no lasting harm. We do not anticipate any severe adverse events. However, we will monitor animals for recognised physical and behavioural changes that indicate ill health. Mice displaying two or more of these will be humanely killed. All experimental mice will be humanely killed at the end of the experimental procedure.

Application of the 3Rs

Replacement

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

Replacement

Due to the multi-cellular interactions involved in the inflammatory response and in inflammatory diseases such as asthma the responses cannot be adequately or fully mimicked by in vitro studies alone.It is critical to perform these studies in mammals since there are significant differences between the biological systems of frogs and fish to that of humans. Where possible we will complement the in vivo work with experiments using in vitro culture systems taking advantage of isolated human cells and cell lines to investigate selected pathways identified in the in vivo models. Throughout the project, where possible, non-animal experiments will be employed. This will include developing in vitro systems such as ‘lung on a chip’ and exploiting in silico technologies and databases where appropriate.

Reduction

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

Reduction

We have developed our experiments so that we can measure multiple parameters in each animal, thus maximising the information gained from each experimental group and minimising the number of animals used. Combining tests in the same mice allows the data gained to be correlated directly, rather than inferred. Based on previous experience we have calculated the minimum number of animals needed to see desired effects using robust statistical analysis. Also, all experiments will be conducted in accordance with the NC3Rs' ARRIVE guidelines. All of which help to minimise variation and avoid unnecessary repeats.

Refinement

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

Refinement

We have chosen the smallest animal possible to represent human disease. While the models chosen closely represent the features of the human disease in the treated animals, they are the least severe and do not promote undue distress to the mice. We constantly monitor animals for signs of ill health and work closely with animal care staff and veterinary surgeons to ensure the best possible husbandry and welfare for mice under procedure. Analgesia is routinely provided to all animals when required.

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	Neuronal cell development and survival
Key Words	Motor neurone disease, Axonal transport, RNA metabolism, Cell biology
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Our objectives are: 1) To understand how defects in the components of the intraneuronal transport and signalling systems lead to the death of motor neurons in motor neuron diseases. 2) To investigate the underlying mechanisms of the roles of proteins implicated in motor neuron disease in response to DNA damage and to elucidate how defects in these proteins could affect the expression of other genes.

Our study will contribute towards our understanding of the mechanisms of motor neuron death caused by defective intraneuronal transport or response to DNA damage. Therefore, our findings will benefit the scientific community with a broad range of interests in neurological conditions. Moreover, working from the mouse models of motor neuron disease to mouse primary cells and neurones derived from reprogrammed mouse skin cells, will aid the understanding of the mechanisms of disease onset and progression. Using this knowledge in human derived fibroblasts and neurones and applying this information back to human conditions and for cross species comparisons at the cellular and neuronal tissue levels will set a paradigm for the effective use of both the mouse and human-derived cells as valuable model systems.

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

This research will benefit patients and their families, who have been affected by motor neurone disease, hereditary motor neuropathies, and some cases of intellectual disability; and health professionals, who work with the above mentioned patient groups. The benefit from the outcomes of this study could be immediate, as our findings could inform the beneficiaries about the causes and basic mechanisms of the disease. In the longer term, this project will contribute to: 1) our understanding of the relationships between defective axonal transport or DNA repair response with abnormal neuronal cell function and development; 2) and hence, discovering novel drugs and more effective treatment of the above mentioned diseases and perhaps

other related disorders; 3) validating the promising drug targets in preliminary pre-clinical settings; 4) informing patients and ensuring best possible care planning.

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

Mice,6000 over five years

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

A proportion of the animals used in this study are transgenic mice that start showing signs of a progressive muscle weakness in their limbs. The level of severity of the phenotype in these mice is substantial, as this is a progressive condition which leads to paralysis and it is crucial for this research to obtain tissues from all stages of the disease in order to pinpoint the correct pathway that is impacted, or the efficacy of the drug. To minimise the animal suffering we monitor these mice twice a week between from the pre-symptomatic stage. Mice with signs of paralysis will be given dry mash and gel blocks and their food and water intake will be monitored daily. Mice will be weighed once and checked twice every day till end point (righting reflex within 30s is not observed; or 15% loss of body weight) is established. End-stage mice will be monitored 9am-5pm. If the mouse shows sever symptoms, then it will not be kept and will be culled humanely as specified by the Home Office. No mice with severe symptoms will be kept overnight. Another group of mice showing adverse effects in this study exhibit an abnormal gait but have normal feeding behaviour and life span.

Application of the 3Rs

Replacement

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

Replacement

Motor neuron disease targets neurons in the brain and spinal cord and thus it is impossible to have access to these tissues during the development of the disease before the post-mortem stage. This would provide us with data about the very late stages of the disease. Although we will be using skin fibroblasts isolated from patients and reprogrammed cells, we will still need mouse models to have access to tissues at all stages of life and for culturing primary neurons.

Reduction

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

Reduction

We will maintain and breed just enough animals for providing us with required tissues and cells for generation of data which are statistically sound.

Refinement

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

Refinement

Sequencing of the mouse genome has revealed that ~99% of mouse genes have a homologue in the human genome and that for ~80% of mouse genes an analogous (orthologues) gene exists in the human genome. In addition, human and mouse have common biochemical pathways.

Because of the above properties several large international mutagenesis programmes have been generating mutant mice that could serve as model systems for late onset human disorders such as motor neuron disease.

The mouse clearly does not have the same physiology as humans, but does, largely, share the same biochemical pathways as well as genes. Thus we can work with mutant mouse models of human motor neuron degeneration to highlight and interrogate the proteins and pathways that are involved in motor neuron disease.

To minimise the animal suffering we monitor the animals which show signs of muscle weakness or paralysis twice a week between 100 – 120 days of age. Mice with signs of paralysis will be given dry mash and gel blocks and their food and water intake will be monitored daily. Mice will be weighed once and checked twice every day till end point (righting reflex within 30s is not observed; or 15% loss of body weight) is established. End-stage mice will be monitored 9am-5pm. If the mouse shows sever symptoms then it will not be kept and will be culled humanely as specified by the Home Office. No mice with severe symptoms will be kept overnight.

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	Mechanisms of metabolic regulation in health and disease
Key Words	cancer, metabolism, obesity, diet, 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):

Little is known about the ways various organs in the animal body communicate with each other using nutrients. However, there is increasing evidence suggesting that metabolic communication between cells and tissues is important for healthy tissue functions and is perturbed in disease. The aim of this project is to elucidate the mechanisms that allow cells exchange nutrients in order to support each other's functions and thereby tissue homeostasis. The project will also investigate how these operations fail or contribute to diseases such as metabolic syndrome and cancer.

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

The benefits of this project will be fourfold. Firstly, it will elucidate fundamental mechanisms of non-cell autonomous metabolic communication; secondly, it will reveal metabolic pathways that can be targeted for therapeutic intervention in human disease; thirdly, it will validate the use of specific compounds as therapeutic or diagnostic modalities in both non-human and human disease; and fourthly, it will generate and validate new mouse models for human disease.

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

Up to approximately 4500 mice per year will be bred and used under the auspices of this project.

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

Experimental procedures proposed for this project have either been established or will be refined to minimise the possibility of adverse effects. Possible adverse effects expected may include weight loss, appetite loss, hunching, or temporary shivering. None of the procedures, on their own or in combination are expected to breach the moderate severity threshold. In case of unexpected adverse effects an animal care

and welfare officer and a veterinary surgeon will be consulted. Any animal showing more than a moderate level of harm will be killed by an approved method.

Application of the 3Rs

Replacement

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

Replacement

In order to generate data that is relevant to the way in which cells and organs interact with one another inside the body, it is necessary to utilise animals. For example the complex ways in which tumour cells interact with a multitude of different types of healthy host cells *in vivo* is key to understanding cancer progression but this can only be studied in a living animal. However, the knowledge acquired through this project will be used to inform suitable in vitro experiments that will aid replacement in the future.

Reduction

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

Reduction

We will carefully plan our experiments so that to attain the best statistical power with the minimum number of animals. We will also aim to maximise the amount of information that can be acquired per animal within the confines of this licence. We will also develop or validate new, non-terminal methods that will allow longitudinal monitoring of biological parameters (e.g. liver function) in a non-invasive manner, such as *in vivo* imaging.

Refinement

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

Refinement

Mice have been selected because of their advanced genetics, readily available disease models and well established laboratory procedures. In all cases, animal suffering will be minimised by following strict guidelines in accordance with the Home Office and by regularly monitoring animals in consultation with an animal care and welfare officer and a veterinary surgeon. Any animal showing unexpected adverse effects of any procedure will be killed immediately by an approved method. Animal

use will be minimized wherever possible by employing the lowest numbers necessary to achieve statistically significant results.

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	Evaluation of anticancer agents and combinations in orthotopic 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):

As the understanding of the mechanisms behind cancer progression continues to increase, so does the requirement to develop and validate relevant models in parallel to test new strategies. The aims of this project are to provide the scientific community with accessible expertise in terms of available clinically relevant cancer models, knowledge and technical capability to improve decision making on which agents should progress to the clinic and which patients will benefit from the treatment. This project focuses specifically on solid tumours arising in organs of the prostate, pancreas and bladder, all of which are very different in terms of their origin, growth rate, progression and response to treatment.

The objectives of this project are:
1) To develop, validate and optimise patient relevant organ specific pre-clinical models of prostate, pancreatic and bladder cancer to enable the testing of anti-cancer agents. .

2) To evaluate anti-cancer agents and combination therapies using models developed in objective 1.

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

In order to benefit the clients/collaborators who develop the anticancer agents and ultimately patients who are treated with these agents, the development of pre-clinical cancer models that exhibit greater patient relevance by implanting them in relevant organs will allow these novel agents to be tested in more relevant conditions where environmental factors such blood supply, spatial arrangement, interaction with supporting cells and structures will be better represented. These models require expertise in surgery as well as generating the cells that emit light and then applying the imaging technology to capture the right data and analysis, which is not readily available in most institutions and companies. These models will enable decision on moving programmes forward into clinical trials or in some cases this may result in a

specific anticancer programme being cancelled which may seem a negative benefit, but identifying anticancer agents that are either ineffective or unsuitable for further development can be considered a positive benefit in the longer term as it prevents the unnecessary progression of ineffective therapies to early phase clinical trials and allows the redirection of resources and patients to other projects. Once validated, all models are added to the proprietary databases; access to which is free to all users, as well as abstract submission to national and international scientific conferences.

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

Mice will be used for this project as they are the lowest species of animal that allow the modelling of human cancer, and offer the opportunity for genetic manipulation to generate specific models relevant to human cancer. Substantial numbers of cancer relevant models are already validated in-house (100+) including prostate, pancreatic and bladder cancer making this species most suitable for this project. Over the course of this project we'd expect to use 7,200 animals to model these cancer types, the different stages of cancer and novel anti-cancer agents and combinations.

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

The majority of mice will undergo surgery to implant tumours in the site of origin in the prostate, pancreas and bladder under anaesthesia which are then measured once/twice weekly (or up to three times weekly dependent on growth) throughout the study by imaging under anaesthesia to track internal size before it becomes too large. Imaging is non-invasive and not expected to affect the wellbeing of mice. Dosing of candidate anticancer agents by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response (as assessed by imaging) or humane endpoints as guided by imaging before the onset of any adverse effects. Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects is uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs will result in humane killing regardless of body weight measures. The highest level of severity will be moderate. All mice will be killed at the end of the studies with tumour, blood and tissue collected which will allow further characterisation of treatment effect providing additional information such as how the cancer has spread or whether the drug has reached its target.

Application of the 3Rs

Replacement

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

Replacement

Research conducted in a test tube or artificial environment (In vitro) has replaced animal use in early development phases, particularly in the development of screening assays to refine compound selection, target identification, off-target toxicity or toxicity versus normal tissue cell lines, which increase our understanding of the target and candidate agent and therefore guide and refine the steps prior to moving into in vivo, and minimise subsequent use. However, there is still a requirement to use animals for this project as in vitro assays still do not optimally mimic all interactions between cells and tissues, such as blood vessel formation, specific organ environment, spread to other organs and thereby relevant drug access or the many homeostatic mechanisms in play in an in vivo environment that allows relevant tumour biology drug evaluation.

Reduction

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

Reduction

The use of in vitro studies can be used to identify lead compounds, evaluate dose ranges confirming target modulation/expression and relative off-target toxicity which can be used to inform on relevant doses for use in the equivalent animal models to evaluate drug distribution, target modulation and toxic effects. The use of complex 3-dimensional in vitro assays can be applied to pre-screen studies and compound selection prior to advancement into animal testing (thus reducing animal use). The model development stage of this project will be used to determine statistically powering so the minimum number of mice are used in a study design but still achieve scientific endpoints. The use of imaging technologies can also reduce the number of animals required to generate study outcomes as model variation can be improved by eliminating mice which do not develop the disease appropriately or refining the model so this is minimised.

Refinement

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

Refinement

Mice are the lowest species in which the knock out of the immune system allows growth of human tumours. Mice with a fully functioning immune system also provide the opportunity to investigate the immune system interplay with a mouse tumour. The mice will have tumours implanted into the prostate, pancreas or bladder i.e. at the site of origin which are more relevant to patients but are more complex and

require imaging to track the growth inside the mouse by using prostate/bladder/pancreatic cancer cells that are altered to emit light which is then captured by an imaging system specifically designed for small animals. Organ-specific models are known to better model cancer in patients as tumour grows in the correct environment which facilitates spread to other organs as seen in the clinic and show a reduced response to chemotherapy therefore providing more relevant information on the drug. The use of imaging is also a refinement as data from the internal tumours can be captured in real time, provided additional data that wouldn't normally be visible, only using animals that show tumour, and minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled killing of the mice, thus reducing the duration of model and regulated procedures.
The development of relevant pre-clinical models of oncology in objective 1 is a key stage for the evaluation candidate anti-cancer agents to ensure the right models are being used to answer the questions being asked in objective 2. The following will be undertaken to minimise animal suffering.

- Pilot studies for the establishment of new tumour lines and refinements to surgical techniques will be carried out on an ongoing basis under the advice of the vet and/or the named animal care and welfare officer will be sought in this respect.

All surgical procedures will be conducted in line with established welfare guidelines on aseptic surgery using suitable anaesthesia along with peri and post-operative analgesia.

- Any in-life sampling will be in line with established welfare guidelines and micro-sampling regimens will be utilised where study design supports this.
- The frequency of dosing will be such that animals fully recover between injections and will not suffer more than transient pain and distress and no lasting harm and there will be no cumulative effect from repeated injections.
- Use of pilot tolerability studies to ensure there are no unexpected adverse effects associated with new models or unexpected toxicity because of tumour: drug interactions and to ensure the drug levels used are not associated with any cumulative effects.
- Using cells that emit light to allow imaging to be used to recruit only those mice that have been identified to have the right tumour location and to reduce model duration

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	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 131. 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	Physiological biomarkers of poultry welfare.
Key Words	Neuroscience, acute affective state, electrophysiology, chicken brains
Expected duration of the project	5 year(s) 0 months

Purpose of 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.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

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

Increasing welfare of poultry (and other farm animals) is high on the public agenda. However, understanding which conditions or management processes affect the animals more negatively is difficult to ascertain. We should not assume that, just because humans might (not) like certain conditions, that chickens would respond accordingly.

We therefore have to ask the chickens. This can sometimes be done with behavioural tests, but there are a number of situations in which it is impossible to use behaviour, because the animal is unable to behave normally (e.g. when being picked up and put into crates for transport).

This project aims to use physiological (neurobiological) indicators as potential measures of the animals' welfare state. We aim to develop short-term, immediate measures, which give us an idea of an animal's immediate emotional state. For this, we are looking at brain activity in brain areas that are known to process emotions.

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

If we can validate these markers, we can use them to assess the welfare impact of different management practices on the animals. If we can assess this, then we can make practical recommendations as to which methods are higher welfare than which others. Because we are doing this research in collaboration with a company that designs processing systems for broiler chickens, our findings will be implemented quickly.

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

We will use broiler chickens of up to 3.5 kg in body mass. Over the 5-year length of the project, we anticipate using 100 animals.

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

Because we need to validate that our measures can identify negative experiences, we need to induce these experiences in the animals. However, these are unlikely to be any worse than those they might encounter had they been kept in commercial establishments. For a few animals, some aspects of their environment may be worse than they would normally have experience. However, the potential benefit to the millions of chickens being housed and then killed every year in the UK alone will outweigh the slightly increased negative experiences of a small number of birds. Birds will undergo surgery under general anaesthetic to implant electrodes into the brain. They will be allowed to recover from anaesthesia and heal from surgery before being recorded. Post-surgical pain will be treated with routine analgesics. The recording will be conducted by attaching a wireless recording device to the implant. This will be designed to be as light as possible and to impair the animals' movements as little as possible, so that the impact on the animal is minimal, and we can focus on the impact of the different stimuli we present to it. These stimuli may include negative stimuli, such as brief restraint, brief periods of pain or bad-tasting food; or the can be positive stimuli, such as re-uniting them with flock mates, providing dust baths or providing them with preferred food types. If necessary, the animal will be given time to habituate to the equipment before we start recording. At the end of each experiment, the animals will be humanely killed.

Application of the 3Rs

Replacement

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

Replacement

Because we are interested in the physiological responses of live animals to different environments and conditions, we have to study this in live animals. No in-vitro or computer model can mimic the response of real animals. However, early optimization of the implant surgery will be done first with cadavers, and then with non-recovery anaesthesia before moving to recovery surgeries.

Reduction

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

Reduction

We will use the most powerful statistics available to make sure we can use the fewest possible animals for the most possible outcome. Whenever possible, we will conduct power analyses to estimate the minimum effective sample size needed. The

experiments will be done within-bird, allowing us to control for a lot of inter-individual variability, and therefore to reduce the sample size needed to obtain meaningful results.

Refinement

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

Refinement

Because our question is about chickens, we have to answer it using chickens.

Except for the experimentally required negative experiences, we will minimize the animals' negative experiences by closely monitoring them for distress and disease, and by administering antibiotics and/or analgesics when necessary to the animals recovering from surgery. We will use wireless recording methods for the electrophysiology, as this reduces the stress on the animal of being physically connected with a wire.

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	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 133. 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	Nucleic Acid Sensing by Innate Immune Receptors
Key Words	immune response, vaccination, 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):

Viruses are infectious agents that cause a variety of diseases, ranging from a common cold to AIDS. The immune system can eliminate viruses, and we are trying to understand how the immune response is kick-started upon infection.

The first step is that the cells in our body recognise the presence of a virus. We know that cells have specialized proteins called receptors that detect viruses. However, how these antennas sense viruses is largely unknown. By investigating the mechanisms of detection we hope to understand how the immune response is initiated during virus infection.

One of the hallmarks of this anti-viral immune response is the production of a group of molecules called interferons. The name stems from the property of interferons to interfere and block the replication of viruses. Interferons achieve this by instructing cells to switch on their antiviral defences. Interestingly, interferons are not only essential as central players in antiviral immune responses. They are also produced during vaccination and are necessary for the development of protective immunity. Moreover, interferons are involved in cancer and may help our immune system to fight malignant cells. These new areas of research hold great promise for the development of new vaccines and novel cancer treatments. We want to obtain a better understanding of the underlying biology, which will be required for the development of new medicines.

Despite all these beneficial functions of the immune system, it is a double-edged sword and can cause problems, too. Patients suffering from autoimmunity are not infected with viruses or other pathogens, but their cells activate a long-lasting immune response that damages the body. Our hypothesis is that the immune antennas are not tuned to the right signal. We hope to reveal why the immune system is tricked into this false alarm.

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

The primary potential benefit of this work relates to new knowledge in the area of immunology. Our findings may allow us to develop new ways of boosting immune responses to more effectively eliminate dangerous viruses. Being able to better activate immune responses may also advance vaccination strategies and instruct ways to develop new treatments for cancer. In addition, we envisage inhibition of immune sensors as a treatment in autoimmunity, and our work may provide insights towards such approaches.

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

We will use mice as an animal model, including genetically modified mice that lack specific immune receptors or related molecules (up to ~24,000 animals over 5 years).

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

We will breed genetically modified mice. In most cases, the genetic modification will not cause any adverse effects. In some instances, genetic modification may lead to the development of autoimmunity. Manifestations of this include behavioural changes and weight loss. These will be carefully monitored, with clearly defined thresholds such as 15% weight loss, and animals will be killed immediately if these thresholds are reached. The majority of animals will be humanely killed without undergoing procedures and tissue will be used for experimentation. In addition, some animals will be used in models of virus infection, autoimmunity, vaccination and cancer. These models involve administration of viruses, substances or cancer cells. We will use injection, inhalation and the drinking water to administer these agents. Most animals will not suffer at all or will experience only mild and transient adverse effects such as tenderness around the injection site, which typically self-resolves within 24 hours. In the infection, autoimmunity and vaccination models, a small number of mice (less than 10%) may suffer adverse effects that last longer, and this will include weight loss and behavioural changes. In our cancer model, tumour development will occur in all animals. We will regularly monitor animals undergoing procedures and will record and measure adverse effects. Weight loss will not exceed 15% and tumour diameter will not exceed 1.2cm. Animals will be humanely killed immediately if these thresholds are reached, or before if scientifically possible.

Application of the 3Rs

Replacement

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

Replacement

To answer our scientific questions, our project integrates multiple scientific approaches. This includes biochemistry and molecular biology in the test tube wherever possible to dissect individual aspects of immune recognition. For example, this involves using cells isolated from animals humanely killed by Schedule 1 methods and using existing cell lines. However, we also need to use an animal model because the immune response is a complex process involving many different types of cells and molecular mediators. There is no feasible alternative that would entirely replace the use of a living animal. Where work not involving protected animals is insufficient to achieve our research goals, we will use mice as an animal model, including genetically modified mice that lack specific immune receptors or related molecules.

Reduction

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

Reduction

We will use statistical models to determine the minimum number of required animals. We will also design experiments in such a way that many data points can be collected from the same animal. We will use male and female animals, which reduces the number of surplus animals. We will use a breeding strategy - managed by staff trained specifically in maintenance and breeding of mouse colonies - that keeps the number of mice to a minimum. Unwanted genetic changes will be prevented by regular crosses to a reference mouse strain. Finally, experiments will be blinded as much as possible to avoid bias. Taken together, these measures will allow us to obtain robust and reproducible data from a minimum number of mice.

Refinement

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

Refinement

We chose mice given that their immune system is sufficiently similar to the one in humans to draw conclusions that are likely to equally apply to man. Once the scientific objective of any procedure has been attained the animal will be disposed of humanely. Specific humane endpoints will be applied. We have chosen those models of virus infection, vaccination, cancer and autoimmunity that are most refined

and cause the least possible harm. We will are fully monitor all animals involved in the study and continuously seek to identify new methods for refinement.

Specifically, our virus infection models interrogate the early stages of infection. At these time points, the innate immune system becomes activated and we will study this process. However, at these time points, virus replication has not yet resulted in tissue damage that causes profound disease. Animals will be culled before they reach this later stage. Similarly, in our autoimmunity studies, we are using slowly developing disease models instead of acute onset, severe models. This allows us again to focus on early stage of innate immune activation and to stop procedures before animals become more strongly affected.

Other examples of refinement applied in our work are: (a) Freund's adjuvant will not be used. This is a component of vaccine formulations that has been used in the past and caused adverse effects such ulcerations. We will use other adjuvants that do not cause adverse effects. (b) We will use tumour models that are easy to monitor and do not form secondary tumours (metastasis). (c) Footpad injections will not be used and will be replace with a refined model (hock injection) that is much less painful but achieves similar scientific aims.

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	Neuronal communication in the brain of mice
Key Words	Brain, cortex, synapse, neuron, tau
Expected duration of the project	5 year(s) 0 months

Purpose of 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 central goal of neuroscience research is to understand how the brain processes external and internal stimuli to coordinate cognitive and behavioural processes. Ultimately, this understanding must relate behaviour to the activity patterns of neurons and their synaptic connections within key circuits of the brain. Elucidating how the activity of these circuits becomes abnormal is also crucial to understanding pathological situations such as neurodegeneration. This project deals with these quetions in an unusually direct way - by observing the activity of neurons and synapses in the brains of live mice as they process sensory stimuli (1-4). Optical methods such as multiphoton microscopy now provide the resolution required to image neuronal and synaptic activity in awake animals and we will use these methods to make a circuit-level analysis of sensory processing and neuropathologies such as Alzheimer's Disease.

Our aim is to understand how nerve cells and their synaptic connections convey information (e.g visual or spatial) during health and disease.

Our key questions are:

1. What is the nature of the neural signals by which information is processed and transmitted in the visual system and hippocampus in awake behaving mice?

2. How is the transmission of neural signals at the synapse altered by changes in behavioral state of the animal?

3. How is the processing and transmission of neural signals altered in neurodegenerative disease states

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

This work is expected to have benefits in three broad areas: 1) By elucidating how different types of neuron respond to different types of visual stimuli, it will yield a

greater understanding of how the brain executes vision. 2) By concentrating on imaging the activity of synaptic connections, it will reveal how these key neuronal compartments alter visual signals and how they alter the transfer of neuronal signals when the brain enters different states, such as switiching from "sleep' to "alert". 3) By studying how the operation of neurons and synapses is altered by the accumulation of proteins that are known to cause neurodegeneration, we hope to suggest novel therapeutic targets and strategies.

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

This project will use mice. Approximately 3000 will be used over the course of the project.

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

The large majority of the genetically-altered mice used in this project usually show no adverse effects, particularly at the ages used in our experiments. However, about 5% of mice will be mutants that develop protein depositions in the brain leading to neurodegenerative changes of moderate severity. Some animals undergo surgery to allow us to implant a window through which we can image their brains. They may require post-operative pain-killers but are usually fully-recovered and alert a few minutes after the procedure. Occasionally, this surgery may also include the implantation of an optical fibre or cannula. After recovery from surgery, animals are gradually habituated to the experimental equipment, on which they have their head fixed in place but can run on a ball. They will be rewarded, for example with sucrose, and stress will be minimised by accustoming them gradually to the apparatus, but nevertheless, this can sometimes be somewhat stressful for the mice. At the end of all experiments animals will be humanely killed and where applicable tissues collected and analysed. If animals are suffering for any reason before the end of the experiment and do not respond to treatment, they will also be humanely killed.

Application of the 3Rs

Replacement

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

Replacement

The research proposed here requires the use of animals and animal tissue. The mechanisms underlying the transmission of signals in the brain are complex and involve interactions between different cell types. Studies in cell culture are uninformative as to the physiological properties of these processes, as the properties of the various neurons and their interactions are altered by the tissue culture

process. Nevertheless, we will use a number of different experimental preparations, minimising the use of living animals where possible. Where experimentally relevant, studies will use *ex vivo* brain slice preparations to study how sugnals are transmitted across synapses.

But to investigate how synapses in the brain contribute to the processing of information (e.g visual information in the visual cortex or spatial information in the hippocampus), we will need to work *in vivo*. This is the essence of our approach: to use the actual, unperturbed, neural circuit as far as possible. Cultures of neurons cannot see or navigate in space or carry out behavioural tasks that reflect the normal functions of the brain, and are therefore not an adequate substitute to understand how the retina or brain works.

As the function of neural circuits is profoundly altered by anaesthesia, many experiments will study unanaesthetised animals. This is also required to study processes such as motivation states. We will always seek to minimise animal use, however, and to maximise the information gleaned from every animal used

Reduction

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

Reduction

We will collect multiple data from each animal, thus minimising the number of animals required. For example, in vivo imaging data of neuronal and synaptic activity will be collected from many hundreds of neurons in the one animal, from several regions of the cortex or hippocampus. The numbers of animals to be tested will be the minimum number required to obtain statistically reliable results, based on previous experience in the laboratory, and from published findings.

To preserve important mouse genetic lines without having to hold stocks of live animals for extended periods we will instead freeze embryos that can later be implanted into a female mouse. The surgical procedures for implantation are demanding and require practise so on occasion it may be necessary to train with re-implantation of un-manipulated oocytes, embryos or blastocysts.

We are also using carefully designed studies that are statistically sound to minimise the number of animals used.

Refinement

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

Refinement

Mice have similar brain structures and functions with humans and can be bred to express proteins that allow us to see different cell types and the amount of brain activity. They are also small enough to be able to image their brains under a microscope.

The experimental and disease models have been designed to provide robust data while minimising animal suffering. Firstly, they use surgeries from which the animals rapidly recover. Secondly, they study the onset of disease processes. This allows the key triggers to be identified before multiple other processes go wrong, and also means that at these early stages, animal well-being is barely affected.

Animal welfare is monitored throughout the experiments, and animals are humanely killed where necessary.

Importantly for this project, a number of transgenic mouse lines are available, which will allow visualisation of specific types of neuron in the brain and the measurement of fluorescent signals when neurons are activated . Mice have been well-studied to investigate brain function and the sense of vision, as well as diseases such as Alzheimer's Disease, so that these results will be readily integratable within the field and should prove more translatable to humans than studies using lower order vertebrates. Finally, the small size of mice means that more of the brain can be observed using current microscopes than is possible in larger species.

Using a chronic cranial window for imaging involves surgery from which animals quickly recover, and then are stable for several months with usually no decrease in life expectancy or quality of life due to the surgery. Animals will be gradually accustomed to the imaging apparatus and will be rewarded (e.g. with sucrose solution) while under the microscope to decrease any aversion and stress associated with the apparatus. The transgenic mouse models to be used either have no adverse phenotype or, as in mouse models of neurodegeneration, they present some of the symptoms seen in human conditions such as Alzheimer's Disease. The use of such mouse models of human diseases is making possible the scientific studies from which cures will be found.

Where surgeries are carried out, peri- and post-operative analgesia will be used to minimise pain. Animals will be housed with appropriate environmental enrichment, and post-operatively this will be adapted to ensure that there is no chance of catching the cranial implants, while maintaining an interesting environment.

PROJECT 135. NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Information processing in the mammalian brain
Key Words	Neuron, Brain, Behaviour, Imaging, Electrophysiology
Expected duration of the project	5 year(s) 0 months

Purpose of 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);

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No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Neurons are the basic cellular units of the brain, and are connected via synapses to form neuronal networks. The properties of single neurons and the synapses that functionally connect them to each other are thought to provide the basis for processing and storing information about an animal's experiences and needs. One of the central questions in neuroscience is how particular tasks, or “computations”, are implemented by neural networks to generate animal behaviour, and how patterns of neuronal activity are stored during learning.

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

The results of this project will extend our basic knowledge of the fundamental mechanisms underlying information processing and memory storage in mammalian central neurons. This is essential if we are to understand how neurons communicate with each other and how information is transformed and stored by networks of neurons in the intact brain. In the long term the results of these experiments, and the techniques we will have developed, will provide new approaches of potential value for understanding and treating disorders of brain function such as occur in stroke, hereditary movement disorders, epilepsy and dementia.

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

Up to 19,000 mice and 850 rats will be used over 5 years

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

Most animals will undergo only mild procedures with a maximum expected severity of moderate. In these animals, adverse effects may include some post-operative pain, controlled by analgesia and some initial stress on head-fixation which will be limited by gradual introduction of head restraint and provision of ample water, sugar-

water or food rewards. Animals that are head-restrained in order to image brain activity accurately but supported on a treadmill are free to run or rest voluntarily and do not show signs of stress from the head restraint. Animals may lose weight initially but will be supplied with supplementary gels to aid recovery and supportive food and treats throughout. Any postoperative pain or complications that are not improved or resolved within a timeframe approved by the veterinary surgeon will be killed by and approved method. At the end of the experiments, an approved method of killing will be used and animal brains will be removed for further study.

Application of the 3Rs

Replacement

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

Replacement

Animals are needed in order to study intact brain circuits and their involvement in encoding sensory responses and driving behaviour. There is no lower mammalian species that could be used for addressing the scientific plan.

Reduction

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

Reduction

Breeding will be carefully controlled so that only animals required for experiments are generated. We will use advanced statistical tests (e.g. Kolmogorov-Smirnov, Wilcoxon matched-pair tests) in order to use the minimal number of data points to provide statistically significant results. Comparisons across multiple experimental groups will be made using the appropriate tests (e.g. ANOVA).

Refinement

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

Refinement

Rodents are probably the lowest species for which direct comparison can be made with the structure and functioning of the human brain. Mice are currently the species of choice in most areas of biomedical research, as they allow the use of powerful techniques such as the generation of transgenic animals. By using transgenic animals, we can express non-harmful molecules, such as fluorescent proteins, in neuron types of interest, that allow us to look, in a very targeted way, at the activity

of cell types most relevant to our research questions, rather than just sampling all cells randomly. This greatly enhances the scientific value of the work as well as reducing the number of animals needed for experiments. As more sophisticated genetic targeting methods are introduced, we will use them to further refine our scientific approach in order to gather data even more efficiently.

We have continued to refine head restraint systems, reducing the weight of the head plate and shaping them so as to minimise any physical impediment on the mouse in its home cage. The introduction of sound proof boxes for behavioural training, providing a quiet environment, and the continued use of treadmills to allow animals to run or rest voluntarily, has substantially reduced stress.

We will continue optimising and adding to these refinements minimise the welfare costs to the animals.

PROJECT 136. NON-TECHNICAL SUMMARY (NTS)

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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);

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No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Despite recent advances in cancer immunotherapy, there are still many unknowns that limit our ability to harness the power of the immune system in the fight against cancer. The tumour environment can use different mechanisms to promote their growth and evade anti-cancer immune cells. We need to understand these complex interactions in more detail to design better cancer therapies.

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

We will explore the function of immune-regulating immune cells on the development and spread of cancer. Our research will specifically investigate the function of immune-regulatory cells, which are critical for controlling a type of inflammation that promotes cancer. More specifically, the proposed research will investigate the role of specific immune cells in different stages of cancer, with the ultimate aim of developing new therapies to combat or control this deadly disease. We will use several physiological models of cancer induction, including surgical administration of cancer cells or cancer-inducing reagents. We will also investigate the effect of radiotherapy, commonly used in humans, on how local radiation-induced inflammation influences the immune response in cancer. Furthermore, we will perform intra-vital live-imaging studies to visualize these actual interactions. Importantly, we will aim to translate these results to human disease. Already there are safe treatments in the clinic that target these immune cells for different diseases, and our work may lead to the “repurposing” of these available therapies for cancer treatment.

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

Mouse. Maximum 5,240 per year.

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

Adverse effects relating to tumour establishment, development and the assessment of tumours and the administration of substances and sampling procedures are mild. Our severity limit is 'Moderate'. The majority of mice will experience mild to moderate symptoms. All tumour-bearing animals will be closely monitored and will be killed should clinical indications develop, such as loss of condition, a greater than 20-25% loss in normal body weight, significant abdominal distension, dyspnoea, digestive disturbances or neurological/behavioural abnormalities. Animals will also be killed if the tumour ulcerates or if tumour burden impedes any vital function (such as locomotion, vision, eating or excretion). In all cases, knowledge of the models will be used to guide health observations and to inform decisions on killing of animals before they become severely ill. Animals will also be observed to best ensure the detection of tumour development at unexpected sites. At the end of experiment, all animals will be killed

Application of the 3Rs

Replacement

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

Replacement

My research dictates the use of animals, as the process of cancer development and spread is currently most accurately and efficiently modeled in mice. Specifically, the role of the immune system for carcinogenesis is best studied in mice for several reasons: 1) We can answer detailed questions about cancer immunology by genetically modulating immune cells in mice. This is still impossible to achieve in humans, or in a petri dish. 2) The complex interactions in immunology and cancer are impossible to model accurately outside of the body. 3) Mice still represent the best model system for studying cancer.

Nevertheless, I have previously developed techniques to model very specific aspects of the immune system in a petri dish. I will employ this philosophy to my future studies, with the aims of substituting animal experiments and/or reducing the number of animals in experiments whenever appropriate.

Reduction

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

Reduction

Power calculations will be used to determine how many mice are required for studies to show statistical and biological significance.

As mentioned above, we plan to employ (and develop) techniques that reduce the number of mice. These techniques include ‘organ in a dish’ cultures.

Furthermore, I will collaborate with imaging experts to accurately monitor tumour development over time. This allows for the longitudinal analysis of single animals, leading to more robust control parameters and statistics that will ultimately reduce the number of animals required.

Refinement

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

Refinement

Mice are the best animal species for my research. As specified before, the many parallels between mice and humans are exploited in normal and transgenic animals. Moreover, as mentioned before, I will employ some of the best established and characterised murine cancer models with state-of-the-art immunology reagents to address questions with important implications in human disease. We will continuously refine these models to more accurately address relevant questions in human cancer research. For example, we amended our PPL to allow targeted radiotherapy treatment, which is known to involve immune responses in the cancer. Also, to accurately study the development of cancer, we will surgically inject tumour cells or cancer inducing reagents locally, which is critical for mimicking how humans develop disease.

We have optimised the procedures to minimise potential pain, suffering or distress, and enhance animal welfare. For example, new types of soft bedding material will be used for recovery from some procedures where the animal will experience pain. Also, we have developed new more refined genetic mouse models, which avoid the previous need for more harmful procedures such as cell-transfusions and irradiation. We continue to strive to develop new refinements that help us address important scientific questions with more refined (and therefore fewer and more humane) animal experiments.

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	Assessing biomaterial and cell transplant strategies for bone formation
Key Words	Biomaterials, Scaffold degradation, Bone formation, 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:
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):

Tissue engineering is a newly developed scientific field which aims to develop new therapies for the treatment of diseases which cannot currently be adequately treated with existing therapies, for example, osteoporosis or fracture non-union. Tissue engineering techniques use patients' own cells, which are grown within a scaffold (template) made from biomaterials to enable the cells to grow and develop into functional tissues. These biomaterials for scaffolds play an important role in guiding the cells' growth and also initially provide mechanical, structural support. However, the scaffolds have to be degradable. In other words, as the new tissues produce, the scaffolds should break down naturally d. It is therefore essential that the breakdown rate of the biomaterials utilised within a tissue engineering therapy, are well defined. The objective of this project is to assess our newly developed biomaterials which enable real-time, non-invasive, non-destructive monitoring of the scaffold degradation rate as well as assessment of bone formation rates. This project will also address whether the incorporation of stem cells to enhance bone formation and therefore promotes better fracture healing.

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

The ultimate goal of this project is to develop tissue engineering based therapies for patients with bone disease. The successful prediction of the effect of scaffold degradation rate and stem cells' incorporation on bone formation will help to speed up the new therapy development. Our project could accelerate the development of new biomaterials and establish a new technique to test the implants' stability and degradation in real time, non-invasively and non-destructively.

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

We will use mice and rats for the experiments. We estimate use up to total 2,000 animals over 5 years.

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

Both mice and rats will undergo procedures that involve incision in skin or creation of a defect in the non load bearing, non-articular surfaces of the skull. Scaffolds will be placed into small pockets created in the flank of the animals to assess biocompatibility. Small holes will be made in the skull of rats and repaired with newly designed and developed scaffolds, and the healing rates will be tested and characterised. These animals may experience moderate discomfort due to the surgery and the introduction of foreign materials, but anaesthesia, and pre and post operative analgesia will be provided to minimise this. In addition, the animal may experience local inflammation at the site of implantation. This is rare but could cause signs of distress to the animals. At the end of studies animals will be humanely killed so that further analysis can be performed to assess the performance of the grafts and the level of graft integration into the host, and ultimately the potential for the grafts to facilitate and promote bone healing.

Application of the 3Rs

Replacement

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

Replacement

Biomaterials degradation can be initiated by multiple mechanisms. The body fluid, enzymes and mechanic force can all cause the degradation. It is also known that the formation of bone by cells within the biomaterials (scaffolds) depends on the degradation rate of the scaffolds. This complex process cannot be mimicked *in vitro* hence it is necessary to assess the degradation rate and its effects on bone formation in animal models. Although *in vitro* models can give valuable information, they are unable to completely replace *in vivo* models.

We have created ex-vivo models to pre-assess these effects and thus the animal number to be used has been reduced.

Reduction

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

Reduction

Our experience with the experimental protocols will be applied to ensure appropriate group sizes are used to identify statistically significant differences between groups, whilst minimising the numbers of animals undergoing the protocol. Group sizes are

constantly reviewed and experts in statistics are consulted to ensure the minimum numbers of animals are used.

We will run pilot experiments with a relatively small numbers of animals where necessary, to establish initial biocompatibility, fluorescence tagging intensity and cell-seeding densities, from which the bony healing rate, scaffold degradation rate and imaging quality can be acquired appropriately. This strategy will minimize the chance of an experiment having to be repeated because it was incorrectly designed.

Refinement

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

Refinement

To minimise animal suffering, procedures in this application have been designed and selected to be the least invasive and traumatic and as surgically simple as possible. The subcutaneous model will be used to pre-screen scaffolds prior to testing in the cranial model. Only scaffolds showing favourable outcome in the subcutaneous model will progress to the cranial model. The cranial model was chosen as the bone is not jointed or load bearing and has relatively sparse nervous supply, compared to the long bones or the face, for example. This makes it one of the least painful models of bony injury. For most subcutaneous experiments, mice will be used as these are less sentient than rats, but will provide reliable data for assessing the biocompatibility of the scaffolds. For the cranial model, the mouse skull is too small and thin to be used. Such defects are likely to cause significant harm to the animal, and the thin surface is not sufficient for union with the scaffold matrix. For the cranial model, rats have been selected as the most suitable model. Where possible two scaffolds will be used per animal to allow a within animal control/comparison. Non-invasive imaging and analysis of scaffold degradation products in urine will be used for a longitudinal study, rather than sacrificing multiple animals at various time points. These measures will minimise the welfare costs to the animals.

PROJECT 138. 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 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 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	Repair and regeneration of the injured heart
Key Words	heart, mouse, fish, myocardial infarction, 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.
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):

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

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

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

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

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

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

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

Application of the 3Rs

Replacement

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

Replacement

The heart is a complex organ containing many cell types of which arguably the most important are the muscle cells, responsible for the pumping function of the heart and the endothelial and smooth muscle cells, which make up the blood vessels of the heart. Many of the experiments we propose will be carried out on isolated pieces of cardiac tissue or cell cultures of heart muscle, blood vessel and epicardial cells studied in the laboratory. However cells in a test tube or in a tissue culture dish cannot be used to study the complex changes occurring in the complete heart, nor how it functions in a living animal. Equally, isolated cell populations in

tissue culture transform to adopt different functional characteristics, compared to the equivalent cells as they reside in the heart proper, which confounds any experiments to determine the effect of externally-added factors on heart injury and repair.

Reduction

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

Reduction

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

Refinement

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

Refinement

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

induced anaesthesia is combined with a suitable inhalation agent to ease potential discomfort upon recovery and body warming; working closely with an in-house anaesthetic expert.

PROJECT 140. 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.

We are also interested in understanding the effects of respiratory infection on the rest of the body, such as how infection can lead to muscle wasting.

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

Our project will increase our basic understanding of how the body defends itself against lung infections. In doing so we will contribute to the field of immunology and respiratory diseases. In the longer term this basic understanding should lead to new therapies which promote protective immune responses, or prevent unwanted and potentially damaging inflammation. This may include the development of new vaccines for respiratory infections.

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

We estimate that we will use approximately 16 000 mice over 5 years.

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

In a typical experiment, we would aim to understand the role of a particular component of the immune system in protecting against infection or causing pathology in the lung. This will typically involve altering the immune response using different means, such as using genetically modified mice or by vaccinating the animals, before infection with a respiratory virus. Lung infections can lead to illness in mice and we expect some symptoms of infection including some weight loss. However, this is not severe and mice typically regain weight within a few days. There may be circumstances, for example in some genetically deficient mice, where disease can be worse. We will carefully monitor mice for illness throughout infection. Animals will be humanely killed at the end of the experimental procedure.

Application of the 3Rs

Replacement

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

Replacement

Immune responses to infection are complex and involve an interplay between the bacteria or virus causing the infection, the infected organ and the immune system in ways that cannot be reproduced in culture systems. We need to use a mammalian species due to the similarities in the immune and respiratory systems between these animals and humans.

Reduction

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

Reduction

We always endeavour to obtain maximal information from each animal we use and take many different tissues from each infected animal in order to gain many different readouts of the immune response to infection. Group numbers are kept to a minimum, but are sufficient to gain meaningful data.

Numbers of genetically modified animals bred will be kept to the minimum numbers required for experiments.

Refinement

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

Refinement

We believe mice to be the lowest animal which accurately reflects disease in humans. We have studied lung infections in mice for many years and continuously refine our techniques to minimise distress and suffering of the animals. Appropriate doses of pathogen are used so as not to cause severe disease. Mice are continuously monitored for signs of disease throughout infection. In particular, mice are weighed daily, as excessive weight loss is a sign of more severe disease. Any animals showing signs of severe disease are euthanized. Whenever procedures could cause pain or severe discomfort, analgesia is used or animals are anaesthetised. Good, sympathetic, animal handling, injection and blood sampling techniques will minimize discomfort. When pups are used, we scent our gloves with bedding from the cage before handling and limit the time away from the mother. Animals are housed with appropriate bedding, nesting material, with individually ventilated cages.

PROJECT 141. 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 evolution of food hoarding
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):

Evolutionary selection on behaviour has changed brains. However, we know very little about which changes in brains can lead to changes in behaviour. Here we study which changes in brain structure and/or function have led to the evolution of food-hoarding behaviour from ancestral animals that did not hoard food.

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

A better understanding of brain evolution, and what kind of changes in the brain lead to evolutionary changes in behaviour. This has a larger relevance for understanding ourselves and our own evolution.

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

Coal tits (Periparus ater), blue tits (Cyanistes caeruleus), and great tits (Parus major); 100 in the first protocol, although this has been increased to 160 since more funding has been obtained. Maximum 300 birds over 5 years.

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

We will house coal tits (hoarding birds) and blue and/or great tits (non-hoarding close relatives) in conditions which we expect to increase hoarding motivation (half the birds) or conditions which should minimize motivation to hoard (the other half of the birds). These conditions mainly consist of an unpredictable food supply (high motivation) vs. predictable ad libitum food (low motivation). We will also look at the effect of social rank (being dominant or subordinate) on the motivation to hoard. We will then verify the success of our environmental manipulation by measuring both consumption and hoarding behaviour in the animals. Because we believe that the mechanisms that control hoarding motivation work through the stress hormone corticosterone, we will also monitor corticosterone levels in the blood stream of the

animals. The adverse effects are minimal: - Stress of captivity: mild; minimized by habituating them to captivity in a large aviary with many hiding places and by housing them in pairs in relatively large cages. - Effects of temporary food restriction: mild; minimized by never food restricting for more than 90 minutes at a time, and providing enough food through the day; body mass monitoring - Effects of blood sampling: mild: small possibility of too much blood loss. Minimized by taking very small samples and stopping the bleeding with cotton wool. We always check bleeding has stopped before the birds are returned to their cages. At the end of the study, the birds will be humanely killed to collect brain and other tissues for further examination and comparison between the two species.

Application of the 3Rs

Replacement

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

Replacement

We are interested in the physiological basis of animal behaviour. Only animals can behave and we therefore need to use live animals.

Reduction

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

Reduction

The experimental design is well balanced and multifactorial in order to increase statistical power. We are using the minimum number of animals required to pick up expected effect sizes.

Refinement

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

Refinement

Coal tits are the most common food-hoarding birds in the REDACTED. The only alternative that is relatively abundant as well are rooks and magpies, and these would be much harder to work with, and would probably be affected more by the studies. Great and blue tits are the most common close relatives of the coal tits and therefore provide the best non-hoarding comparison species for the physiological responses of the coal tits to the environmental manipulations we will perform.

The refinement measures are how we habituate the birds to captivity (large aviary, places to hide); how we house them (in pairs); and how we avoid handling the birds as much as possible. For example, to shuttle birds back and forth to the behaviour testing aviary, we let them fly from the home cage to the room, and train them to fly back by turning off the light in the aviary and on in the home cage.

PROJECT 142. 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	Ischaemia-Reperfusion of Thoracic Organs
Key Words	myocardial infarction, ischaemia-reperfusion
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Ischaemic heart disease, characterised by a reduced blood supply to the heart, is the most common cause of death in Western countries. Considerable research effort has so far failed to adequately determine the essential cellular mechanisms responsible for myocardial cell death when deprived of an adequate blood supply (ischaemia) or, paradoxically, when blood is returned (reperfusion). The cellular processes involved in ischaemia/reperfusion injury form the overall objective of the studies outlined in this application, and the information will be used to determine ways in which this injury process can be ameliorated. The proposed studies will address some potentially fundamental and interesting differences between these processes regarding how heart cells respond and adapt to injury. From this information, it is anticipated that new therapeutic targets for the amelioration of cardiovascular disease may be generated.

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

This project will investigate the mechanisms of tissue damage following ischaemia and subsequent tissue reperfusion. It is feasible that these studies may result in the development of a novel methods for the detection of myocardial infarction (cell death due to ischaemia) and for the treatment of acute myocardial infarction and ischaemic heart disease (i.e. patients who survive the initial event), both of which are major causes of premature mortality in the UK.

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

Mice – 7,500 over 5 years Rat – 5,500 over 5 years Guinea pig – 1100 over 5 years Rabbit – 600 over 5 years Note that these numbers represent the expected use between 20+ active researchers working on this project.

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

This project will to investigate the mechanisms of injury and death following ischaemia and subsequent tissue reperfusion. Studies will be conducted primarily in tissues isolated under terminal anaesthesia and with little or no suffering. In a limited number of studies, animals will undergo pre-treatment with pharmacological agents, or by modifying their diet, with few or no adverse effects expected. Animals will be given appropriate care, including pain relief, to limit any pain and suffering. Animals will be continuously monitored for signs of distress and, if necessary, humanely euthanized. Few adverse events are expected with this approach.

Application of the 3Rs

Replacement

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

Replacement

Cardiac ischaemia, and reperfusion injury, are complex and incompletely understood phenomenon, involving the interaction of multiple factors, and, as such, cannot currently be studied without animal models. Our understanding of the processes involved, and their relative importance, limits our ability to use computer modelling, though this is a goal we are working towards.

Reduction

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

Reduction

Experimental data will be continuously analysed and assessed to achieve the aims of the project with the minimum number of animals. All protocols will be refined and conducted by trained individuals, to reduce errors and experiment numbers. Studies will conform to the NC3Rs ARRIVE guidelines.

Refinement

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

Refinement

Mice and rats are the main species used in this project, and are well-established models for the study of ischaemia and reperfusion. The basic mechanisms of cell

death and injury following a reduction in blood supply are similar between small laboratory species and man. In some experiments (particularly those examining arrhythmias mechanisms) larger species such as the guinea pig and rabbit are required mainly because these are the smallest species that share the basic electrophysiological features of the human action potential.

Our experimental protocols have been developed to limit harm to the animals, being as short as reasonably possible and mainly conducted under general anaesthesia. We will continue to make efforts to refine protocols and further reduce the welfare costs.

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	Safety Testing of Medicinal Products Using Small Animal Species
Key Words	Regulatory, Small Animal, Safety 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.
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 expansion of scientific and medical and knowledge has led to the development of drugs which can treat or alleviate the symptoms of many illnesses. Whilst progress has been made there is still a need to develop medicinal products to diagnose and treat many human conditions such as Cancer, Ischaemic Heart Disease, Sepsis, Stroke and Alzhiemer's disease. When these needs are combined with the growing threat from antibiotic resistant bacteria the advancement of science and the development of new products are as important as ever. This project licence authorises the conduct of studies in laboratory small animal species to evaluate the hazard profile of pharmaceuticals in terms of general toxicity and potential life time exposure.

The primary aims of this project are to support the development of these new medicinal products through acquisition of data to 1) Support selection of new candidate molecules for further evaluation and development. 2) Demonstrate the safety-hazard profile of a new medicinal product prior to the initiation of clinical trials involving humans 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of tissues from surplus stock animals to support drug development and the validation of non-animal alternative methodology.

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

People are exposed to medicinal products as patients, medical professionals, carers and during their manufacture. The products can be biological or non-biological materials and include diagnostic agents or substances associated with drug candidates eg metabolites, impurities and drug degradants. The principal benefit of this project is the generation of safety data to allow regulatory decisions regarding human exposure. Without these studies, progression of new medicines to early human studies and to patients could not occur safely. Validation and refinement of

test methods may be completed for specific techniques and may be published to the wider scientific community.

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

Over the 5 year life of this Project Licence, it is estimated that 55,000 mice, 82,000 rats, 8,000 hamsters and 2,760 rabbits will be used.

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

Animals will be given the “test material” under investigation in a way which mimics the intended human exposure. As most therapies at taken orally the majority of animals will receive the test material either mixed in their food or directly by insertion of a flexible rubber catheter in to the oesophagus. Most animals are treated daily in this way, occasionally studies may require two or three doses within 24 hours. For some test materials the oral route of administration may not be appropriate for example it may be broken down, not tolerated or not absorbed during the digestive process. In these situations, alternative administration routes such as sub-cutaneous or intravenous injection may be more appropriate. For test materials which will be administered to humans intravenously over extended period of time due to their inherent toxicity or fast clearance from the body it may be more appropriate to surgically implant a permanent catheter (with appropriate anaesthesia and analgesia) to allow the animal free movement whilst being dosed in their home enclosure. Test materials intended to treat a localised condition may be administered to that body part or area for example to the skin. Blood and urine samples may be taken to measure the level of test material or its metabolites within an animal’s circulatory system. These may also be analysed to detect any effects on body systems and organs for example liver or kidney function. Study animals are closely observed several times a day by highly trained technologists who monitor for any signs of discomfort. Other measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour such as reduced activity. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects. Many toxicological effects of the test material are not evident during the in-life phase of a study and do

not impact the animals wellbeing. Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia.

Application of the 3Rs

Replacement

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

Replacement

At present there are no scientific and legally acceptable evaluations of systemic toxicity which will satisfy regulatory requirements and provide sufficient safety data other than use of animals. Validated *in vitro* tests for specific organs are available and used to replace or refine procedures wherever possible. As new *in vitro* methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace *in vivo* procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.

Reduction

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

Reduction

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

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

Wherever practicable, and by looking across studies, the combination of endpoints eg general toxicity, reproduction and developmental toxicity, safety pharmacology, mutagenicity etc in studies is considered, to reduce overall animal usage.

As most studied involve the examination of tissues following treatment opportunities for re-use are very limited. Tissues are collected to support drug and in vivo developments from any surplus stock animals.

Refinement

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

Refinement

Species choice and use of specific animal models is determined by the need to generate regulatory acceptable data. Where a choice of species is possible, care is taken to select the most biologically appropriate species, and the species which most closely relates to man. Typically studies are performed on small animal species before testing progresses to larger animals such as dogs, minipigs and primates.

Generally the rat is the rodent species of choice in safety assessment. There is wide knowledge of the response of rats to various substances and a wealth of background literature. Rats are large enough to provide repeated blood samples, thus requiring significantly fewer rats than mice to achieve the same objective. Mice (or hamsters) may be used when considered a more appropriate species, for example, if they more readily absorb the test material, are more relevant biologically or improved tolerance depending upon objective of the study.

Rabbits may be used when considered a more appropriate species, for example non-pregnant range finding studies prior to conducting reproductive toxicology studies in pregnant rabbits; local tolerance or vaccine development studies as the actual intra muscular or subcutaneous human dose volume can be administered

Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints.

Socially compatible species are routinely group housed with environmental enrichment which encourages species specific behaviours without not adversely impacting study outcomes. Occasionally it may be necessary to single house animals for example to collect urine samples of for the administration of test substances. All such occurrences are conducted in accordance with project licence limitations and under the oversight of the local Animal Welfare and Ethical Review Body.

Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare. Any confinement or restraint is restricted to the minimum required, under guidance issued by the site’s Animal Welfare and Ethical Review Body (AWERB).

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	Provision of a service for production and maintenance GA animals and antibody production.
Key Words	Service, Genetically altered animals
Expected duration of the project	5 year(s) 0 months

Purpose of 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 aim of this licence is to provide a comprehensive service for the production and maintenance of genetically altered animals. In addition, the licence covers small scale production of serum and antibodies.

Genetically altered animals provide complex systems for the study of biological processes. The procedures and protocols that constitute this PPL will result in genetically altered animals being made available for use in a range of project licences involved in medical research. Most of the work is carried out on mice, but rats, frogs and fish are also covered. Protocols used are established and are constantly refined, and the work done under the licence contributes to a huge range of 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)?

By providing these services centrally we prevent avoidable animal wastage and ensure experienced personnel do the work. A number of highly trained staff provide the expertise in the production of these animals, meaning that the smallest numbers of animals are used, and that staff are experts in this area, constantly looking to refine practices such as reducing the need for surgery for embryo transfers and using stool samples for genotyping. The service also ensures these genetically altered animals are archived by cryopreserving embryo and sperm to enable their use as a future resource, and to help reduce animal numbers when they are no longer required for active research as well as to enable easy sharing of these animals with scientists across the world. We will also produce sophisticated and refined genetically altered models for research using best practice and novel methods.

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

Mice >100,000 Rats ~1000 Fish >40,000 Frogs >10,000 Are anticipated to be used over the course of the 5 year 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?

Most animals used on this licence will be either bred under a mild severity and show no adverse effects or will be used in a mild or moderate surgical procedures resulting in no expected harm due to the nature of the aseptic techniques used and the pre, peri and post operative care they receive. Where animals undergo a moderate procedure we have clear end points and control measures to stop suffering as soon as possible. Animals are killed by a schedule 1 method at the end of procedures or kept alive for breeding.

Application of the 3Rs

Replacement

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

Replacement

This service PPL by its nature requires the use of animals, and will result in GAA being made available for use in most of the PPLs used at the Institute, for which the benefits are clearly described within each PPL and will be published via the scientific groups holding these PPLs.

Reduction

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

Reduction

Numbers are kept to a minimum by training staff to high standards, quality control, database tools allowing us to track animals and any adverse effects accurately, through centralising the supply of animals we can reduce wastage and by using specialised staff success rates are high again reducing numbers. The Cryopreservation part of the work is key in reducing the long term 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

Rodents, Fish and Frogs are the most frequently used laboratory animals and we have services with best practice established techniques that allow us to provide services for these species to the scientists onsite. We make every effort to refine our procedures wherever possible, and there is a definite culture of care within the service. Modification of our surgical techniques is happening constantly in line with new developments and best practice to improve welfare. Cryopreservation is encouraged, as a means of reducing the welfare issues involved in animal shipment. We keep up to date of new genetic tools which reduce the severity of phenotypes in animals. Breeding will be kept mild wherever possible, by keeping lines with moderate or severe phenotypes breeding heterozgously wherever possible. New environmental enrichment products are trialled continuously by animal care staff, and there is an active training and development programme for animal care staff ensuring best practice.

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	Development of new biological anticancer agents
Key Words	Cancer, Virotherapy, Targeted, Biologic
Expected duration of the project	5 year(s) 0 months

Purpose of 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. We aim to develop a new type of cancer therapy based on cancer-killing ‘oncolytic’ viruses that may be given by intravenous delivery to metastatic cancer.

2. As part of this, we aim to define factors that limit the ability of viruses to replicate and spread through solid tumour tissues. Current clinical trials show that inadequate spread through tumours can be a limiting challenge for oncolytic viruses

3. We will also develop ‘armed’ viruses capable of targeted expression of therapeutic biologics within cancer

4. We will assess whether other 'armed' micro-organisms (such as bacteria that are normally found in the gut) and 'armed' mammalian cells such as monocytes may also be useful for targeted cancer therapy

5. Finally, we will explore the potential synergy between oncolytic virus and external beam radiation.

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

Oncolytic viruses promise very potent new cancer therapies that improve on state of the art in two ways – they are more cancer-selective than most anticancer agents, and they are more powerful than most anticancer agents. The first oncolytic virus received its product licence in the USA in 2015, and now we are trying to improve on that agent to develop viruses that can be used to treat advanced cancer of various types.

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

All of our work will be performed in mice. Over a period of 5 years we would expect to use up to 7000 mice. This number of animals is necessary to ensure our

experiments are done properly and reach statistically significant results – and therefore never need 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?

Animals we use should not experience obvious pain or discomfort, and if any animal is found to be suffering it will be immediately put down using a humane technique. Many of our mice will be used to grow subcutaneous human tumours, but this appears to cause the mice no discomfort and they behave perfectly normally. They are all humanely killed at the end of the experiment, and before they undergo any suffering.

Application of the 3Rs

Replacement

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

Replacement

Our work exploits the complex cancer ‘microenvironment’ – including many non-cancer cell types that appear to support tumour growth. Some aspects of this also involve the innate and adaptive immune system, and these features cannot be properly modelled in vitro. Nevertheless we do make extensive use of cells in vitro and human tumour biopsies, direct from the cancer surgery, to explore the possibility of using non-mouse alternatives

Reduction

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

Reduction

Experiments are designed carefully to achieve their goals using minimum animal numbers. We also make extensive use of in vitro models to try and minimise the numbers of animals we need 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

Mice are the animal species of lowest neurological sensitivity that can support the complex tumour architecture and microenvironment similar to human disease. We make extensive use of imaging approaches to maximise the amount of information we can obtain from each animal, without subjecting it to any invasive procedures. Imaging readouts provide an important technological advance, because individual animals can be imaged at different times, minimising variation due to the use of different animals at different times, and provide much better quality data (with far less variation) than could be achieved before such techniques were available. Where imaging is carried out on non-anaesthetised animals, restraint will be brief and potential techniques of improving animal comfort will be explored, for example using a restrainer with a cut out to allow probe positioning, or acclimatising the animal to handling prior to commencing experiments. Where imaging is carried out on anaesthetised animals, monitoring of breathing rate, temperature and ECG will be utilised to minimise potential adverse effects. Some animals will be administered human cells, to provide surrogate immune systems, and these animals may show signs of graft-versus host disease if the human cells react to the mouse. This can be minimised by only grafting cells from human donors that are known not to cause the problem, and that will be our approach. Animal suffering will be minimised by ensuring they are subjected to the minimum disturbance possible, and by close liaison with the Named Veterinary Officer and use of pain relief (eg. analgesics) if required.

PROJECT 146. 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	Sperm-mediated genetic and epigenetic effects in zebrafish
Key Words	reproduction, fertility, genetics, epigenetics, gametic 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:
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 currently know very little about the importance and mechanisms involved in selection acting in the gametes (sperm and eggs) of animals. The aim of our research is to verify the importance of selection acting on sperm characteristics and also the mechanisms that are involved in determining these characteristics. Understanding which sperm are the ones that ultimetaley fertilise the egg of the many millions produced by a male, and what makes these better than other sperm will greatly help us to understand the the possible consequences of our use of assisted fertilisation technology in animal breeding and not least human reproduction and fertility.

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

Understanding processes involved in reproduction are fundamental to understanding life and it is surprising how little we know about these processes. We here focus on the possible impact of environmental conditions on the male zebrafish, its sperm and its offspring over one or two generations. Our research ultimately helps to understand what makes a good sperm and how such sperm are selected under natural conditions to fertilise eggs. Our findings are of very broad significance and have direct implications for the study of human fertility and the use of artificial fertilisation technologies.

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

We will be using zebrafish from early embryo stages into adulthood. We expect to use a total of 2100 fish over the duration of five years. These numbers are based on the sample sizes and effect sizes calculated in previous experiments using the same setups.

In the context of what you propose to do to 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 expose adult animals to three different environmental conditions: elevated temperature, food restriction (starvation) and varying social environments. These animals will also be used tfor collection of gametes to perform in vitro fertilisations. All treatments will last for a maximum of 2.5 weeks. Expected adverse affects may be abnormal swimming behaviour, weight loss and injuries due to fighting among animals. We will monitor all fish daily and any fish that cannot recover from treatment induced suboptimal conditions within one week will be killed according to Schedule 1. The overall level of severity is estimated as mild. Control animals that suffered mildly will be re-used for gamete collection. All other animals will be killed according to Schedule 1 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

It is not possible to study complex processes such as how traits in fathers are inherited by offspring without the use of animals. Zebrafish have been extensively utilised as a genetic model for the study of development and thus a large number of resources are available. Large-scale phenotypic assays are more feasible in this species than in rat or mouse. Thus they are the vertebrate species with the lowest neurophysiological sensitivity likely to yield results relevant to the human condition.

Reduction

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

Reduction

Design of experiments are based on previous research data where protocols for experiments in male competition and dietary restriction protocols have been tested, validated and used multiple times for other projects, These existing protocols and data have been used to conduct statistical power tests to calculate the smallest number of animals required in order to achieve our objectives as stated in project plan. In addition, the use of repeated measures and split-clutch designs further allow the reduction of sample size due to increased statistical power. These designs are only possible in externally fertilising species.

Refinement

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

Refinement

Zebrafish are non-mammalian vertebrates that have been shown to have stress response. Zebrafish have been extensively utilised as a genetic model for the study of development and thus a large number of resources are available and the mutagenesis and transgenesis protocols are well established. Thus they are the vertebrate species with neurophysiological sensitivity likely to yield results relevant to the human condition.

We have chosen some of the mildest forms of stresses, which are however robust and well documented to have distinct effects that are expected to be transmitted to the subsequent generations. We are conscious of the fact that repeated daily exposure can be considered less invasive than chronic exposure to a drug and thus design our experiments accordingly. We avoid use adverse stimulus that may have lasting physiological impact in the adults.

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	Cell Therapy for Diabetes
Key Words	Type 1 Diabetes, Regenerative Medicine, Islet Transplantation, Reprogramming, 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):

The purpose of this project is to generate an alternative supply of islets for transplantation for the treatment of diabetes. This will be achieved by reprogramming the exocrine part of the pancreas that is normally left over at the end of the islet purification procedure. The reprogrammed cells will be characterised in vitro for insulin content and ability to secrete insulin in response to graded changes in glucose concentration in the culture media. However, on occasions it will important to characterise how the cells behave in a diabetic animal. We propose to use an immunodeficient mouse model so that the human cells will not be rejected. The mouse will be rendered diabetic following treatment with the drug streptozotocin. Following engraftment of the reprogrammed cells we will test for the presence of human insulin (C-peptide)in the blood of the mice as well as for blood glucose.

What 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 around 300,000 people with type 1 diabetes (T1D) in the UK. They are all dependent on exogenous insulin injections. However, it is very difficult, if not impossible to completely normalise blood glucose levels in this way. Many people with T1D suffer daily large excursions in blood glucose; either large increases (hyperglycaemia) or decreases (hypoglcaemia). Both have a huge impact on their well-being and an associated burden on the National Health Service. The only tested method that can normalise blood glucose levels without these excursions is islet transplantation. We have developed a protocol whereby the pancreatic tissue left over from the islet purification procedure is efficiently reprogrammed into functional islets. We calculate that one pancreas could generate in the region of 10-20 transplantable islet units. This would therefore increase the number of transplants taking place in REDACTED from around 20 per year to over 200. This would have a huge impact on the well-being of people with T1D, and in particular the substantial number who are seriously ill through the consequences of persistent hypoglycaemia.

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

We propose to use mice as a model to study whether our reprogrammed cells function in a manner similar to purified human islets in an in vivo environment. We expect to use up to 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?

We will purchase mice that have been genetically modified to disrupt their immune system. These mice will not reject transplanted human cells. We propose to render the mice diabetic by injecting them with a drug that destroys their islets. The resultant diabetic mice have elevated blood glucose levels but other than that are healthy with a normal life-span. In a typical experiment some mice will undergo surgical procedures, including implantation of cells beneath the kidney capsule, injection of cells into the hepatic vein, injection of cells subcutaneously or intraperitoneally, and kidney removal. At regular intervals blood samples will be taken to determine whether the grafted cells can normalise the elevated blood glucose levels. At the end of the experiment the mice will be humanely killed. The severity of the protocol is moderate.

Application of the 3Rs

Replacement

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

Replacement

Before we can take this novel cell therapy for diabetes into the clinic we need to show that the cells work in a living environment. The mouse is the most appropriate animal to use since there are a number of immunodeficient strains available and the streptozotocin diabetic mouse is well–researched model. About 90% of the work undertaken in this project will be done in tissue culture dishes. However, as we make major advances in developing the protocol we need to ensure that the resultant cells also function in animals.

Reduction

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

Reduction

The end-points are well characterised and this will allow us to use the minimum number of animals per group. The minimum number has been calculated on the basis of sound statistical principles, i.e. the minimum number of mice required to

show normalisation of blood glucose levels and circulating human insulin when transplanted with reprogrammed cells. The figure of 500 arises on the basis that 100 mice will be used per year: 5 mice per group, 4 groups per experiment and 5 experiments per year.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 diabetic mice will be closely observed with regular recording of body weight; usually around the same time each day. Although healthy in many respects the streptozotocin-treated mice exhibit increased urination. Consequently the mice are given additional dry bedding and this is replaced regularly.

Surgery will be carried out aseptically by experienced personnel. The animals will receive appropriate analgesia/anaesthesia and peri-operative care as advised by the veterinary surgeon.

PROJECT 148. NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	The role of movement in prenatal skeletal development
Key Words	Skeletal development, Fetal movements, Biomechanics, Spine, Joint
Expected duration of the project	5 year(s) 0 months

Purpose of 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 understand how mechanical forces due to fetal movements (the movements of a baby in the womb) affect development of the skeleton, and in particular the bones, joints and spine. There are a number of conditions affecting the skeletons of newborn babies that are linked with abnormal fetal movements, such as hip dysplasia (where the hip joint is unstable), arthrogryposis (where multiple joints are malformed and bent) and congenital scoliosis (abnormal spinal curvature and/or vertebral shape). Hip dysplasia and congenital scoliosis occur in about 1 in 1,000 births and arthrogryposis occurs in 1 in 3,000 births. However, the role of fetal movements in skeletal development is poorly understood, for example, when is the most critical time for movement, and can we artificially increase movement to reduce the effects on the skeleton.

What are 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 from this project are that it will: a) Give us a better understanding of skeletal development. This is relevant to tissue engineering of bone and cartilage. b) Provide insight into developmental conditions affecting the skeletal system in newborn babies, such as hip dysplasia, congenital scoliosis and arthrogryposis. In particular we hope to be able to offer insight to patients and their parents on why they might have developed the condition c) Identify possible means of reducing the effects of abnormal fetal movements on skeletal development by investigating the effects of applying massage, or introducing an exercise regime, in pregnant mice of embryos without movement. This could lead in the future to preventative treatments for conditions affecting human babies, such as hip dysplasia.

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

Only mice will be used. We use genetically modified lines in which the fetal movements of homozygous (having an identical pair of the gene of interest) embryos

are absent or abnormal. I expect that 1,500 or less genetically modified animals will be humanely killed over the course of the 5 year term.

In the context of what you propose to do to 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 heterozygous (having a dissimilar pair of the gene of interest) animals have no phenotype, and all homozygous animals will be harvested prior to being born, the vast majority of animals are expected to suffer no adverse effects, with the severity being sub-threshold. At the end, all 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

Most of my research on joint shape and spine is actually performed on an unprotected animal alternative, namely chick embryos younger than two thirds through gestation. The advantage of this model is that a) there is no mother that needs to be sacrificed, and b) the chicks are harvested prior to the maturation of the pain receptors and so there should be no suffering involved.

However, the chick has no intervertebral disc (a key structure of the human spine) and so any studies of this disc must be conducted in a mammal, of which the mouse is the most suitable. Furthermore, in order to be able to fully understand the importance of fetal movements for human diseases, we also need to study an animal that develops in a womb, as the environment of the egg is very different to that of the uterus.

Joint shape data gathered under this license will contribute to a computer simulation of joint growth and shape change, which will help us understand how mechanical forces direct and affect joint development. . This stage of the project will use data from the previous three stages, and will therefore not necessitate any further animal use.

Reduction

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

Reduction

We save all skeletal tissues from the embryos, so each embryo is used for at least two different studies (joint and spine). We use inbred strains to minimise variation. We do 3D imaging so that shape is easily characterised, reducing the number of

animals needed. Experiments will be planned so they can be published in accordance with the NC3Rs' ARRIVE guidelines.

Refinement

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

Refinement

Bearing in mind that we need to use a mammal for studying the disc, and the mechanical environment of the uterus), the mouse is the most refined animal model for fulfilling the objectives of the research.

Our breeding protocols are designed so that embryos with a harmful mutation are harvested prior to being born.

Procedures other than breeding will cause minimum harm to the animals. The biggest welfare cost for any individual animal will be two injections, with general anaesthetic up to a maximum of four times, which will only apply to a small subset of the animals

Animals exhibiting any unexpected harmful characteristics will be killed using a humane method or in the case of individual animals of particular scientific interest, advice will be sought promptly from the local Home Office Inspector. Any animal showing signs of suffering that are greater than minor and transient or in any way compromise normal behaviour will be immediately killed using a humane method.

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	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.

Another strategy to fight flu infection is to use antibodies which can bind and neutralise the virus. We shall isolate and make such antibodies from immune pigs and test their efficacy in the pig influenza model.

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. Generating broadly neutralising antibodies against influenza and establishing the pig as a model to test such antibodies will facilitate the selection of antibodies to be taken to human clinical trials.

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 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	Breeding and maintenance of genetically altered mice
Key Words	Transgenic, 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:
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):

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

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

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

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

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

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

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

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

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

Application of the 3Rs

Replacement

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

Replacement

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

Reduction

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

Reduction

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

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

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

Refinement

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

Refinement

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

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

PROJECT 151. NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Understanding mechanisms that regulate tumourigenesis and metastasis
Key Words	Mouse, Cancer, Metastasis, 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):

Almost 1 in 4 people in the UK will develop cancer at some point in their lives. Although great advances have been made in cancer biology with many patients now being cured, it is still a devastating disease that can be hard to treat for some cancer types, especially if the cancer cells have spread to other organs of the body. Critical to improved patient care is a deeper understanding of the biology of cancer, which can pave the way for the development of new therapies.

The specific aspects of cancer we are focussing on are ‘tumourigenesis’ (the initial formation of the tumour) and ‘metastasis’ (the tumour cells ability to grow at a secondary site). Both these events are multi-step processes that depend on the accumulation of mutations within the cells that allow them to become cancerous. Thus knowledge of the key genes that control this processes is critical – genes that when mutated result in a cancerous cell.

However, this is only part of the story, as factors ‘outside’ of the tumour cells, i.e., what is going on in the body, also have a key role to play in both tumourigenesis and metastasis. This can include the normal cells around the tumour cells and critically the immune system, as well as factors such as the age of the person. Thus understanding of the way the body can ‘control’ the ability of the tumour cells to grow and spread to other organs provides avenues for potential therapies, as highlighted by the success of “Ipilimumab” – a drug that works to activate the specific cells of the immune system that are able to kill off cancer cells.

The aims of this work are:

1. To identify genes found in cancer cells that when changed/mutated can affect tumour growth and metastasis.

2. To identify how factors such as the patient’s immune system influence tumour growth and metastasis. What are the potential benefits likely to derive from this

project (how science could be advanced or humans or animals could benefit from the project)?

An understanding of the genes that are altered in cancer cells, and how they work to ‘alter’ the normal functioning of the cell, is critical if we are to have any hope of identifying ways in which we can ‘kill’ the cancer cells, i.e., the development of drugs/therapies that are able to target these ‘altered’ cells and leave normal ‘healthy’ cells alone. Similarly, if we can find ways in which the body is able to control the growth of tumour cells or prevent their growth at new tissue sites, then this information can be utilised by pharmaceutical companies to develop drugs that target the tumour cells or help the ability of the body to fight them.

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

It is expected that a maximum of 150,000 mice will be used over the course of 5 years, with 50,000 of these being used solely for breeding to generate mice for analysis.

In the context of what you propose to do to 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 licence, 60% of the mice we propose to use will experience no more than at most a transient feeling of pain, such as when they are administered substances via an injection into the tail vein or when a blood sample is taken and are considered to be 'mild' in terms of severity. The remaining mice (40%) will undergo more experimental procedures and thus are classified as 'moderate' severity. These mice may carry an altered gene and are monitored to see if they develop a tumour. Alternatively mice can be administered tumour cells and their ability to control the tumour or the spread of the tumour is investigated. When administering a 'new' tumour cell line, pilot studies (on 2-3 mice) will be performed to determine the size to which the tumour may grow to allow sufficient time for metastasis (spread of the tumour cells to another tissue site) to occur. Tumour cells may also be administered to 'aged mice' (~1 year old) to determine the effects of age on tumour growth and spread. We also characterise the immune system of the mice. All mice are monitored daily for any signs of a developing tumour or signs that the animal may be starting to experience abnormal clinical features that suggest it is no longer able to tolerate the presence of the tumour (be it a swollen spleen due to the development of lymphoma or metastatic tumour cell growth in the lungs starting to make breathing laboured). Since animals can also develop tumours internally (i.e., where the tumour growth/mass cannot be directly observed), we use other signs to indicate the mouse is starting to become unwell, such as coat condition, pale and cold extremities, reduced movement and/or social interaction. At the point when the mouse starts to display these clinical signs, it will be humanely killed and the mouse examined to

determine why it was displaying these symptoms, as well as tissue samples collected 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

Humans and mice share many similarities, both in terms of our basic organs (as we are both mammals) and our genetic make up (there is a very high degree of similarity in the actual genes that both mice and humans share). When mice develop cancer (either due to our alteration/mutation of their genes or due to the administration of tumour cells to them), their tumours are very similar to that seen in humans (in terms of the way the develop and their actual characteristics). Also, using the mouse means we can look at the way the body reacts to the cancer cells and how factors such as the immune system try to control them. This is something that simply cannot be performed by growing cancer cells in a dish in the laboratory.

Thus mice are a very good model for human cancer, and allow us to perform studies that cannot ethically be done using human subjects. Importantly, mouse studies have enabled the development of clinically relevant agents in cancer treatment, such as the development of targeting antibodies that are currently being used to cure patients with advanced melanoma (antibodies that target two proteins on the surface of the immune cells that are able to kill the cancer cells). Indeed, although these are only two examples virtually every compound used in the oncology clinic was developed or validated using mouse model systems and mouse models have also contributed significantly to our fundamental understanding of the mechanisms of cancer.

Reduction

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

Reduction

Where possible we shall always import existing mice rather than generating new ones.

In some circumstances, such as when certain mouse lines have been only recently created, less published data will be available and in these instances we propose to perform small pilot experiments to determine the final experimental design. All mouse lines will be archived so that they may be distributed to other researchers

worldwide. This will reduce the number of animals used globally, as fewer animals will be required to re-generate these archived lines.

Data will be generated from the statistically determined minimum number of animals, and wherever possible, experiments will be designed to avoid the known sources of variability that can arise.

Wherever possible, multiple experiments will be performed on the tissues collected from an individual mouse so as to maximise the use of the mouse.

All data generated from our research on the mice will be published in scientific journals available to the whole scientific community, reducing duplication of production resources and phenotyping procedures elsewhere. Wherever possible, the results of experiments that involve large datasets will be made publically available to serve as a resource for other scientists and clinicians

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 constantly refining our experimental processes to minimise harm and reduce adverse effects on the mice without affecting the experimental data. For example, when taking blood samples we have refined our protocols such that only a drop of blood is needed to be able to complete our analysis, thus minimising the distress to the animal.

When mice are to be irradiated (given gamma-radiation to wipe out their bone marrow prior to transplant of donor bone marrow), we have instituted a new policy whereby the mice must be weighed 24 hours beforehand and their condition thoroughly observed. This allows us to avoid irradiating mice that may be rather small in body weight and/or may have started scratching (for example) and would be less likely to tolerate the irradiation procedure. We also ensure the mice are placed on antibiotics for 2 weeks after the irradiation, whilst their immune system is compromised, and we also provide mash in dishes on the cage floor for the first week after irradiation to ensure that should they feel slightly weak/tired (as some patients can feel after irradiation therapy), they are still easily able to access food.Mice are social animals and thus wherever possible we try not to house them on their own. However, in cases where we observe fighting in a particular cage of mice, the aggressive mouse will be removed and solo-housed, so as to prevent further harm to the rest of the cage. We have highly trained technicians looking after the mice, and the mice are checked every single day to ensure they are healthy.

Those that are being observed for the development of tumours are observed twice daily and humanely sacrificed if they are starting to show any signs of discomfort.

The implementation of a sophisticated database system and animal tracking system ensures that data on procedures and welfare assessments can be readily accessed and analysed.

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	Frontal cortex: learning and decision making
Key Words	prefrontal cortex, cingulate cortex, decision making, learning, behavioural change
Expected duration of the project	5 year(s) 0 months

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

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

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

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

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

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

We measure the activity and structure of macaque brains before, after, and while they learn and make simple decisions between objects or actions to obtain food and juice rewards. Measurements are made with magnetic resonance imaging (MRI). MRI is non-invasive. Nevertheless macaques may potentially be distressed by the

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

Application of the 3Rs

Replacement

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

Replacement

Much of the laboratory's work is Nevertheless sometimes we use animals because we also want to examine what happens if interventions are made in the brain that

change how it functions. Some techniques, such as TMS, are available for carrying out tests with human volunteers but they can only be used to examine brain areas close to the top and sides of the head and their impact is short lived. To look at brain areas that are some distance from the scalp and to examine the longer term impact of an intervention in one area on brain networks and behaviour we use animal models.

Reduction

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

Reduction

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

Refinement

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

Refinement

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

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

We examine brain activity while animals perform behavioural tasks in order to understand the role that the brain areas play in mediating the behaviour. We motivate the animals to perform the tasks by rewarding them with small juice rewards or food rewards. Every day, however, we ensure that the animals have a

period of free access to water and they are given additional food when they have finished performing the tasks.

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

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

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	Early Safety Assessment and Enabling Studies
Key Words	Investigative toxicology, method 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:
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):

New medicines have the potential for benefit to man in terms of new or improved disease treatments. Before potential new medicines are administered to humans their safety must be evaluated by screening for harmful effects. This screening is done by toxicologists using animal models since these provide the best prediction of what might happen in people.

Following assessment of scientific articles, non-animal methods, a small number of potential new medicines will be tested in short-term toxicity studies in order to select the potential new medicine with the greatest chance of successful progression into the first clinical trials in 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)?

Medicine development is a long and costly process. It is therefore desirable to avoid wasting money, resources and animals on substances which are not suitable for development as potential medicines. Achievement of the objectives of this licence will enable safe development candidates to progress and will also help to remove unsuitable candidates from the development pipeline at an early stage, increasing the chances of those medicines entering human clinical trials having an acceptable safety profile.

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

Rats and mice will be used. Early studies are likely to use relatively small numbers of animals (less than 60). It is anticipated up to 50 studies will be run a 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?

Studies will be conducted by dosing routes similar to those used in man, e.g. by mouth (orally) or by injection. The animals are then observed regularly to monitor changes in appearance and behaviour. Procedures carried out during these studies are: a) Weighing: as a loss in body weight is often an early sign of harmful effects in animals b) Blood sampling or collection of urine for measurement of different components as changes in these may serve as early indicators of toxicity. Doctors for similar reasons often take blood and urine samples from humans. c) ECG monitoring to assess changes in heart function (e.g. number of heart beats per minute). This technique is also used by doctors to assess heart function in humans. d) Examination of the eyes using a similar device to that used by opticians A degree of restraint or confinement may be required for some of the various dosing, sampling or assessment procedures. At the end of the study the animals are humanely killed by an overdose of anaesthetic. Samples of various organs are taken and examined under a microscope to ascertain whether the potential new medicine has caused changes that would prevent administration to humans. From experience, the majority of animals are expected to have mild adverse effects such as slight weight loss. A small percentage of animals may show more significant adverse effects e.g. more marked weight loss, or changes in appearance (e.g. ruffled fur in rodents) or behaviour (e.g. reduced activity) indicative of moderate severity. Humane end-points are applied, under veterinary guidance as necessary.

Application of the 3Rs

Replacement

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

Replacement

At present there are no alternatives that don’t use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment. Specific types of hazards can be detected using methods that don’t require the use of animals and these are used where appropriate.

Reduction

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

Reduction

The numbers of animals will be set after consultation with professional statisticians. Animal numbers are kept to the minimum commensurate with meeting the objective of each 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 rat is the rodent species of choice. There is a wide knowledge of the response of rats to various chemical entities and a wealth of knowledge and published information. Rats are big enough to provide repeated blood samples, thus requiring significantly fewer rats than mice to achieve the same objective.

Adverse effects will be kept to the minimum to achieve the objectives. Animals will be housed together and provided with specific materials to provide enrichment.

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	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 aggressive 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 155. 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 abuse potential and evaluation of novel entities to treat substance abuse.
Key Words	Drugs, Abuse, Rodents, Safety, 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.
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):

Psychoactive substance abuse poses a significant threat to health and the social and economic fabric of families, communities and nations. In an attempt to limit and control the drug abuse potential of novel drug candidates entering the market place, specific preclinical studies are mandated by the world regulatory authorities (eg European Medicines Agency EMA and USA Food and Drug Administration FDA) for all novel compounds in development which enter into the CNS irrespective of their therapeutic indication. In addition, novel drugs for the treatment of substance abuse are urgently required.

The overall project aim is to provide highly specialised preclinical services to the pharmaceutical and biotech industry to evaluate the abuse liability of drugs which enter into the CNS irrespective of their therapeutic focus and evaluate the efficacy of novel drugs for the treatment of substance abuse. This is achieved by the following three objectives:

1. To assess the abuse liability of novel drug candidates in development which enter the CNS regardless of therapeutic indication, as mandated by the regulators.This includes determination of the pharmacokinetics of the drug candidate, as required by the regulators, so the exposure in the rodent model can be related to the human exposure.These studies will allow the regulator to determine if the drug candidate should be scheduled (yes or no) and if yes what schedule it should be placed into thereby limiting its distribution in the wider population.
2. To assess the abuse liability of new chemical entities in preclinical development for abuse liability. These studies will enable clients to determine if their new chemical entity is advantaged over existing therapies and hence worthy of further preclinical and clinical development.
3. To assess the efficacy of new chemical entities to treat drug abuse. These studies will enable clients to determine if their new chemical entity is worthy of further preclinical and ultimately clinical 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)?

To assist sponsors to develop, perform and report a pre-clinical Regulatory Abuse Liability package which will meet the world-wide regulators requirements, and provide the appropriate scientific support throughout the process. This will allow the sponsor to move rapidly through the clinical development programme without the assessment of abuse potential becoming a rate limiting factor. In addition, these studies will allow the regulators to decide whether a drug candidate should be scheduled or not and if scheduled which schedule the drug candidate should be placed into thereby limiting its distribution to the wider population. In addition, to allow clients to make decisions in regard to their novel compounds in preclinical development. Does the compound show reduced potential for abuse potential over a marketed product or compound in clinical development? Does the novel compound exhibit efficacy in a model predictive of the ability to treated substance abuse. The medium benefit is the discovery of compounds with the propensity for reduced abuse potential or to treat substance abuse and the long term benefit (likely to be subsequent to completion of the licence) may be a clinically effective drug (since regulatory agencies expect a drug's sponsor to have screened the new molecule for pharmacological activity, prior to assessing its therapeutic potential in humans).

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

Approximately 12,500 rats over 5 years. The exact number of animals used will be dependent upon external factors such as the number of clients and the success of those clients in designing suitable drugs.

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

The majority of the animals are likely to have a mild severity experience but the severity classification of the Protocols is moderate. The majority of studies will involve the administration of drugs which enter the CNS (predominantly orally or via an indwelling cannulae) for behavioural testing. Drug treatment might be once or repeated. Some compounds will have been extensively evaluated prior to assessment. In such circumstances no adverse effects are expected. Some compounds may not have been tested extensively in animals and unexpected toxic effects might arise which could cause pain, suffering, lasting harm or in extreme cases death if humane end points were not applied. The IVSA studies involve anaesthesia. The animal models employed may involve training in specialised equipment which may produce transient discomfort/stress. Upon completion of procedures 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

The project objectives each require investigation of candidate substances to be tested in an integrated behavioural/physiological/pharmacological model that requires the whole animal which cannot be replaced by in vitro or ex vivo studies. Prior to testing, it is expected that candidate substances will have been selected on the basis of extensive efficacy and safety evaluation (compounds assessed as part of the abuse liability regulatory package) or *in silico*, *in vitro*, *ex vivo* and in vivo experiments for more novel compounds undergoing evaluation prior to moving into development.

Reduction

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

Reduction

The number of animals will be minimised by:

1) The expertise of the company's statisticians who are able to perform power calculations to ensure that studies are suitably powered to detect the difference of interest.

2) Use of animals where
appropriate to evaluate multiple test compounds or re-inforcing drugs (eg heroin, coc aine, etc). This not only reduces the number of animals used but has the following advantages: allows comparison of effects of related drugs in the same animals (including pharmacokinetic evaluation), minimises the number of animals that need training (IVSA and drug discrimination) in a particular task and surgical intervention (IVSA).

3) When evaluating drugs that potentially possess sedative euphoriant properties, the rats need to be trained to self-administer a positive control. Opiates, eg heroin, remifentanil and oxycodone, are often used as the positive control when training rats to self-administer opiates. These opiate drugs cross the blood-brain barrier very easily and very quickly, and consequently, are highly rewarding (reinforcing) which means that a large proportion of the rats given access to these opiates develop strong self-administration responding. Morphine is not an ideal choice as a training reinforcer because it does not cross the blood-brain barrier quickly and it has

relatively poor brain penetration. Thus, in comparison with opiates like heroin, remifentanil and oxycodone, morphine has much less rewarding effect. Therefore, it takes more sessions to train rats to self-administer morphine and the proportion of

rats in an experimental study that achieve successful acquisition of drug-self-administration is much
lower (40-50% typical responder rate with morphine, 80-90% typical responder rates with heroin).

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 has been chosen since its anatomy, physiology, behaviour and genetics has been well-documented. Rats display many of the behavioural and cognitive characteristics of humans and are easier to train than mice and, therefore, they are typically used in the more complex behavioural models, such as those included in this project licence. There is a large literature detailing the use of rats in these abuse models and methods proposed in this licence have all been validated in these species. Rats are social animals and will be group housed unless single housing is believed to be preferable for the animal's wellbeing, or the scientific validity of the study.

The models detailed in this project licence have been established, used and refined by the company over the past 15 years. They have all been validated using suitable compounds and widely used by the pharmaceutical industry.

In order to minimise adverse events during the conduct of the studies outlined in this Project Licence two strategies are adopted (pilot studies and dose finding). In spite of extensive characterisation of candidate drugs that are evaluated in the tolerance and dependence studies we employ a small pilot cohort of rats (maximum of 3 rats per dose) that run 7 days in advance of the main study. If adverse events are observed the doses in the main study are revised as appropriate. In intravenous self-administration, drug discrimination and reinstatement self-administration studies dose finding experiments are typically conducted before the main phase of the study to determine suitable starting doses of all drug candidates and novel compounds to be evaluated. The aim is to determine doses that produce a clear pharmacological effect, without causing significant impairment of lever pressing or pronounced behavioural effects or adverse effects on general health. Due to careful selection of doses we infrequently observe any adverse effects of training drugs, reference comparators, drug candidates or novel drugs.

Surgical procedures will only be used if alternatives are not available. Anaesthesia will be maintained at a suitable depth to avoid the animal feeling pain. Aseptic operating procedures, topical application of antiseptics and dressing will be used to

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

In studies where rats administer substances of abuse to themselves (intravenous self-administration studies) at low doses the rats are singly housed. We have attempted to house the rats in pairs during these studies, however this was unsuccessful. The rats are allowed to socialise in a large cage for 15 minutes each day in groups of 3.

The jugular catheters (a flexible tubular instrument passed through blood vessels for the introduction of fluids into the blood stream) for intravenous self-administration studies are now made in-house which has reduced the failure rate to <1%. The failure rate of the commercial alternative was around 5%. If a catheter fails the animal must be removed from the study and data is lost.

Rats in IVSA studies must be able to differentiate between a substance of abuse and saline. We have found that if the saline extinction phase of intravenous self-administration studies is run 7 days per week, more rats meet the criteria for extinction and extinction is achieved more quickly. This ensures more rats successfully complete the study and the study is of shorter duration.

For the intravenous self-administration studies rats are generally placed in the testing boxes once per day for a maximum of 2 hours (week days only). However, the rats are removed from the testing boxes as soon as the session is completed which can be as short as 20 mins in order to reduce the time animals spend in the boxes without water. The only exceptions are during the saline extinction phase where the animals spend the whole session (2 hours each day) in the testing boxes and are tested 7 days per week and during the progressive ratio (PR) testing session when they spend between 2.5 and 4 hours in the boxes (maximum number of PR sessions per rat per study 8). It is worthy of note that rats tend to drink only around 10% of their totally daily water intake during the light phase.

We have performed a research package which has assisted in the replacement of primates with rats in IVSA studies.

Rats do not typically like to self-administer benzodiazepines: there are only a handful of old and poorly designed IVSA studies in the scientific literature using benzodiazepines. Previous studies in rats have been conducted using non-challenging, low schedules of reinforcement over very prolonged test sessions (e.g. 24 hours). However, even in non-human primates, the percentage of animals that will self-administer a benzodiazepine drug is very low at ~25%. We have assessed the reinforcing properties of two benzodiazepines, midazolam and diazepam, using

male Sprague Dawley rats to select the most reinforcing drug for use as a Reference Comparator in our IVSA tests. In a second study, the most reinforcing of the two drugs was re-evaluated in rats to determine the reproducibility of its reinforcing effect. Midazolam and diazepam were reinforcing in male Sprague Dawley rats using very low intravenous doses The percentage of responders at the most reinforcing drug doses was 30-75% for midazolam and 25-50% for diazepam. This compares favourably with the ~25% responders for benzodiazepine drugs generally obtained in non-human primate studies. The most reinforcing of the two drugs, midazolam, was used in our second study. The results for the reinforcing effect of midazolam were reproducible between our two studies. Over these two IVSA studies, we have refined the methodology to use benzodiazepines, midazolam and diazepam, as reference drugs in rat IVSA tests. We are committed to the dissemination of information into the scientific community with regard to refinement of methods and models. We have presented these results at international scientific conferences in 2017 REDACTED. Take up of this test by the wider scientific community could reduce the numbers of non human primates used in IVSA tests for evaluation of benzodiazepine drug effect. We have now performed several studies for clients using these drugs as comparators in the rat IVSA model. The regulators now do NOT recommend the use of non-human primates for these studies.

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	Infection Model Validation and Development
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 allow us to develop optimize and refine models of microbial infection for subsequent use in an efficacy licence which together enable us to provide vital support services to the pharmaceutical and biotechnology industries and academia plus support internal research programs to accelerate the development of novel 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 infections that do not respond to available antimicrobials due to developing antimicrobial resistance and newly-emerging diseases. Development of new antimicrobials is of clear benefit to humans and animals. Models of infection developed or optimised in this licence will allow the development of novel antibacterials, antivirals, antifungals, antiparasitics, disease treatments and vaccines which are vital elements of drug discovery required by pharmaceutical and biotechnology industries and academia, and regulatory authorities.

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 based on our experience over the last 5 years will be approximately 6,000 Mice, 4,000 Rats and 1,000 cotton rats 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 housed in social groups in a purpose built facility. Animals will be infected with microbes (bacteria, fungal, viral and parasites) to mirror human infections. Typical symptoms after infection will be loss of appetite, lethargy, changes

in body temperature, mild pain or changes in the breathing pattern. The aim for the studies is to replicate the clinical disease observed in humans so disease specific symptoms are likely to be present such as diarrhoea following infection with a food poisoning organism or a chest infection following a challenge with the cold virus. Infections will be designed so that only mild disease develops similar to the first symptoms a patient experiences following infection. To ensure only mild disease occurs a range of assessments and monitoring strategies developed over 25 years are used, including use of pain relief drugs, regular clinical assessments, measuring body weight and temperature. These measures enable us to stop experiments before animals show significant signs of the infection. Based on experience over the last 5 years, ~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. At the end of the studies animals will be humanely euthanized and infected site/tissues removed for culture to quantify the infection burden or immune response.

Application of the 3Rs

Replacement

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

Replacement

We use an extensive panel of insect and non-animal alternatives to reduce animal usage

Insect Hosts. We routinely use Wax moth larva to initially determine if microbes are virulent and whether infections respond to treatment. Whilst wax moths are not a complete replacement for animal models they significantly reduce the number of animals required.

Biofilm and Hollow fibre Models.

We run studies using *in vitro* biofilm and hollow fibre models as replacements for animals.

Biofilm models are used to measure the ability of drugs to break up colonies of bacteria and fungi that stick to implants and catheters used to treat patients. Specific testing of biofilms is required as they are normally highly resistant to treatment.

Hollow fibre models are used measure the impact of drugs on microbes and are a vital part of determining the treatments used in early clinical trials. These models can

also simulate the changes in drug concentrations in the blood of humans that occurs following single and multiple doses.

Reduction

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

Reduction

To obtain good quality data and to use the minimum number of animals, statistical expertise will be applied to work undertaken on this licence.

The following guidelines will be used to minimise the number of animals required:

- Use the same strain of animals, and purchase from a single supplier with minimum variance in weight.
- Use validated microbial strains, obtained from patients with appropriate infections or strains from culture collections.
- Run experiments using the same experienced experimental team where possible.
- Design model endpoints to occur at times when variance is minimal.
- Calculate sample size based on available date before the experiment.
- Where possible we blind treatments.

For all experiments, within the Work Order and protocol (as appropriate) 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 SOPs and reference used etc.
- Deliverable statement including how data will be presented and statistical analysis.
- No experiment will be performed until approved by senior staff.
- A report, detailing the outcome, is written and circulated to 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

The overall plan of the work is to support the discovery and development of novel drugs to benefit humans with a range of infections.

Exclusively using non-mammalian species is not possible because they lack relevant tissue physiology and therefore cannot replicate human physiology.

Only rats, mice and cotton rats will be used on this licence.

Mice will be used in the majority of studies unless there is a scientifically relevant reason that mice cannot be used.

The most appropriate species and strain of mice and/or rats will be chosen based on previous or published data and choice of experimental model.

Best practice and use of the most refined methods will be applied to all experiments.

Animals are observed by trained staff, with referral to the Named Animal Care and Welfare Officer, Named Veterinary Surgeon and Project Licence Holder as necessary.

All animals will be regularly monitored for weight loss and general condition.

For anaesthetic protocols the optimal anaesthetic regime relevant for the species/strain will used in conjunction with the Named Veterinary Surgeon.

Where necessary, painkillers will be used under the guidance of the Named Veterinary Surgeon.

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	Gene therapy for neurodegenerative diseases
Key Words	Gene therapy, neurodegeneration, mice, zinc finger proteins
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):

Huntington's disease is a terrible lethal brain disease with no cure. We have developed a way to switch-off the mutant gene that causes the disease. This is based on a small artificial protein, called a zinc finger, that we have designed to stick to the disease gene's DNA. After about 5 years of trials developing the off-switch in cells, we progressed to testing in mice. We were able to inject modified (safe) viruses containing the off-switch into mice that had the Huntington's disease gene. We managed to switch off the gene, resulting in symptom improvement, and the effects lasted up to 6 months, but with reduced efficiency at later time points. We are therefore working to improve delivery in the brain and other tissues for longer times. Unfortunately, this can only be done using mice. Cell culture cannot mimic the factors in the brain which are currently stopping the treatment from working long term, such as the immune system attacking gene-therapy treated cells. We will be expanding also our therapeutic approach to other brain diseases.

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

We want to develop a safe effective human therapy for a disease that causes a slow and unpleasant death. Huntington's disease and other similar diseases are inherited and devastate affected families, with 100% of carriers (people with a genetic mutation) developing the disease.

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

We will use genetically modified mice that mimic the features of brain disorders in humans. We expect to use less than 8000 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?

One of the mouse models used develops neurological symptoms such as tremoring, loss of weight and premature death. We have set a plan of careful monitoring and humane endpoints that will lead to humane killing of the animals when these symptoms reach severe harm to the animals. The other mouse model does not have any disease symptoms. The surgical procedures and experimental approaches cause moderate distress to the animals. All the mice will be humanely killed at the end of the experiment.

Application of the 3Rs

Replacement

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

Replacement

There is no feasible alternative that would entirely replace the use of a living animal. The main scientific issue we are tackling with this project is to achieve sustained therapeutic effects. This can only be carried out in mice, because cell cultures cannot mimic the complex environment found in a living organism, with an immune system and a multicellular environment affecting the outcome of the gene therapy.

Reduction

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

Reduction

The proposed experimental designs are based on previously validated experiments by others and us, and supported by careful statistical analysis to reduce the number of animals used to a minimum (a statistical method called a power analysis is used to calculate the minimum number of animals for meaningful results). Before our treatments for brain diseases are tested in animals, we will test them in cell cultures to select only the most promising ones to be applied to mice. We also wish to freeze and store (cryopreserve) mouse lines in order to avoid continuous breeding. Thus we will reduce the number of animals used during experimental gaps.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 mouse models of Huntington’s disease: each displays different degrees of disease severity. These mice are commercially available, extensively

characterized, and have been used successfully used in scientific studies. This helps us to get reproducible results which allows minimizing the number of animals required for a given protocol. The same strategy will apply to other degenerative brain disorders. We aim mainly to use mice without neurological symptoms.

The careful selection of analgesia and anaesthesia methods under the advice of the veterinaries, the monitoring of the condition of the animals with standardised protocols, the appropriate training of the experimenters and technicians in mouse handling techniques and humane endpoints will minimize the adverse effects. We wish to explore less invasive delivery approaches in order to fulfil the refinement policy.

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	Cell and circuitry replacement in Parkinson's disease
Key Words	Parkinson's disease, cell transplantation
Expected duration of the project	5 year(s) 0 months

Purpose of 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 human central nervous system (CNS - the brain and spinal cord) commonly suffers diseases that destroy cells ("neurons"). The overall aim of the project here is to identify cells that can replace the neurons destroyed by the neurodegenerative disease Parkinson's disease. Parkinson's disease is a disorder where the neurons that help us start a movement degenerate. Once these cells are destroyed by the disease, the patient has little ability to move, and they become very slow or completely immobile. The objective of the research plan is to:
(1) identify cells that can mimic the actions of neurons destroyed by Parkinson's disease; and (2) test the potential for these cells to recover the motor capabilities of an animal model of Parkinson's disease.

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

We aim to determine if cells we have studied extensively in culture should progress toward clinical use. More specifically, the benefits of the experiments proposed here are that they will determine how effective the cells are at improving the functioning of an area of the brain affected by Parkinson's disease.

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

The studies proposed in the research plan here will make use of rats. To make statistical comparisons between animals that receive cell transplants and those that do not, the studies will use approximately 250 animals in total over the 5-year course of the project. This is the minimum number possible to get a meaningful assessment of the cells' ability to recover the fine motor functioning of a living animal.

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

The studies conducted here will involve surgery on animals, and this has innate risks. Traditional risks of surgery are mainly from anaesthetics and infection, and these will be limited by the vast experience of the scientist, and careful monitoring during the operative and post-operative period. Any signs of infection (i.e., poor wound recovery) will be treated with appropriate antibiotics, and the animal closely monitor until recovered. The animals are given appropriate analgesic both pre- and post- operatively, and should have no discomfort beyond that caused by the incision made in the skin during surgery. Any animals which display discomfort beyond 1 week post- operatively, will be reviewed (for infections or adverse events) and given additional analgesics as needed. The lesion model does not greatly affect the animal's ability to move, forage, feed or think. Any animals which do not feed, forage or move suitably after surgery will be fully assessed by trained staff (including a veterinarian, if necessary), and appropriate action taken.

Application of the 3Rs

Replacement

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

Replacement

The experiments are designed to be a final stage of testing to determine whether cells we have extensively screened *in vitro* have the potential for clinical use. Animal numbers are reduced to a minimum by the extensive use of *in vitro* testing to assess cell viability, potential for tumorigenicity, their ability to differentiate and grow in brain tissue. These will be performed before any *in vivo* work is conducted. The significant *in vitro* testing of the cells, however, cannot replicate the many biological systems (e.g., immunological, local tissue responses, protein - protein interactions) that can alter the effectiveness of cells transplants, and none can reveal the overall impact the cells have on improving movement in a living animal. Hence, the final stage of testing necessitates the use of living animals.

Reduction

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

Reduction

When ultimate (*in vivo*) testing is warranted (i.e., when cells have shown promise in *in vitro* analyses), the size of animal groups for testing is determined through power calculations to identify the minimum number required to compare control and experimental animals. This is done through the advice of an on-site statistician that remains involved in the data analysis of such projects.

Refinement

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

Refinement

Rodents (instead of other animals, such as flies) are used for these studies as they are the lowest form of animal that we can use to obtain human-relevant (motor) behavioural measures. Human relatable motor skills cannot be meaningfully assessed in lower vertebrates, and there are no suitable alternatives that have significantly similar motor circuitry to humans. The animals (rodents) used, and the models produced, have been modified for more than 40 years to allow for a meaningful model of Parkinson's disease while having the least cost to the animals.

PROJECT 159. 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 therapeutic approaches for inflammatory joint disorders
Key Words	Inflammation, Arthritis, New 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;
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 of this project are to:

· Identify efficacy of test drugs and side effects not predicted by cell culture based model systems.

· Study the effects of the drug on the body, and also the effects of the body on the drug.

· Demonstrate that anti-inflammatory activity can be shown at specified doses.

· Identify the best arthritis models to use pre-clinically that correspond with a specific therapeutic target.

· Test a drug's efficacy to affect leucocyte recruitment and identify associated mechanisms that may be shared with other

autoimmune diseases

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

The target of this programme of work is to provide pre-clinical supporting information for clinical trial applications. A drug requiring evaluation will be supplied to us by clients in the biotech and pharmaceutical industry, along with evidence supporting the rationale for testing the agent. We aim to investigate the efficacy and mechanism of action of a drug to help our clients make a more informed decision on whether to proceed into clinical trials. This reduces the risk of later stage failures and hopefully predict on side effects associated with a particular therapy. Information supplied by us will speed up the clinical trial process and make it less financially prohibitive. The benefit is, therefore, a reduced number of unproductive human volunteer studies (and a reduced risk of adverse effects) and most importantly the development of improved and more effective therapeutics targeting inflammatory joint diseases and

potentially other inflammatory and/or autoimmune diseases with shared mechanism of action. The benefit to patients will be the identification of new anti-inflammatory drugs. This programme of work will help identify the best potential drugs early in the drug development process or aid in refining drugs that have not been efficacious in the clinic due to poor historic efficacy data and pre-clinical design.

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

We would expect to run approximately 100 studies on behalf of sponsors using approximately 3500 mice and 1000 rats over the 5 year duration of this project licence.

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

Animals injected with inflammatory stimuli to induce an arthritic disease state will, over time, develop inflammation in their hind and front paws which affect the digits, footpad area and sometimes the ankles. Joint swelling is an expected outcome of this protocol and is a primary measurement of disease progress or disease regress with a potential therapeutic. Animals may suffer from discomfort associated with inflamed joints but will be monitored daily for any additional, but unlikely, signs of discomfort associated with an arthritis diseased state such as weakness or a hunched appearance. General assessment of pain is not accurate but includes close monitoring of the animal’s behaviour and feeding, the use of facial expression scoring system (grimace scale) and responses upon handling. The use of pain relievers by nature have anti-inflammatory effects which may compromise the underlying pathology of the disease, rendering the testing of potential anti-inflammatory therapeutics and the resultant need to use animals for the assessment of new therapies futile. Therefore, the use of analgesics to reduce pain and discomfort will be considered carefully and used where an analgesic regime will not interfere with the scientific endpoints being considered. General welfare checks and humane endpoints of a severe protocol will be observed at all times. Whilst no weight loss is associated with arthritis models, any animal which has lost 15% of its body weight will be monitored, and if this weight loss is combined with any other signs of discomfort mentioned above, the animal will be removed from the study. If the animal loses 20% of body weight, this is an endpoint and it will be removed from the study. Any animal which fails to put weight on one of its limbs for more than 72 hours will be removed from the study. A 72 hour window is a crucial to allow a potential therapeutic to manifest its effects against a control (non treated animal). Our experience with this model and historical data suggest that most therapies will show statistically reduced disease parameters in the time period when disease is allowed to plateau (usually towards the end of the study design, which has been refined over the years). The white blood cell (leucocyte) migration models are short term moderate models where the injection of an inflammatory stimulus recruits cells

to the site of injection. There is no expected peripheral effects associated with these models but, depending on the route of administration, there may be low-grade systemic inflammation. However, this is not expected to affect the wellbeing of the animal due to the length of these models. Animals will be monitored regularly for any unusual signs of discomfort such those mentioned above. Such symptoms very rarely appear with leucocyte migration models and therefore the risk of significantly affecting an animal's wellbeing is not expected. Any animal which has lost 15% of its body weight will be monitored, and if this weight loss is combined with any other signs of discomfort mentioned above, the animal will be removed from the study. If the animal loses 20% of body weight, this is an endpoint and it will be removed from the study. In all of the protocols and models described in this application, we plan to provide as much data as possible from every animal. This includes in-life assessment of disease progress (e.g. Manual measurement of joint swelling or imaging disease progress) as well as post-mortem analyses of whole organs and the cells/factors associated with an inflammatory response which may be specific to a particular organ (e.g. the local lymph nodes) or are systemic (blood, spleen, other organs). When possible and when confidentiality of data is not an issue, we aim to publish our results in peer reviewed journals and scientific conferences.

Application of the 3Rs

Replacement

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

Replacement

The programme requires that the models used are ones which closely mirror human disease. All compounds to be tested would have previously been screened in relevant cell culture based models to determine those candidates suitable for animal testing. Rodents (rats and predominantly mice) are suitable for these studies as the work cannot be conducted in lower vertebrates, invertebrates or cell lines due to the poor resemblance of these options to the clinical setting. Animal models address issues which current non-animal based tests cannot accurately determine.

Reduction

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

Reduction

Animal models will be restricted to the minimum number needed for a statistically valid result. The number of animals used will be the minimum safely necessary to allow meaningful statistical analysis of the data generated.

The most important aspect of the proposed programme of work that will reduce the number of animals used is careful selection of drugs, on the basis of preclinical and in vitro data. Only those potential drugs that offer a realistic prospect of therapeutic exploitation will be investigated.

Most importantly, in the past 6 years, we have validated and established several non-animal based platforms that allow a potential client to test a particular aspect of their drug, such as toxicity, the mechanism of action and / or target cell type. Such platforms are either cell culture based, relying on commercially validated and available immortal cell lines or human blood, artificial 3-D tissue equivalents, or more physiological platforms which are based on consensually or ethically derived human tissue. In fact, by installing such assays, I have managed to reduce the contract expectation under this programme from 100% use of animals (forecasted 5 years ago) to 40% (based on contracts from 2012-2017).

The investment of REDACTED allowed for a more thorough assessment of the inflammatory pathways and cells associated with disease, thus bolstering statistical significance by offering additional readouts of drug efficacy and reducing the number of animals required. We have also recently acquired small animal imaging technology which may allow for monitoring of disease development in each animal over time, lessening the need to humanely kill satellite groups to examine disease progress internally, and thereby reducing total animal numbers. These techniques should maximise output and provide a more thorough assessment, with an aim to help in selecting the best models. Certain aspects of disease assessment, particularly in the in-life phase, are fairly subjective. Therefore, there is a demand to standardise and refine this. The use of the imager has potential to not only be beneficial in further assessment of the disease, but also in providing more measureable and standardised outcomes of disease progression, and the subsequent valuation of a therapeutic.

Rodents provide an effective and physiologically relevant platform in general for most pre-clinical testing. For the purposes of drugs targeting inflammatory pathways, the use of higher species is not required because there is a wealth of knowledge on different types of models in rodents, as well as historical in-house expertise with such models.

Refinement

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

Refinement

The majority of pre-clinical testing for arthritis and joint inflammatory disorders has been validated in mice, followed by rats. Only the most relevant models will be chosen based on the expected mechanism of action of a potential new therapeutic, or the information gained from testing that therapeutic in other parallel inflammatory disorders which has provided efficacy. More importantly, animals will only be used when all other non-animal based models will fail to provide the necessary information to progress the potential therapeutic into clinical testing stages. Models of arthritis, and joint inflammation, are expected to result in swelling of one or multiple joints in both the hind and front paws of animals. The swelling may prevent the animal from bearing weight on one or more paws, and refinement measures will be employed to allow the animal to be comfortable and able to access food and water. For example, drinking bottles with long nozzles will be provided, food may be placed at the bottom of the cage, and softer bedding will be considered. There are clear end points which allow for the least harm to be experienced by the animals such as weight loss (unlikely) or the inability to bear weight on a single limb for more than 72 hours. The animals will be handled minimally, but they will be monitored daily for changes in weight, well being, and joint swelling. The use of pain relief will be considered when it does not affect the outcome of the study.

PROJECT 160. 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 Particle Toxicity
Key Words	Mesothelioma, Tumour Suppressor Genes, Carcinogenesis, Nanoparticles, Nanotoxicity
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

During our lifetime we are exposed to nanomaterials. Due to their useful properties, nanoparticle manufacture is an area of fast industrial growth. In the past decade, both academic communities and the public worldwide have become highly concerned with the adverse effects of nanomaterials and their potential hazard to humans. Recent studies showed that nanoparticles may drive toxic effects leading to disease. The potential for human exposure, both occupational and public, and subsequent disease development is of serious concern.

It is not known how, in molecular terms, certain types of nanomaterials affect the body and how fibres of different types compare in their potential hazard.

The Objectives of this project are:

Objective 1 To obtain a detailed adverse outcome pathway for MNP-induced toxicity

Objective 2 To compare the toxicity of different types of MNP using molecular readouts with the focus on Tumour Suppressor Genes

Objective 3 To identify biomarkers of exposure to pathogenic MNP, prior to tumour development.

Objective 4 To gain new insights into therapeutic approaches targeting the Tumour Suppressor Genes and related pathways in the tissues exposed to MNP.

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

Currently, there is no explicit legislation concerning the respiratory exposure limits for nanomaterials and there are gaps in regulation of exposure for people involved in manufacturing or disposal. A better understanding of particle toxicity will help to develop safety regulations and thereby prevent the harmful effects of nanomaterials. Mechanistic data obtained through this research may also serve to improve existing

therapies for patients exposed to asbestos in the past. The data determining toxicity of different types of nanofibres will identify less harmful nanomaterials and thereby promote the “safe by design” approach to manufacturers. In general, any strategy that can reduce the adverse effects of particles would have the potential to benefit people who had been exposed in their lifetime.

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

Our previous in vivo work has been successful REDACTED. We now wish to take the experimental work further and fully describe, in molecular terms, the mechanism of nanofibre toxicity. We are planning to use approximately 5500 animals over 5 years of the project. Mice and rats are proposed for the studies because there is an advantage of using transgenic mouse models; and the rat model of inflammation is considered to be the gold standard for in vivo work and also has higher susceptibility than mouse to mesothelioma.

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

Our main model of fibre toxicity is based on delivery of low doses of particles into mice. The adverse effects related to the routes of delivery (typically injections into the pleural or peritoneal cavity) have a minor impact on animals. This is followed by the exposure period without any symptoms for many months, mimicking a long latency period of disease development in humans. As signs of disease start presenting themselves, animals are monitored for their health and humanely killed when disease is manifested (e.g. respiratory distress, weight loss). The severity level expected is moderate. In the tumour initiation experiments mice are injected with tumour cells; that is tolerated very well. Animals are monitored after injection and killed before tumour has greater than a minor impact on mice. The severity level expected is moderate. All the animals are killed by Schedule 1 at the end of the experiments.

Application of the 3Rs

Replacement

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

Replacement

To address the mechanistic questions we will utilize *in-vitro* based approaches as well as analysis of whole tissues and systems from mice. The pleura is the main target for fibre-shaped particles and it produces a complicated response to fibres. Exposure-induced toxic effects are generated *via* participation of different cell types. Additionally, these effects differ during the time-course of disease development. *In*

vitro assays can't possibly mimic such complexity. Broad and accurate evaluation of fibre toxicity requires using tissues which can accurately model toxic response. Further, for translational outcomes there is no substitute for animal experimentation. We have generated a substantial collection of primary mesothelioma cells, both human and mouse, that provide a valuable tool for replacing animals. 80% of our work is done *ex vivo* and all current mechanistic studies are conducted in cellular systems before we attempt modulation *in vivo*. Where possible we will use primary cell lines and 3D explants for evaluating potential biomarkers and modulators. However, the clinical validity of these and their relevance to human disease requires validation in animal models.

Reduction

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

Reduction

All study designs will be based on 3Rs principles and minimum number of mice will be calculated using Power Analysis. Experiments will be designed utilising Experimental Design Assistant software that facilitates using a minimum number of animals consistent with predicted statistical significance. Through our strong collaboration with the surgeons we have access to patient mesothelioma tissues and have established a substantial collection of primary mesothelioma cell lines. We use patient-derived samples for *in vitro* studies to narrow down the research options required to be studied *in vivo*. We developed a method of mesothelioma cell isolation from the mice with fibre-induced tumours and now have numerous cell lines to utilize in mechanistic studies. This approach allows us to use earlier experimental endpoints and reduce the number of animals in intervention studies. The colonies of mice will only be continued until the consequence of experiments has been confirmed and will be kept to the minimum size, consistent with good practice on breeding genetically altered mice. In addition, mechanistic and morphological testing will be conducted in human and mouse cell lines before modulation in animals. This not only refines, reduces and replaces animal work, but ensures that animals are only used in a targeted way to verify the role of molecules shown to be significant *in vitro*.

Refinement

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

Refinement

The mouse and rat have been chosen as an animal model because of the similarities between their physiology and human physiology. Biochemically and physiologically

many of the mechanisms of toxicity are very similar to those in humans. The fibre toxicity models, based on injections into the pleural and peritoneal cavities, have an advantage of more accurate dosing compared to inhalation studies, where there is a concern about what proportion of particles is reaching the pleura. The doses we use are relevant to potential human exposure. Mice have a great flexibility for targeted genetic modifications, particularly for molecular mechanistic studies. Use of genetically modified animals will accelerate experiments and facilitate in-depth mechanistic studies. We use a model-specific scoring system that helps to assess the health of experimental animals. Rats are known to be susceptible to fibre toxicity and specifically to mesothelioma development in the pleural and peritoneal cavity. The incidence of mesothelioma in rats exposed to fibres via intrapleural or intraperitoneal injection is higher than in mice and develops after ~1 year of exposure. Intratracheal instillation of fibres in rats provides a good model for studying the lung response to fibre toxicity.

We use published NC3Rs and LASA guidelines for maximum volumes of injections and blood samples. We excluded the intra-muscular delivery route from our experiments due to painful effect of these injections on animals.

To avoid/minimise single-housed animals at the end of prolonged study we design experiments in such way that 5 mice are group-housed in the beginning of the study so very few animals end up being singly housed. In an unlikely scenario of having two mice on their own, we will use mirrors to re-introduce singly-housed males.

We also introduce additional refining factors specific to the strains (e.g. for animals that have skin sensitive to infection, we optimised husbandry by introducing paper-based bedding, which is autoclaved in the cage and the cages changed frequently, as well as feeding their diet on the cage floor to prevent any trauma to the nose).

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	Functions of siglecs in the immune system
Key Words	Innate immunity, macrophages, neutrophils, pathogen
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 different cell types in the immune system have to communicate with each other if the response is to be effective in fighting off infections, without becoming dangerously over-active. Much of this communication involves molecules being presented by one cell and interacting with a “receptor” on the surface of another. By using strains of mice that have been bred to either lack individual receptors or express forms of the receptor that don’t work anymore, we can compare their responses to those of normal mice and work out the function of each receptor. Using this approach we have demonstrated that the receptors are important in controlling immune responses to infectious agents such as flu and in inflammatory responses such as asthma and septic shock. The proposed project will continue this work and will provide additional new information and knowledge that will allow us to understand how the immune system is regulated in health and disease. Not only will this research lead to better understanding of disease processes, but it is also expected to result in better treatments for these important human diseases in the future.

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

Regulation of inflammatory responses is crucial in human diseases such as acute lung injury, sepsis and recovery from influenza infection. Our work expected to give important insights into the signalling pathways involved which may lead to new therapeutic approaches to treating human disease. We also expect to reveal new insights into how the immune system defends itself against the influenza virus, especially during the first few hours after infection. Therefore this research could also lead to better treatments for infectious diseases.

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

Mice will be used exclusively. We expect to use approximately 2000 mice over the 2 year period of this project. Only a small proportion of these will undergo any treatment. The majority are used in breeding programmes and humanely killed to produce the tissues such as bone marrow which are a vital source of specialised cell types for in vitro experiments.

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

Apart from breeding and maintenance, some of the mice will be treated with agents that induce mild inflammatory and immune responses. They will also be inoculated with influenza virus to study how the receptors of interest control the response to infection. In all of the studies of inflammation and flu infection, the majority of animals will only undergo short-lasting feelings of malaise and possibly mild fever. This is very similar to how we feel when we have a cold. For our scientific studies, we do not need the disease in mice to develop beyond this point. These treatments are not expected to lead to long-lasting harm or suffering of the animals. At the end of experiments or at the end of their useful breeding life, mice are humanely killed.

Application of the 3Rs

Replacement

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

Replacement

The immune system is a highly complex, integrated network of cells, secreted molecules and tissues. Although individual cell types can be isolated and studied *in vitro*, in most cases it is not possible to extrapolate these *in vitro* findings to how the whole network responds to infectious agents and inflammation. Therefore, *in vivo* studies are essential if one is to obtain a complete understanding of the role of a given molecule(s) in the immune system. The mouse provides an excellent model system for understanding how the mammalian immune system works and the use of gene targeted mice has greatly increased our knowledge of the functions of specific proteins involved in host immunity. In this project, we propose to use mainly ‘Knock-Out’ and ‘Knock-In’ mice to continue our functional analysis of cell surface receptors involved in these important functions.

Reduction

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

Reduction

We have developed cell culture methods for expanding large numbers of cells from the stem cells present in mouse bone marrow. These include bone marrow-derived macrophage cultures using CSF-1 and dendritic cell cultures using Flt-3 ligand. We are also currently refining expansion of other cell types such as T regulatory cells. These *in vitro* cultured cells mimic their natural counterparts very closely and are therefore an effective replacement for animals in biochemical studies. We will continue to exploit immortalised cell lines wherever possible to extend findings made with the *in vivo* mouse models of inflammation and infectious disease.

Animal numbers are also minimised by the use of good statistical tests. The number of mice used in this study will be calculated according to four components; 1) the nature of immune response expected in control groups of mice; 2) the anticipated effect of the loss of a particular immune cell/molecule on the immune response 3) the significance level; and 4) the error rate (acceptable false negative) that is judged to be reasonable.

Refinement

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

Refinement

The mouse provides an excellent model in which to study the relationship between the immune system and disease induction, since mice are well characterised immunologically, their immune systems closely resemble those of humans, and the majority of these models have been extensively studied and have pre-determined correlates of disease regulation. This allows us to reduce the number of unknown factors in any given experiment and increase the probability of obtaining interpretable and meaningful data.

In addition, multiple genetically modified mice lacking various immune molecules/cells have been generated, and can provide a very refined approach to performing detailed analyses of the role of receptors in immunological functions.

We aim to minimise welfare costs, yet at the same time maximise the output of data from animal experiments, by using sophisticated *in vitro* assays on tissues and cells in order to evaluate how the mice have responded to the various challenges used. We aim to stop experiments with inflammatory or infectious agents at the earliest possible point where scientific validity is reached, thus reducing or preventing unnecessary welfare problems. Animals that undergo challenge with infectious agents are supported, for example, with a high-energy diet, much as human ‘flu patients would be.

PROJECT 162. 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	Immunity in chickens; modulation by pathogens, human intervention and breeding
Key Words	birds, immunity, infection, vaccine, resistance
Expected duration of the project	5 year(s) 0 months

Purpose of 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 of the gut and lung are significant problems in poultry produced for eggs and meat. This is a welfare issue for these birds resulting in suffering and death, as well as being a human disease risk as some of these conditions are transferrable to humans (zoonotic disease).

In addition, there is a considerable financial impact. The estimated disease effects and control costs of, for example coccidiosis (a disease in the chicken gut) and infectious bronchitis virus (a respiratory disease) in the UK are over £12M and £23M respectively. The costs of zoonotic diseases run into billions per year, e.g. acute gastroenteritis in humans in the United Kingdom each year at a recurring cost of ca. £2Bn. At present we try to control these diseases using a variety of vaccination strategies but these are not highly effective.

In this project we want to understand how birds react to infections and how we can improve disease control in poultry, thereby reducing the risk of human infection. The major gaps in our knowledge include the understanding of (i) how immune responses at different stages of infection work and how the immune response can be sustained for longer, (ii) how infectious agents such as bacteria and viruses avoid detection by the bird and (iii) how we can best improve the immune response of the birds to defend against diseases.

The objectives of this PPL are to

1. Define the interaction between the early and late immune responses after exposure to infectious agents such as viruses, bacteria, and parasites.

2. Define how infectious agents can change the immune response in the bird

3. Investigate and test substances to improve host protection against infection.

4. Investigate new strategies for prevention and treatment of 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 project aims to study how the immune system of birds functions in order to understand how we can improve poultry health and welfare. Most of the UK flock of 850 million meat chickens and egg laying birds receive many vaccines and this is seen as preferable to having to use therapeutic drugs to treat infections, which might impact on the human food chain. Several poultry diseases pose a threat to human health and outbreaks of bacterial food borne diseases frequently arise. Reducing the prevalence of poultry diseases will improve animal welfare and lower the incidence of human infection. The work under this licence will fill gaps in our knowledge on avian immune responses, escape mechanisms of disease causing agents and new treatments and will lead to a better understanding of host-pathogen interactions. The findings will steer future work on developing vaccines and identify markers of host immunity to support selective breeding of animals with improved resistance.

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

Birds, mostly chickens, are used, but other birds e.g. ducks and turkeys are used for influenza related research. The number of birds will depend on the grants obtained in the near future, and will be not more than 3400 birds over the duration of the licence, 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?

When using live viruses, bacteria or parasites the birds may show wheezing, sneezing, lower activity and diarrhoea. The animals will be humanely killed at the end of the experiment or earlier if unexpected or more than mild effects occur. The birds are monitored frequently.

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 necessary because it is not feasible to study the complex interactions between infectious agents such as viruses and the immune system of the host in plastic dishes.

To replace some use of animals we have developed cell culture system to screen for interactions of pathogens or vaccines with host cells.. We grow parts of organs in culture such as rings of windpipes, mini-guts and bone marrow derived cells. We use

these systems to obtain a lot of information that determines which experiments we conduct in the live birds.

Reduction

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

Reduction

To reduce the number of animals we will use genetically altered chickens that are resistant or susceptible to certain diseases. Existing knowledge of the genetics of these birds enables us to focus on the specific aspects of the complex immune responses. On the other hand we also use commercial birds to be as close to the poultry industry standard as possible.

In addition we share tissues, DNA and RNA taken after the experiment with other scientists to ensure best use of animals. The minimal number of birds required will be estimated and studies designed and interpreted in collaboration with in house statisticians and will be based on experience, literature and statistical predictions.

Refinement

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

Refinement

Poultry are key reservoirs of pathogens understudy and alternative cell- and mammalian based models do not provide the most relevant data. Wherever possible we will meet experimental objectives before animals exhibit disease symptoms or humane end-points are reached.

Use of commercial birds reared on site will enable us to perform experiments that are closest to the real world, the poultry industry, as we can thereby ensuring that the translation to the field situation is realistic.

PROJECT 163. 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 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 164. 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 drugs with activity in the CNS: determination of novel targets, efficacy and safety
Key Words	Drugs, CNS, Efficacy, Side-Effects
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:
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):

Mental health problems are a primary cause of the overall disease burden worldwide. Drugs currently available to treat CNS disorders are often limited in their effectiveness and may be associated with unacceptable side effects. As a result, more efficacious and safer medicines to treat CNS disorders are urgently required. The overall project aim is to provide highly specialised preclinical services to the pharmaceutical and biotech industry to evaluate the efficacy, mode of action and side effects of novel drugs for the treatment of CNS disorders. These specialised techniques may occasionally be employed to evaluate centrally-mediated side effects of drugs developed to treat other conditions.

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

The evaluation of novel drugs provided by clients in suitable tests/models in this project is expected to facilitate decision making by the client in regard to the progression and development of a compound as a treatment for a CNS disorder. Accordingly, experiments performed in this project are expected to expedite the development of better drugs to treat CNS disorders.

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

Approximately 6000 rats and 2500 mice over 5 years. The exact number of animals used will be dependent upon external factors such as the number of clients and the success of those clients in designing suitable drugs.

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

The majority of studies will involve the administration of centrally-acting drugs (usually orally) with blood or tissue sampling or behavioural/physiological testing. Drug treatment might be once or repeated. Most of the procedures are anticipated to

be mild. Some drugs will have been tested in vivo prior to being sent to us for assessment in assays the clients do not have validated. In such circumstances no adverse effects are expected. Occasionally, substances will be evaluated which may not have been tested in animals and unexpected toxic effects might arise which could cause pain, suffering, lasting harm or in extreme cases death if humane end points were not applied. Occasionally, drugs will be given centrally or by infusion via small pumps implanted under the skin (so avoiding daily dosing). Such procedures will involve anaesthesia. In some cases, the animal models employed may involve induction of specific pharmacological responses and/or involve training in specialised equipment which may produce transient discomfort/stress. In some instances, animals that are fully recovered at the end of procedures may be kept alive at the establishment (with the agreement of the vet), with a view to their re-use on procedures if appropriate and licensed. Upon completion of procedures animals will be killed. For these reasons, the likely/expected level of severity of the licence is moderate.

Application of the 3Rs

Replacement

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

Replacement

There are no alternatives to the models employed as they are used to assess the integrated behavioural and/or physiological/pharmacological responses of the whole animal to different treatments. Although the decision to test compounds in the project is generally based on responses in cell lines, cells and tissues, such assays cannot replace *ex vivo* or *in vivo* testing. Such animal testing is a fundamental requirement for progressing novel agents in man.

Reduction

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

Reduction

Qualified and highly experienced biostatisticians advise on experimental design and ensure that the correct number of animals is used to produce meaningful statistical comparisons. Animals will also be minimised by measuring several parameters in the same animals where possible (where animal welfare and experimental data will not be compromised). Where animal welfare is not compromised animals may be re-used in certain tests (on up to 8 occasions).

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 since their central nervous systems are well documented and they are the lowest form of mammal that can provide meaningful data about man. All studies will employ healthy adult animals. A variety of established, fully-validated animal models and assays will be employed. These have been widely used by the pharmaceutical industry to predict the effects of drugs in man. Where substance classes have not been given to animals before, pilot studies will be performed. Where substances are given as part of the procedure to induce an animal model of a CNS disorder, or a specific pharmacological response, doses will be carefully chosen and experiments designed so that any adverse effects and/or the duration that animals are exposed to the adverse effects are the minimum required for the scientific objectives to be met. Surgical procedures will only be used if alternatives are not available. Anaesthesia will be maintained at a suitable depth to avoid the animal feeling pain. Aseptic operating procedures, topical application of antiseptics and dressing will be used to reduce the possibility of infection. Post-operative analgesia will be used as advised by the vet to reduce pain and suffering. All animals will receive the highest possible standard of post-operative care. The project is supported by a dedicated animal husbandry and technical support team. Studies will be conducted by staff highly experienced in animal handling.

PROJECT 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	Novel and advanced therapies for heart failure
Key Words	cardiac, gene therapy, cell therapy, heart failure, myocardial infarction
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Myocardial damage (such as heart attacks, genetic defects or damage from chemotherapy) not only reduces the power of the heart to contract but leads to further ongoing damage as the body overstimulates the remaining heart muscle. This leads to heart failure, a condition with severe symptoms and a prognosis as poor as some of the worst cancers. Patients die from failure of the heart to beat strongly enough or, in about half the cases, to sudden disturbances of rhythm (arrhythmias). Drugs in use presently for heart failure concentrate on preventing further damage but do not reverse the condition. Advanced therapies such as new kinds of drugs, or gene and cell therapy aim to produce greater benefits and ultimately restore full function to 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)?

For gene or drug therapy, the aim is to restore full function to the undamaged muscle in a safe way, and to reduce arrhythmia. Clinical trials are already in progress as a result of our work, and the aim for the animal studies now is to improve the therapy as a result of what we learn from these trials. For cell therapy, the more ambitious aim is to give new muscle back to the heart using stem cells. For this, tissue engineering strategies using materials are likely to be the best way to deliver the stem cells

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

Over 5 years we will use an absolute maximum of 8200 mice; 4800 rats; 410 guinea-pigs and 1250 rabbits. The total is likely to be considerably less than this as we have listed a number of alternative 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?

For the operations to induce heart failure (aortic banding and myocardial infarction), or to introduce gene or cells, there will be an open chest procedure. This has the potential to have similar pain levels to a human heart operation, and will be performed in the same way with full anaesthetic, and painkillers given during and after the operation. Mimicking the damage caused by chemotherapy is done by tail vein injection under anaesthetic: however the drugs can be irritant so pilot studies have been done to reduce these effects as far as possible. Animals will be followed for weeks to months to determine the development of heart failure and the effects of drug, gene or cell therapy to reduce it: they will be imaged serially during this time. Around 50% of the animals may undergo sudden cardiac death due to arrhythmia when heart failure occurs. Loss of consciousness is thought to be rapid, so that this death is relatively fast and painless. Others can develop symptoms of heart failure which are breathlessness, fatigue, water retention causing some paw swelling and blue extremities due to poor oxygen supply to tissues. Usually, we can predict when the heart is starting to fail from the imaging studies, and so few of the animals reach the point where symptoms are seen. At the end they will be killed by approved humane methods and hearts taken for further studies where live tissue is investigated using state-of-the-art imaging.

Application of the 3Rs

Replacement

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

Replacement

Heart failure is a condition where the responses of the body drive forward the disease process: this cannot be mimicked in an isolated heart outside the body. Similarly, the effect of gene or cell therapy to improve the function and prevent further damage must be done in a live animal. However, we gain a great deal of further information by using tissue and cells taken from the animal and used outside the body. We also perform similar experiments using human tissue from surgical specimens when we can, but these are available only 5-10 times per year and never include healthy tissue. We have been developing, as a replacement, heart muscle cells derived from human stem cells. Many of our experiments are now done on these cells, which we can also reconstitute into a functional muscle strip. We are using patient material as a source to derive these stem cells in order to investigate the effect of genetic modification on heart muscle cell function.

Reduction

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

Reduction

For the protocols listed, funding has been obtained or will be applied for before going forward. Funders require power calculations to match animal numbers to the outcome, and these are peer reviewed by expert scientists. The Boards of many funders now include statistical advisors to assess these power calculations. Randomisation and blinding to reduce bias and improve reproducibility of models will be used at appropriate states in the experiments. We commit to publication in line with the NC3Rs ARRIVE guidelines

Refinement

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

Refinement

It terms of the objectives, there are different specific advantages of the various species. Mouse gives the power of the transgenic models for investigating human cardiac mutations. Rat produces a reproducible human-like myocardial infarction and develops heart failure gradually over a controllable period. Guinea-pig and rabbit both have myocardial electrical characteristics closer to human heart, which is important for the integration of human stem cells. Rabbit heart thickness is more suitable for testing larger material/cell constructs intended for man. We wish to develop this as an alternative to pig or primate as a pre-clinical model.

In general we will match the monitoring frequency to maximise the chance of adverse effects being captured. In all cases where adverse effects are noted, advice will be sought from the named animal welfare officer and/or named veterinary surgeon and humane euthanasia will rapidly be used for any animals not responding to treatment given. All surgery will be done under general anaesthesia: pre and post-operatively the pain will be controlled with analgesics as for human. For the chemotherapy protocol, tail vein injection will be done under light anaesthesia and analgesics use afterwards. Thorough flushing and washing will remove the irritant drug from the site of injection. Heart failure as a result of the various strategies is seen in symptoms such as fatigue, paw swelling from water retention or breathlessness. Imaging of the heart will be done to catch the animal just before these symptoms emerge, but as soon as they are seen this is considered a humane end point. Some animals will die suddenly from disturbances of heart rhythm, as with heart failure patients. The human experience from resuscitated patients (as well as our own observations of mice) suggests that this occurs in minutes with immediate loss of consciousness, so the distress has a limited duration.

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	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. REDACTEDTogether 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 167. 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	Immunogenicity of Crimean-Congo Haemorrhagic Fever virus vaccines
Key Words	Crimean Congo Haemorrhagic Fever Sheep
Expected duration of the project	5 year(s) 0 months

Purpose of 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.
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 is an unmet need for safe and effective vaccines for livestock against Crimean Congo Haemorrhagic Fever (CCHF). CCHF is a disease spread by ticks from livestock to humans, and has a fatality rate of 15-70%. It is present in many countries, including Asia, Eastern Europe and southern Africa. It is, at present, an increasing problem in Western Europe including areas such as Greece and Turkey. Assessment of a vaccine which can prevent and control CCHF in Sheep is the aim of this study.

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

The information gathered from this study will allow the vaccine to be used in sheep in countries where CCHF is present. Preventing and controlling CCHF in livestock in these countries will help to control CCHF infection of humans, and therefore ultimately help prevent the loss of human life associated with this disease.

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 around 100 sheep 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?

Following vaccination of the sheep with the CCHF vaccine, no adverse effects are likely / expected other than shot lived discomfort / elevated temperature as is normal with any vaccination, and is considered to be of a mild level of severity. All animals will be euthanized 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

Sheep are the target species for this vaccine, in that they will be the species in which this vaccine will be used out on farms and in the field in countries where CCHF is present. Due to the complex nature of the immune system, it is not currently possible to assess with certainty how good an immune response a vaccine will illicit from pure in vitro analyses. Furthermore, before this vaccine is permitted to be used on farms and in the field in endemic countries, information on how effective it is in sheep is required by the regulatory authorities of the country in question.

Reduction

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

Reduction

We will use statistical tools to determine the minimum sample size that allows determination of our study endpoints with appropriate 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

This vaccine has already been shown to be effective in mice, however due to sheep being the species in which this vaccine will be used in to control CCHF in endemic countries, it is most appropriate to use sheep as the animal model for this programme of work. Animals will be housed together with bedding and other items of enrichment. Highly trained animal technicians will monitor these animals throughout the day, ensuring they are comfortable and to maximise their welfare status.

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	Development of novel cancer immunotherapeutics
Key Words	Cancer, 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;
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):

Cancer immunotherapy has emerged as the first genuine breakthrough in cancer treatment since chemotherapy. Immunotherapies treat cancer by harnessing the immune system to target tumours, rather than using cytotoxic drugs that are poorly efficacious against advanced disease and often have severe side effects. Despite these new immunotherapies, the current drugs are associated with significant side effects and are effective in a minority of patients. Therefore, new immunotherapies that are less toxic and efficacious in a greater number of patients are urgently required. This project aims to speed the development of a new cancer immunotherapeutic, which has the potential to treat patients that do not benefit from current therapies. The objectives of the project are to: 1) fully elucidate how the new immunotherapy alters the immune system to attack tumours to help design human clinical trials, 2) to identify other anti-cancer agents to combine the drug with to bring maximum benefit to 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)?

We are developing a completely novel cancer immunotherapy that has the potential to treat patients who do not respond to current immunotherapies and with less side effects. The novel immunotherapy is also combinable with existing immunotherapies, potentially boosting their efficacy. The project will enable the most effective clinical trials in man, by identifying the underlying mechanisms by which the immunotherapy destroys tumours, allowing the best tests to be performed when assessing efficacy in human trials. Furthermore, the project will identify other drugs that the immunotherapy can be combined with for maximum effect, directing the combinations patients should be treated with in future clinical trials.

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

A transgenic mouse model that replicates human immunology will be used. The project is expected to last 5 years and will use approximately 6000 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 immunised by intraperitoneal injection to induce the production of an antibody that is normally only expressed by humans, a procedure has been performed many times and has been optimised to minimise severity. Tumour cells will then be injected subcutaneously into the flank(s) of the mice induce tumours, which will be then be treated with the immunotherapy. The tumours are expected to grow, but they are not expected to interfere with feeding, eating, drinking, movement or to ulcerate, and if they do the animal will be killed. The immunotherapy is administered by direct injection into the tumour, mirroring how the drug will be administered in humans, and this procedure has been performed many times previously without any adverse effects. All procedures in the programme of work have been previously optimised to maximise effectiveness and minimise severity. At the end of the study, all mice will be euthanised as control mice will have rapidly growing tumours and, also, detailed analysis will be performed on the tumours and other aspects of the mouse immune system at the end of the study.

Application of the 3Rs

Replacement

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

Replacement

In depth profiling of the novel immunotherapy has been performed using cell culture systems that do not involve the use of animals. These experiments have allowed us to look at individual aspects of the anti-cancer immune response generated by the drug, but they do not allow us to look at the process by which anti-cancer immunity develops.

The immune system is complex and multifaceted, and thus experiments must be performed in animals that have a complete immune system to explore how the immunotherapy stops tumour growth and which other drugs it should be combined with for maximum effect. Importantly, the drug requires the presence of a set of naturally occurring antibodies for efficacy, and these antibodies only exist in humans. This project will use a genetically modified mouse that, like humans, also produces these antibodies. With the need to recreate a uniquely human situation, these specific mice are the only model suitable for these studies. Additionally, mouse immunology is very well understood and has good translatability to human immunology.

Mice are only being used as no viable alternatives are currently available. As the project progresses, emerging techniques that replace the use of animals will be assessed and employed, if suitable.

Reduction

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

Reduction

Animal studies are only being performed when there is no alternative. The studies will be carefully planned and reviewed by a panel of experts before commencement and statistical methods will be employed to ensure that the numbers of mice used in each study are the minimum required to give valid results. Mice will be randomised into the experiments and groups for statistical comparison will be age and weight matched. The numbers of mice to be used per group will be calculated by power analysis, to ensure that animals are not wasted.

As the project progresses, emerging techniques that can replace the use of animals will be assessed and employed if suitable to further reduce 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

The novel immunotherapy requires the presence of an antibody that is pre-existing in humans and Old World monkeys, but not other mammals. To prevent the need for any experiments that involve the use of non-human primates, a transgenic mouse model has been generated that mimics humans and Old World monkeys by producing the required antibody after immunisation.

The mice will be housed in a specialist facility, free from pathogens that could affect their health and will only be handled by experts. All procedures that will be employed have been performed extensively and tumours will not be allowed to reach a size that increases the severity of the procedures. All procedures will be performed by experts.

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	Mouse models of virus and bacteria induced airway disease
Key Words	Virus, Bacteria, Asthma, COPD
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Asthma and chronic obstructive pulmonary disease (COPD) are chronic diseases of the lungs. 5.4 million people in the UK suffer from asthma whilst COPD is the third leading cause of death worldwide. How and why asthma and COPD develop and persist is still relatively poorly understood.
As well more mild illnesses such as the common cold, Viral and bacterial infections of the airways cause life-threatening attacks of asthma and COPD. Again, understanding of how these pathogens cause asthma and COPD attacks, and the range of other diseases associated with them is somewhat limited and treatments are in many cases not available or considered inadequate.
This project aims to increase our understanding of the mechanisms underlying asthma, COPD and other viral and bacterial respiratory disease. This will help us to identify, as well as test, more effective 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 potential benefits of the project will be in both advancement of science and development of new drugs. The project will enhance our understanding of immune responses to common respiratory virus and bacterial pathogens, improve knowledge of how these viruses and bacteria interact in co-infections of the airways and examine their roles in the development of asthma and in attacks of asthma and COPD. This information will help us to identify processes which can be targeted by new treatments and provide us with animal models of disease in which initial testing of such new treatments can be undertaken.

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

All experiments will be performed on mice. Over a 5 year period we expect to use no more than 24,000 mice (4,800 per year). This number includes breeding of up to

10,000 genetically altered mice and purchase of up to 14,000 non-genetically altered animals.

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

The maximum severity of procedures is considered moderate. Breeding protocols are considered of mild severity. Infection with some bacteria and viruses such as influenza and respiratory syncytial virus could cause some obvious signs of disease such as lethargy and weight loss. The administration of substances such as viruses, bacteria, or drugs to mice will be done by the least invasive method and where appropriate under anaesthesia. Because we are concerned with respiratory disease, some conscious animals will be made to breath substances which cause them to become breathless, in order to measure their lung function. This will however be transient and animals are expected to recover fully and rapidly from this. All experiments will result in mice being killed humanely, most commonly by overdose of anaesthetic. In the case of breeding procedures, mice may be either used for experimentation as discussed, used for further breeding, or transferred to other researchers for their studies.

Application of the 3Rs

Replacement

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

Replacement

In addition to animals, we perform studies on human volunteers and in cells and tissues donated by those volunteers. Wherever possible studies are performed on human samples. Questions such as those relating to clarification of complex immune system mechanisms often cannot be studied in cells from humans however and studies of people with disease often cannot for ethical reasons supply the types of sample or frequency of sampling required to answer a research question. In these cases we will use mice for our experiments, in which we can make genetic and drug interventions, or manipulate the immune system, allowing us to clarify disease mechanisms and demonstrate cause and effect, with the ultimate goal of developing new, more effective therapies for man.

Reduction

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

Reduction

We will always make sure by surveying the scientific literature that studies are not unnecessarily repeated. Statisticians will be consulted to ensure that the appropriate number of animals is used to ensure statistically, as well as biologically meaningful results. Appropriate negative and positive controls for a given treatment are essential to avoid unnecessary repetition of experiments, but where pilot experiments demonstrate that a control is redundant, study designs will be refined accordingly in future work. Finally, breeding strategies will be optimised to help reduce the number of animals used. All studies will be carried out in a way that enable publishing in adherence with 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 will always make sure by surveying the scientific literature that studies are not unnecessarily repeated. Statisticians will be consulted to ensure that the appropriate number of animals is used to ensure statistically, as well as biologically meaningful results. Appropriate negative and positive controls for a given treatment are essential to avoid unnecessary repetition of experiments, but where pilot experiments demonstrate that a control is redundant, study designs will be refined accordingly in future work. Finally, breeding strategies will be optimised to help reduce the number of animals used. All studies will be carried out in a way that enable publishing in adherence with the ARRIVE guidelines.

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	Cell Death and Inflammation in Tissue Repair and Cancer
Key Words	Cell Death, Inflammation, Cancer, Anti-cancer 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):

Anti-cancer chemo- and radiotherapy work by activating cellular processes whose purpose is to kill the cell. A key problem is, however, the acquired resistance of cancer cells to bypass the cell death programmes. Cell death acts as part of a quality-control and repair mechanism that eliminates potentially harmful cells, and failure to do so is linked to cancer. However, it is now recognised that killing cancer cells with chemotherapeutics, while important, is not sufficient to provide long-lasting protection from the tumour growing back.

One promising approach to improve cancer therapies is to stimulate the patient's own immune responses against breast tumour cells. This can be achieved by inducing tumour cells to undergo immunogenic cell death, meaning that the patient's dying cancer cells stimulate a specific anti-tumour immune response, which in turn can control, and sometimes eradicate, residual cancer cells.

Our projects are aimed at harnessing the complex relationship between cell death and immunity to elicit robust immunogenic cell death.

Therefore, understanding the mechanisms that regulate immunogenic cell death might allow us to kill cancer cells, and at the same time activate a specific anti-tumour immune response to generate a clinically durable anti- cancer response.

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

Our research will assist the design of future clinical trials and the development of novel anti-cancer treatment combinations. We will identify new and more effective treatment combinations to treat solid tumours in humans. Moreover, this work will benefit the basic research community by increasing our fundamental knowledge of how our body defends itself from pathogens and cancer.

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

This project will require the use of various strains of mice genetically modified for proteins that regulate the body's defence against pathogens and cancer. We anticipate using around 24,300 mice in total for the duration of this five-year period.

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

We will conduct research into how the body defends itself from cancer. Animals are not expected to show an overtly harmful phenotype, other than localised inflammation and tumour development. Tumours will be primarily subcutaneous tumours, tumours of the lung, mammary gland and intestine. In certain cases, animals may also develop distant tumour colonies in lung, liver and bone. Tumour burden will be limited to the minimum required. Occasionally, tumours may ulcerate and very rarely they will compromise locomotion. Depending on the tumour model, cancer cells may migrate to distant organs (metastasis). Metastasis may present as (e.g.) weight loss, palpable internal tumours or lymph nodes or compromised respiration. Animal suffering will be minimised by making every effort to keep the tumour models employed at the subclinical levels. Tumour burden will be assessed also with the help of imaging. Other adverse effects associated to the experimental manipulations described in this project include risk of infection and minor pain or discomfort that will be dealt with using aseptic techniques, antibiotics and analgesics. Toxicity may arise from the use of anticancer agents and radiation. This is not expected to be a regular occurrence as they are delivered at previously determined well-tolerated doses. To minimise any possible adverse effects, we will closely monitor animals undergoing experimental procedures and pay attention to any signs of suffering. Also for genetically altered mice that are not yet the subject of a specific experiment, common problems affecting each genetic background will be monitored. Animals will be humanely killed at the end of each procedure. We have also indicated several guidelines that regulate when animals should be humanely killed before the end of the procedure.

Application of the 3Rs

Replacement

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

Replacement

The ability of tumours to grow depends on interactions with cells of the surrounding tissue. Therefore, it is essential to study tumour biology in animal models in vivo, as only limited information can be obtained from culturing cancer cells in incubators. Additionally, metastasis is a process that can only happen within the whole organism

in vivo and no non-animal alternatives are available for that either. However, I plan to continuously monitor the research in an attempt to replace sections of in vivo work with ex vivo or in vitro alternatives wherever possible.

For example, we will be using heterotypic organoid cultures where we grow mouse-derived tumour organoids in the presence of immune cells. This will allow us to streamline our research and rapidly evaluate tumour-immune reactions in vitro.

We are also using the fruit fly as a model system to study certain aspects of tumour defence mechanisms. This will help us to focus our questions and refine our in vivo experiments in mice.

Reduction

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

Reduction

In some experiments animals will only be used to generate primary tissue cell cultures that avoid invasive procedures and uses fewer mice. We will use well-characterised cancer models that minimises the requirement for pilot experiments to define n numbers. We use statistical power calculations to help determine the most appropriate number of mice to use to test an experimental hypothesis. The use of highly inbred, genetically altered animals will decrease the natural variation and improve signal to noise. Demand for genetically altered mice will be carefully assessed before breeding and crossing, and mouse numbers with unwanted genotypes will be kept to a minimum by optimising crossing designs. Moreover, we will make use of CRISPR/Cas9-mediated genetic engineering to generate one-step genetically modified cancer models. This will dramatically reduce the number of mice needed.

Refinement

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

Refinement

Cancer is analysed in the context of the host and, therefore, these studies need to be mainly performed in vivo. The reasons why mice are the best choice as cancer experimental models can be summarized as follow: (1) the physiology of cancer in mice is consistent with the human disease; (2) the need for working with genetic modification (knock out, transgenic models). In mice, many models are available, as are well-defined techniques for de novo production; (3) they are economic, easy to handle, produce multiple offspring and they have a very short gestation period as

well as a functional survival time. (4) well-defined inbred mouse strains and mouse cancer models minimise variability in the responses between individuals, thus ensuring fewer animals are required. We will continuously refine our model such that we optimally power our experiments and use just the right numbers of animals to generate data that is reliable and robust, yet avoids the need to repeat experiments beyond statistical significance. We will commit to working within the guidelines on tumour growth in animal models, as outlined by the NC3R, and in the guidelines for the welfare and use of animals (British Journal of Cancer. 2010 May 25; 102(11) 1555). All animal work will be performed in close collaboration with skilled animal technicians and trained research staff. Moreover, we are collaborating with leaders in the field so that we can use the respective mouse cancer models with utmost efficiency.

For the adoptive transfer procedure, we will use alternative anesthetizing procedures should they become available.

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	Mediators and effectors of pregnancy complications
Key Words	recurrent miscarriages, preeclampsia, preterm birth, gestational diabetes, 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):

Pregnancy should be a happy and healthy period for every woman. However, many women experience serious pregnancy complications that put at risk their lives and their babies lives. Miscarriages, preeclampsia and preterm delivery are some of these serious pregnancy-related disorders. Unfortunately, there is no available treatment or diagnostic method to determine which women will have complicated pregnancies.

To better understand the mechanism/s responsible for these adverse pregnancy outcomes we will use animal models that mimic the different clinical scenarios. Using these mouse models we previously demonstrated that miscarriages, placental damage and maternal vascular disease observed in preeclamspia and the cervical and uterine changes found in preterm delivery are associated with inflammation and the clotting system.

The aim of this project is to identify the specific molecules and cells responsible for the different pregnancy complications and thus identify targets for therapy and diagnostic methods. In addition, we will test treatments to prevent miscarriages, preeclampsia and delay the onset of preterm birth. We will also identify diagnostic, preventive and therapeutic strategies in congenital heart block and in adverse pregnancy outcomes associated with sickle cell disease. We will also investigate the long term health effects of abnormal pregnancies in the mother and offspring

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

The animal models will allow us the identification of the mediators responsible for adverse pregnancy outcomes and targets for prevention and therapy. If we validate these observations in humans, these molecules and targets could be used to detect pregnancy complications before they happen and develop new therapies to improve

the health of mother and child. Using mice, we recently identified an effective treatment that was proven to be successful in women and several human babies were saved. We will build upon our recent success in preventing preeclampsia to continue our search for cures for other pregnancy complications. We expect to find treatments not only to protect the pregnancy and maternal health but also to allow a healthy development of the foetus and the prevention of long term health consequences for offspring.

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

We estimate to use no more than 10000 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?

The severity of the procedures described in this project will have minimal to moderate effects upon the animals involved. For example, light anaesthesia will be used for the non invasive imaging procedures. Small devices to deliver treatments will be placed under the skin to avoid the stress of daily injections using light anaesthesia. Pain killers will be administered. The vast majority of the animals in this project will not undergo surgery. All the procedures described in the different projects will be performed by skilled personnel and thus adverse effects are not expected. Animals will be closely monitored for any signs of distress. At the end of the experiments the animals will be humanely killed and after death organs will be taken for further studies to evaluate maternal and foetal health.

Application of the 3Rs

Replacement

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

Replacement

Unfortunately, we can not use computer models, or cell cultures to adequately replace animal models for pregnancy complications. Pregnancy is a phenomenon that only occurs in animals. Thus, animal models of pregnancy complications are essential. However, we will use cervical, myometrial and placental human tissue and cell lines to identify the molecules responsible for the changes that lead to adverse pregnancy outcomes.

Using a lower order species is not an option in our project as we need to study mammals with placentas in order to correlate results to humans. To study congenital heart block we will measure the contractility of the heart in zebrafish embryos.

Reduction

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

Reduction

To reduce the number of mice to be used we will use the following strategies:

1. Limit the group size to the minimum needed to obtain statistically significant data. I have extensive experience of using statistics in experimental design and we will make sure we use the minimun number possible to achieve statistical significance
2. We will perform multiple experiments simultaneously so that the same control group can be used for all experiments
3. We will try to collect as many different tissues from each animal so that additional animals are not needed
4. We will try to use new instrumentation/methodology that improves precision and reduces the number of animals. For example imaging techniques that are non invasive and allow the study of one mouse at different times instead of sacrificing them at predetermined times for studies along time

REDACTEDI always work closely with my students, postdoctoral fellows and junior scientists. I do not only supervise them but work with them. In my opinion, this close follow up is a very good way to prevent errors and thus minimize the number of mice 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

Because pregnancies have similar characteristics in humans and mice, mice constitute a good animal model. Mice are quick to reach sexual maturity (7- 8 weeks) and they have a short reproductive recovery (less than a week). Litter-bearing (6-10 pups/liter) is another advantage that allows the follow up of the offspring. Mice have short gestational period (≈ 20 days) and the reproductive tract is relatively small, thus it is possible to analyze the course of pregnancy in exquisite detail.

We looked and continue to look for procedures to reduce pain or distress in the animals that we will use.

1- Most of the studies designed to address the causes of pregnancy loss do not require surgical interventions as used by many other laboratories. Ee will induce preterm birth in mice by intravaginal administration of LPS, a non invasive model in which the maternal health is not compromised.

2- In addition, in the two mouse models of preeclampsia that we will study the relevant key features of preeclampsia appear spontaneously without surgical manipulation (e.g. ligation of uterine arteries) or the administration of any compound to induce the condition. Thus, by using these mouse models, in which preeclampsia develops spontaneously we avoid surgery and we minimise distress and pain

3- In the mouse models of pregnancy loss the embryos die at day 8 of pregnancy and in the preterm delivery model the fetuses born at day 16-17 are non-viable at this stage of development. Mice pups even at term have limited cortical development and hence limited or no perception of pain/distress.

4- Animals will be monitored frequently and closely for symptoms after administration of drugs or substances. skilled personnel will be responsible for the administration of the drugs.

5- We do not anticipate animal distress or suffering as the protocols we will use are very mild. However, if by any reason we detect signs of distress or suffering (e.g. lack of grooming, piloerection, hunched up or significant loss of weight compared to controls animals) mice will be humanely killed by schedule 1 protocol and protocols will be reviewed.

6- We will periodically check the scientific literature for new procedures to reduce pain or distress.

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	Mouse models of tumour growth and progression
Key Words	cancer, metastasis, therapy, transplantation
Expected duration of the project	2 year(s) 6 months

Purpose of 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, a major health issue worldwide, is a multistep disease resulting from a series of genetic mutations in genes referred to as oncogenes and tumour suppressors. Understanding how these genetic lesions change the normal cell to a cancerous one is vital if we are to prevent and treat cancer. Only in the context of the complete living animal can we fully understand how cancers develop, invade and spread to other organs. Using genetically modified (GM) mouse models with the same genetic mutations as in the human disease (so called 'patient-like' animal models) we can investigate the biological consequences of these lesions in cancer progression and identify those genetic events and signalling pathways which work together to drive invasion and metastasis. Such information will enable us to design new and targeted therapeutic approaches.

The ultimate aim of the project is to use mouse models of human cancer in fundamental cancer biology research and in identifying new therapeutic targets. An important aspect to be studied is how the tumour environment influences the growth and progression of the disease. In addition, the impact of changes in cell shape as cells die on the inflammatory and immune responses will be studied, which may affect the way that tumours respond to therapy.

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

This project will deepen our understanding of the underlying causes of cancer in general and of specific types of cancer which currently have a poor prognosis such as pancreatic cancer. Knowledge of the genetic causes will dramatically improve our ability to diagnose, treat and prevent cancer which affects one in three of the human population. We will also use mouse models to identify and test new therapies which will benefit cancer patients. This may involve finding novel ways to treat the disease, for example by altering the morphology of cancer cells as they die by apoptosis to

increase their immunogenicity in order to induce inflammatory and immune responses

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

This project uses mice (including genetically engineered models). We expect to use up to 6,000 mice per year over 5 years. It should be noted that 70% of these will not undergo scientific procedures, but will be used solely for breeding and maintenance of colonies.

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

Animals will be bred to achieve test subjects which may be predisposed to cancer. . Mice that do not show any adverse effects relating to their breeding and that do not undergo any procedures except for ear notching for identification and genetic testing will be humanely killed when they are no longer required for breeding. A proportion of animals 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. Some of these animals (up to 30%) will be given anti-cancer treatments or cancer causing agents (for example chemicals/irradiation) 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 20%, immobility or ruffling of the coat will be humanely killed. At the end of the study all animals will be humanely killed and tissues collected at post-mortem to gather as much information from the study as possible.

Application of the 3Rs

Replacement

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

Replacement

Although many aspects of cancer research can be conducted using cells 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 also share animals between experimental groups where possible - e.g. when we need normal animals for controls, we can often obtain these from our breeding colonies where they would normally not be needed in a study. We constantly optimise our breeding strategies to minimise the number of animals needed to achieve the desired genotypes for our studies and we use tumour transplant models where appropriate, which do not require breeding of genetically altered animals and thus use fewer animals in total per study. To reduce numbers of experiments we also perform studies using cell lines or 3D models so that only our strongest hypotheses are tested in the mouse.

Refinement

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

Refinement

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

We always refer to previous studies for adverse effects of anti-cancer therapies and when a group is given a treatment for the first time, we initiate the study with a small

number of animals (n=3-6) which is closely monitored before extending to a larger number.

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

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 Breeding, Maintenance, Genotyping and Genetic Monitoring of both Genetically Altered and 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 and sudden changes in lighting. Animals

produced will be supplied into the project licence authority of other establishments in the UK and bona fide establishments abroad.

Application of the 3Rs

Replacement

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

Replacement

Non-animal methods are not always able to model or replicate the complete array of behavioural, cellular, molecular and physiological interactions required to fully understand how genetic alterations result in normal or abnormal processes. Mice and rats bred or maintained under this project will be subject to scientific justification in the researcher's protocols demonstrating that the goals cannot be met with the use of non-animal methods.

Reduction

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

Reduction

The use of effective and stringent colony management systems will result in a reduction in the number of animals required in the breeding aspect of this project. Colonies will be planned according to the demand of end user groups and will be subject to continual review to ensure production levels are in line with the forecasted demand. Should usage reduce and remain sporadic, colonies will be closed and embryos frozen to preserve the model of interest.

The supply of high quality animals according to client specifications will result in more effective experimental programs where variability would ultimately impact upon the results obtained. The ability to offer this as a service will also result in a reduced need for duplicate colonies at various establishments, also lowering the number of animals of a similar type needed for breeding programs.

Refinement

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

Refinement

End users will be required by the project licence holder to satisfactorily justify the need for the animals that will be bred and maintained under the authority of this service licence. The choice of species/strain is driven by end user requirements.

Prior to the receipt of any animal model, stringent data collection will be performed to ensure all known traits and observations due to the genetic alteration are known, this will allow for specific refinements to be made in terms of breeding practices, husbandry, nutritional requirements, enrichment and handling. For example, animals that carry a genetic alteration which results in reduced functionality of their immune system will need to be housed within specific barriered environments to maintain their high health status and reduce the risk of infection e.g. with opportunistic bacterial agents.

Stringent colony management systems are in place and controlled by animal technologists fully trained in the breeding, care and husbandry of specialised animal colonies, utilising their experience and expertise as well as guidance from experts in the field of genetically altered animals.

Prior to the start of any breeding program all responsible members of the team will ensure specific details related to the animal model are known and used to set up the specific breeding and maintenance plan. The breeding plan will be subject to changes throughout the lifetime of any colony and will be in line with the forecasted usage. Breeding systems that minimise overproduction of unwanted genetic status animals will be used.

Animals will be housed in optimal social groups, allowing for a reduction in potential aggression or overt dominance behaviours, thus reducing any associated stress.

When determining the genetic status of both genetically altered and wild type colonies, the least invasive and most refined method, ear punch system, will be used for the retrieval of tissue, whilst the most advanced methods and technology will be used for the analysis of DNA to maximise the likelihood of success in this procedure, therefore reducing the need for re-sampling.

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	Toxicity mechanisms of inhaled environmental particles
Key Words	Environment, Lung Health, Asthma, Particles, Allergens
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Air pollution (AP), both indoors and outdoors, is a complex mixture comprising gases and different particle types that when inhaled adversely affect human health, in particular the lung. Indeed, air pollution is the environmental exposure with the greatest impact on health. Recently it has been estimated that the particle component of air pollution results in the equivalent of 29,000 deaths annually in England, making it of particular concern for public health. Inhaled particles are a particular concern for individuals with pre-existing health conditions such as asthma, where compromised lung function can be life-threatening. Research in this area has identified different types of inhalable particles of concern within the indoor and outdoor environment, including those derived from vehicles and their exhaust and household products as well as ‘biological’ particles such as fungal spores and house dust mite particles. Many of these biologically derived particles are allergenic, which is a concern for the majority of asthma sufferers. While many of these particles have been identified as potential triggers and exacerbators of lung disease, little is known about which properties of these particles (such as size, shape and composition) make them more hazardous than others. Little is also understood of how one type of particle may influence the body’s response to another type, specifically the ability to influence an allergic response. It is the aim of this project proposal to investigate sets of these air pollutant particles including allergens for their effects on lung disease with the aim of identifying hazardous properties and important particle interactions in rodent models.

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

The anticipated benefits of this project are wide in scope. The outputs will support local and global research activities to further identify how air pollution environmental exposures and different types of particles affect human health and those susceptible populations more prone to the effects of inhaled pollutants (e.g. those with asthma or

susceptible to developing the condition). Our work will be made publically available to be used by other researchers (within the UK and around the world), policy makers, regulators, health providers, patients, and the general public. The results generated will be also be used to support public health advice, providing the most up to date information on environmental hazards. The information will also be used to support activities that contribute to regulatory refinement, for example internationally accepted OECD test guidelines. This work also has the potential to lead to scientific innovation and development of new technologies and biomarkers that can be used to improve existing risk assessment methods in toxicological assessment. Identifying particle properties of concern as well as important interactions between different types of particles and allergens in the air that we breathe also has the potential to influence interventions and changes in certain behaviours and activities in our daily lives into the future designed to reduce the impact of air pollution particles on health.

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

Mice and rats are the chosen model species since they are well-characterised, and there is substantial literature available on the effect of various environmental exposures in these species. In addition, the animal work under this licence is part of a much larger research project, which also incorporates rodent and human cell-based models, and comprehensive reviews of the literature. This information helps keep animal experiments to a minimum, so that they are only performed when a whole living system is required. It is anticipated that we will use a maximum of 1200 mice and 400 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?

Animals will be exposed to one or more particle types or allergens through one or more of the following ways. 1) Instillation exposure directly into the windpipe using a tube under anaesthetic to minimise any discomfort. 2) Instillation exposure dropwise into the nose via the nostrils also under anaesthetic to minimise any discomfort. 3) Inhalation exposure whilst in a cage or in a specialised tube. For inhalation procedures it is expected that animals will experience some restraint stress, including elevated stress hormones, blood pressure, heart rate and body temperature. However, it is expected that they will quickly acclimatise to restraint. During some inhalation exposures animals do not have access to food or water (for up to 4 hours), and so may experience some hunger/thirst. Following exposure animals may then be placed in cages with a gridded floor to allow collection of urine and faeces, which may cause some minor discomfort on the feet. Some animals may also experience short-lasting pain as a result of blood sampling, or saline injection to treat any dehydration. Animals will be killed after the final exposure and tissues removed for further study. All animals will be monitored during and after exposure and humane endpoints will be applied in order to stop any procedure if necessary.

As a result of exposures to particles and allergens, it is expected that some of the animals will develop some inflammation of the airways that is not clinically apparent. This will be in the large majority of cases a mild severity of procedural harm with a small minority of cases having the potential for moderate severity levels. Therefore the maximum severity classification for this work is expected to be moderate.

Application of the 3Rs

Replacement

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

Replacement

As part of a larger research project we have established and will continue to use and optimise *in vitro* models (animal and human cell-based models) of exposure for the assessment of lung responses to inhaled particles and allergens. These can be useful tools that complement the use of animal experimentation. They cannot however at this time replace complex processes such as the development of an allergic reaction from inhaled material or model inhalation or particle distribution throughout the body. However, with the work proposed in the application, data generated on key mechanisms through which particle properties and interactions may influence disease endpoints, targeted refinement of *in vitro* systems are likely. This will contribute to local and global efforts to work towards useful alternatives to animal testing into the future. Other alternative methods (e.g. fish, worms or flies) do not model the public health relevant exposures or responses for mammalian lung we want to address (e.g. airway inflammation) compared to rats or mice. Again, however with key mechanistic understanding of toxicological responses gained from this project, such alternative methods may become useful in the future. Many of the related safety test guidelines, which this work may benefit, are conducted in rodents and so the use of rats and mice is scientifically valid. We will continually re-evaluate the use of alternatives as the work, and field in general, progresses.

Reduction

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

Reduction

Existing data will be used to calculate the minimum number of animals required to ensure the study is statistically valid. If no information is available for a particular substance, we will use previous experience (both ours and from the literature) to select sample sizes. It is anticipated that 6-9 animals per treatment group will be sufficient. In addition, publically available data will be added to, and compared with,

our own accumulating data. This will help to identify biological processes relevant to public health, and thus reduce animal use by preventing the further investigation of non-relevant processes. Experiments will be designed so that they can be published in accordance with the NC3Rs ARRIVE Guidelines. This includes blinding samples prior to analysis (by a researcher not involved in the analysis) to prevent bias. It is also anticipated that complementary *in vitro* work that will be carried out in parallel to the animal studies in this project will provide information that may reduce the need for testing of particular endpoints in animals, thus having the potential to reduce animal number use 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 rodents as the species to examine the impact of material exposure on lung health and asthma models is based on a number of parameters. Firstly, they are well characterised in terms of biological responses and appropriate measurements reflective of human disease and toxicological endpoints. From the perspective of disease modelling of allergic airway disease, mice are the model of choice for research into this area with similar responses and endpoint measurements as humans to environmental exposures. Balb/c mice are the strain of choice as they mount a robust allergic immune response, important for modelling asthma, not present to the same extent in other mouse strains. This project will involve highly experienced researchers and support staff in all areas of the proposed work, which will continually aim to reduce animal suffering while undergoing procedures. The local veterinary and animal welfare staff will be contacted as needed for their expert advice across all areas of animal welfare, including the choice of the most appropriate anaesthetic and procedural details (e.g. volume size, frequency, needle sizes) to minimise and mitigate harms. Outside of procedural activities, the animals will be maintained in a state of the art and well-resourced facility and will be housed with environmental enrichment. Expert staff will establish initial animal health on arrival at the facility. Risk of infection will be minimised, through rigorous adherence to biosecurity principles and procedures in place within the animal unit.

Refinements to our current approaches to exposures will include the following. Mechanistic profiling at early time points within experiments has the potential to identify markers of short term exposure that may predict repeat exposure long term effects. Should these be identified, subsequent experiments will involve reduced exposures that maintain the same predictive capability, thus refining our approach

and reducing animal discomfort and harm. As animals are handled during removal from restraint they are likely to urinate and or defecate. Where possible, this urine and/or faeces will be collected during handling to reduce time spent in a metabolism cage. We will also look into the use of plastic sheeting and/or filter paper to collect faeces and urine during exposure as an alternative to metabolism cages. The key issue is to prevent contamination of the urine with faeces. If successful, this approach will be used instead of metabolism cages. In addition, we will investigate the use of plastic containers containing multiple small wells in the metabolism cages to separate and collect urine/faeces samples from individual mice housed together in a metabolism cage. This would maximise biological sample replicates while allowing multiple animals to be housed together. For those animals that will be placed in restraint tubes for inhalation exposure we will explore the possibility of acclimatising the animals to the tubes prior to exposure by placing the tubes in the animal home cages. This will have the benefit of reducing stress associated with placement within an unfamiliar object during exposure procedures.

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	Cellular stress and drug metabolism in disease
Key Words	drug metabolism, oxidative stress, cancer
Expected duration of the project	1 year(s) 6 months

Purpose of 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 main aims are to understand the systems that protect us against toxic agents, both from our environment – the air we breathe, the food we eat, the medicines we use – and from within our own cells as a result of natural processes. It has been argued that the most important areas for expression of xenobiotic (drug) metabolising enzymes are those which are in contact with the environment, ie the skin, the gi tract (from top to bottom) and the airways. A number of P450s and GST are highly expressed in the pulmonary epithelium to protect against airborne chemicals, consumed deliberately or as part of our environment. The current health worries from the smogs in Asian cities are testament to the importance of airborne chemicals. By appreciating how these systems work, we want to take this knowledge and use it to enhance how we design and administer anti-cancer drugs, with a view not only to improving efficacy and a personalized response to treatment but also circumventing issues like drug 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)?

The transgenic mouse models used in this Project will be invaluable in acquiring more knowledge concerning the cellular mechanism(s) involved in protection against environmental chemicals, and how these systems relate to disease susceptibility and resistance to drug treatments. This will lead to a greater understanding of individuality in response to drug treatment; one of the major benefits we expect to achieve through this work is the optimisation of existing anti-cancer drug therapies, and the development of novel treatment strategies based on emerging drugs.

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

Our work will be exclusively in mice, and we expect to use approximately 25000 animals over the 5 year period of the licence. The vast majority of these mice will be

used in breeding programmes to generate appropriate genotypes and for the harvest for tissues, and only a small proportion will be subject to treatment.

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

Apart from breeding and maintenance, some of the mice will be treated with agents which may have mild to moderate short-term effects. Some experiments will involve growing human tumours in order to study the effects of drug treatments; however, such tumours will not be allowed to grow to a point where they significantly affect the well-being of the animals. These, and other mice, may have multiple blood samples taken over a period of time, for example to allow measurement of drug levels in the blood; this will be done in a manner that minimizes stress to the mice, and in fact will result in the acquisition of higher quality data from a significantly smaller number of animals (see ‘Reduction’ below). At the end of experiments or at the end of their useful breeding life, mice are humanely killed.

Application of the 3Rs

Replacement

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

Replacement

Where possible, we make extensive use of alternative experimental resources, including computer simulations and work with cell culture models. However, in order to adequately assess drug efficacy in relation to humans it is important to remember that, in addition to drug metabolism, the disposition of drugs, i.e. how they are distributed throughout the body, absorbed from the gut, excreted in bile/urine/faeces, is central to understanding how drugs work and how therapeutic action is related to side-effects. Thus, it is essential to use a multi-compartment experimental model such as the mouse.

Reduction

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

Reduction

At all times, experimental design will be such as to, maximise the information obtained from the minimum number of animals. For example, in our pharmacokinetic work, we have pioneered serial bleeding techniques, which together with simultaneous multi-drug dosing and the use of analytical instruments with increased

sensitivity, has reduced our animal use in many of our experimental protocols by a factor of up to 50-fold, whilst generating data of a significantly increased quality.

Further, we have also designed and validated a number of new mouse lines in which reporter expression can be monitored and measured non-invasively, again significantly reducing animal usage.

We continue to seek ways in which we can reduce animal numbers and refine our protocols without compromising the results obtained. Our experiments are always designed to maximize data output from the lowest possible number of animals without sacrificing scientific benefit.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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, whilst displaying a number of physiological differences to humans, represent a good compromise for experimental purposes. They have a relatively short gestation period, allowing the rapid generation of animals. Mice are also amenable to the sort of genetic manipulations that allow the creation of unique experimental models, including humanization for key enzyme systems, thus generating data with greater relevance to Man. the endpoints are chosen in a similar manner.

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	Molecular regulation of the African trypanosome life cycle
Key Words	Parasite, sleeping sickness, trypanosome, differentiation
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Trypanosomes are parasites that cause fatal human and animal disease in sub Saharan Africa. They are single celled organisms, spread by tsetse flies. In the blood of mammals, the parasites monitor their population size by releasing a signal. When there is a critical level of parasites in the blood, the trypanosomes respond by stopping their cell division and preparing for their transmission by tsetse flies. This involves development to a new life cycle stage called the ‘stumpy’ form that can establish the infection in the tsetse gut when taken in during a bloodmeal. We have discovered a collection of molecules that control the ability of the trypanosomes to respond to their cell number in the blood or undergo development upon entering tsetse flies. This includes molecules that receive the environmental signals (i.e. parasite density, tsetse entry), pass the signal within the parasite, or generate the changes in the parasite necessary for development through changing the patterns of genes and proteins that are expressed. In the work proposed we wish to understand how these molecules function and operate with respect to one another to enable successful development of the parasite as it passes through the different life cycle stages necessary for its virulence and spread.

Trypanosomes also often exist in mammal hosts in mixed infections with other related trypanosome species. We hypothesise that in these coinfections the parasites might detect, and respond to, the signals released by other parasites and that this might shape their infection profile, or select for increased virulence or transmission potential. A further part of our work will explore whether these different trypanosome species can ‘talk to one another’, the mechanisms through which they achieve this and the consequences for their virulence or life cycle 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 discovery of the mechanisms by which trypanosome parasites respond to their environment provides novel routes to controlling the parasite. For example, manipulating the signals that normally drive their cell division arrest could reduce the

virulence of the parasite, as could perturbing the activity of molecules that transmit this signal within the cell and between cells. The gene expression profile of some of the molecules linked to arrest as stumpy forms, or proliferation, might also provide molecular markers that permit the prediction of the virulence of the parasite or their likelihood for transmission. The interactions of different trypanosome species could also be informative in predicting the threat of virulence of parasites that have been selected in coinfected hosts, since they may exhibit particular virulence profiles in the absence of coinfecting competitors. Trypanosomes are amongst the most evolutionarily ancient nucleated cells (eukaryotes). Hence, as well as discovering potential vulnerabilities in their life cycle that can be exploited for therapy, the understanding of their molecular pathways involved in development may provide fundamental knowledge helpful to understand how all cells undergo specialisation, including our own cells.

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

We will predominantly use mice for our experiments. The parasite life cycle cannot be adequately reproduced in cell culture such that much of our analysis of the consequences of molecular disruptions needs to be analysed in a mammal host. However, we have a great deal of experience of growing trypanosomes in mice where the consequences of infection are predictable, allowing us to minimise suffering. Sometimes, we also need to generate very large numbers of parasites (for example for molecular analysis) and this cannot be achieved with cells grown in culture. Where these large amounts of material are needed, growth of the parasites in rats is sometimes preferred. To achieve statistical validity for our experiments and to complete the experiments for which we have received substantial external funding, we anticipate using up to 4200 mice and 80 rats over 5 years of 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?

Trypanosome infections can be fatal in rodents, death being preceded by progression through a predictable series of symptoms on a relatively predictable timescale. Our experience of working with these parasites in mice (over 20 years) means that we have become expert at monitoring and predicting the progression of infections, so that experimental outcomes can acheived without the infection leading to illness or death, which is a rare occurence (less than 5% of infections) . Mice and rats are monitored for their disease progression based on a numerical scoring system and undue suffering prevented by humane killing should the infection progress to a level where death his anticipated within a few hours. As a consequence, the overall severity for our experiments is classed as moderate. At the end of experiments animals are euthanized and parasites are harvested.

Application of the 3Rs

Replacement

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

Replacement

We routinely grow trypanosomes in cell culture. However, their life cycle development does not progress normally in cell culture, such that the production of stumpy forms is best achieved in parasites grown in mice. Furthermore, the evaluation of the effects of disrupting particular parasite molecules and processes can only be accurately determined by studying parasites growing in the natural context of a bloodstream infection in a mammalian host. For the isolation of large numbers of parasites, the ability to harvest tens of millions of parasites from a single infection makes some experimental approaches feasible that would not be possible using cultured parasites that are in an unatural growth medium and isolated from the host (and thereby in the absence of an immune response and where molecular signals are not at natural levels due to their non-physiological accumulation or turnover in culture). Furthermore, parasites grown in culture do not generate synchronous populations of stumpy forms that are required to analyse molecular events in the population that reflect molecular events going on in each individual parasite. Coinfections also require to be studied in the context of a functional immune system. Whilst interactions between parasites in culture can be informative, ultimate validation of any effects observed needs to carried out using parasites growing in a mammalian host where the combination of immunity, signal production and turnover can contribute to the infection dynamic.

Reduction

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

Reduction

Trypanosome growth in mice progresses on a relatively predictable course, such that it is straightforward to generate reproducible and statistically valid datasets that satisfy external scientific scrutiny (i.e. where the probability of the observed outcome being incorrect is less than 5%). We are experienced in analysing and predicting parasite virulence in mice and so can minimise distress whilst deriving the necessary scientific information from infections. Genetic manipulations of trypanosomes often involve use of gene silencing or gene overexpression techniques, which are controllable using chemicals supplied in the rodent drinking water (e.g. doxycyline). This provides well controlled analyses because phenotypic comparisons between induced and uninduced populations provide a robust experimental outcome using the same parasite material. We have access to a ‘statistics and study design Director’ who provides advice on the planning and interpretation of experiments to achieve statistically meaningful outputs.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 to monitor trypanosome infections in laboratories worldwide. This allows comparisons of the infection profile and kinetics of proliferation and differentiation between studies and between laboratories. The symptoms linked to trypanosome infection in mice are also predictable allowing us to track the progression of infections with a scoring system allowing defined humane end-points. A staged approach will be adopted when novel drugs are used.

PROJECT 177. 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 178. 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	Mitochondria and ageing intestinal epithelium
Key Words	Ageing, Mitochondria, Cancer, Intestine
Expected duration of the project	5 year(s) 0 months

Purpose of 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 analyse the molecular mechanisms underlying cellular ageing and the development and progression of colorectal cancer.

Colorectal cancer is the second leading cause of death from cancer in the UK. Age is the biggest risk factor for colorectal cancer development. In order to develop new treatments it is important that the mechanisms underlying colorectal cancer initiation and progression are understood. A common feature of a number of types of cancer cells is that they do not use the normal pathway for energy production which is carried out within the mitochondria, small structures present in most human cell types. Cancer cells use a less efficient system called glycolysis. As we age an increasing number of normal human colon cells also utilise glycolysis as their mitochondria cannot function properly. The project aims to look at the effects of altered energy metabolism on stem cell function and to see if these non-cancer cells with altered energy production are selectively vulnerable to turning into cancer cells

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

The mouse is an essential mammalian system to study changes in the intestine which occur with age and lead to the development of colorectal cancer. We aim to generate important insights into the early stages of this disease which may lead to the identification of new targets for treatment.

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

In order to obtain the control and experimental animals with the correct genotype we will have to generate approximately 4700 animals We will use 550 mice in this study, 275 experimental animals and 275 control mice. We have carried out statistical analysis to ensure the least number of animals are used to obtain meaningful results. Where animals have been bred but (unavoidably) are not the correct genotype, they

will be humanely culled and wherever possible their tissues will be used for optimisation of techniques or shared with other researchers.

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

The mice will be given up to 8 injections into the abdomen; all injection routes may cause momentary needle-stick pain. Wherever possible injection sites will be rotated, to avoid tissue damage. The animals which go on to develop disease will be humanely killed as soon as they begin to show clinical signs of disease, most commonly weight loss (the animals will be weighed at least once per week until they show any clinical symptoms whereupon they will be weighed and checked daily), and anaemia which can be readily identified as paling of the animal's paws.

Application of the 3Rs

Replacement

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

Replacement

In humans we do not know that a cancer is developing until it is reasonably advanced, therefore gaining understanding of the initiation event and what controls initial tumour growth is difficult in human tissues. In addition there are no robust human colon organ systems that can be grown in the laboratory which reliably model what is happening in the entire organ. In mice we can genetically induce a tumour at a defined time point and examine the cells which have turned into cancer cells at very early stages. We can also investigate rates of tumour growth by looking at animals at selected time points after the cancer has been initiated. Alongside the animal model we will use samples of normal human colon and colon tumours to look at the age-related metabolic changes which may affect stem cell function and increase their chance of becoming cancerous. We will also establish a murine organ growth system in the laboratory, which can be propagated for a number of months and stored frozen for future experiments. This will reduce the number of animal experiments in future studies.

Reduction

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

Reduction

We have carried out statistical analyses to estimate the minimum numbers of mice we will need to use in order to obtain meaningful data from 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

As we are interested in the very early stages of cancer development, we anticipate that the majority of animals will not suffer adverse effects during the experiment. All animals will be examined comprehensively throughout their life and any mice exhibiting detrimental effects, most commonly weight loss and anaemia, will be humanely killed.

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	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 veterinary care and home office liaison will be sought for additional advice where an obvious route to minimal welfare costs is not apparent.

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	Cell senescence and life span in mice
Key Words	Ageing, senescence
Expected duration of the project	0 year(s) 6 months

Purpose of 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 want to understand how we can slow down ageing and prolong healthy lifespan in mammals.

When the cells in a body age, they accumulate DNA damage. This in turn completely changes the way cells look, function and communicate with their neighbours – they become *"senescent"*. This limits the ability of tissues to regenerate and to function properly such that individuals become more prone to multiple diseases and death with increasing age. We want to see whether we can extend the average human lifespan in good health with good physical capabilities and good memory function.

We have already identified ways to reduce the effects of senescence in cell culture experiments. We will test their efficacy in mice. For many experiments we will use mice which are manipulated to accumulate senescent cells faster than normal (and thus also age prematurely), because this will improve the sensitivity of our analyses and shorten the time necessary to perform them. Faster accumulation of senescent cells will be achieved by irradiation, implantation of senescent cells or genetic alteration. These mice age faster than normal, and we will identify drugs and/or nutritional interventions that can heal such accelerated ageing.

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

Because of the ongoing dramatic increase in human life expectancy, there are ever more older individuals around with an ever increasing risk of spending more of their later years in ill-health. Ageing is the number one common risk factor for all major diseases in the developed world, including cardiovascular disease, diabetes, dementia and cancer, because it increases the susceptibility to all and any one of these. Understanding the biological basis of ageing and the underlying principles of successful intervention are in the long run the most promising approach to let people grow older with fewer of these devastating diseases. Recent research has shown that senescent cells contribute causally to an acceleration of the ageing process.

However, it is not at all clear how this works and whether and how it will be possible to delay ageing by interfering with signals that are generated in senescent cells. We hope to answer these questions. Ideally, our experiments might identify lead targets for drugs that could in the future improve human healthspan and postpone the incidence of multiple age-related degenerative diseases. Specifically, we expect to identify interventions that may ameliorate accelerated ageing in humans (e.g. in long-term tumour survivors) and may be translated into clinical trials.

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

We use mice because they are the lowest sentient animal model suitable for the study of ageing in mammals and because the existence of gene knockouts and transgenics enables very powerful hypothesis testing. A statistical analysis based on prior experience was used to calculate a total number of 4,000 mice to be needed 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?

Our experiments necessitate observing mice until they develop frailty, loss of neuromuscular and cognitive function, diseases and discomfort associated with old age, and this process will be accelerated in some animals by irradiation, high fat diet or other means. Mice will be intensely monitored for the onset and development of these ailments of old age. Thresholds for these parameters have been defined such that mice reaching those will be humanely killed before these adverse effects of the ageing process reach a severe level. As ageing progresses at variable speeds, it is however possible in few cases that old mice that are apparently fine one day may be found dead by natural causes the next. These are required by the regulatory framework to be automatically graded as severe however we will carry out post mortem examinations to try and get a more accurate severity assessment. The interventions that we perform (drugs to suppress cell senescence, dietary restriction) are intended to prolong healthy lifespan in mice and to slow down the development of adverse effects during ageing. The may have side effects, for instance food restriction causes mild to moderate discomfort and changes behaviour (mice become more active when they expect food, but compensate that by sleeping more deeply afterwards), however it is beneficial for long-term health. Drug treatments intended to reduce age-related health decline may also have side effects on individual organ systems. We will carefully monitor those and humanely kill animals before these become severe in intensity and/or duration. We may perform surgical operations to assess the regenerative capacity of organs. These will be performed by trained personal. Animals will anaesthetised under the operation and will receive painkillers after the operation as long as needed. They will be humanely killed within days after the operation to assess the tissues for analysis. We will also implant senescent cells into various tissues by injection. Again, this will be performed by

trained personal and animals will receive anaesthetics and/or painkillers for as long as needed.

Application of the 3Rs

Replacement

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

Replacement

We have done extensive experiments with human and mouse cells in culture and greatly contributed to the understanding of the mechanisms of cell senescence and their possible relevance. However, ageing is immensely complex and the impact of senescent cells on ageing depends on their interaction with the host cells in a tissue and in the wider organism.

Reduction

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

Reduction

We will continue to address mechanisms by using cells, which will typically be generated from tissue obtained during ear marking without additional discomfort to the animal. In a parallel project in the lab we have begun to analyse ageing in a 3D skin model, made from two different types of human cells grown in a collagen or plastic matrix. Similar models for other tissues are being developed, but it will still be a long way before these models are stable enough to allow realistic studies of ageing processes. We will continuously review these developments and use them to replace animals in our research if 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

Protocols were designed to minimize suffering. All people involved are carefully trained in all procedures and are fully aware of all possibilities to minimize suffering. All relevant guidelines are being followed. When surgery is performed, mice will be under anaesthetics and will receive painkillers afterwards. Ageing research frequently requires to keep mice until old age, when they develop age-associated diseases and syndromes (blindness, various cancers, symptoms of frailty including muscle weakness, weight loss and low physical activity). We carefully monitor the

mice daily for indications that such diseases and syndromes occur. Mice will be humanely killed if we see signs of suffering even from the normal ageing process.

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	The Production of Laboratory Animal Bio-Products
Key Words	Dog, Mouse, Guinea pig, Blood products
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

To use animals to provide blood and tissues to generate data to support the development of effective and safe medicines to treat diseases where there is currently a clinical unmet need e.g. cancer & heart 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)?

Bio-products provided will contribute invaluable scientific information to support and progress potential new medicines where there is currently an unmet clinical need. Conducting investigations using blood and/or tissues taken from animals reduces the number of potential new medicines requiring evaluation in living animals and can be used to establish whether conducting experiments on living animals would be beneficial. The products are also used to aid the development of new medicines in man or animals when it is necessary to calibrate and validate many of the machines or testing systems used to support research. They may also be used to support other methods in research as an alternative to live animals.

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

Over a 5 year period: Dogs: 275 Mice: 50 Guinea pigs: 50

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

More than 85% of the animals used under this licence will be kept under general anaesthesia throughout the sampling procedures and will not be brought back to consciousness. They will be humanely killed while still under anaesthesia with an overdose of anaesthetic. Therefore these animals are not expected nor likely to experience any adverse effects. The remainder (mainly dogs) are trained to donate small volumes of fresh whole blood at weekly or monthly intervals and these donors are not expected to suffer adverse effects as a result of the project (similar to taking

a blood sample from a human). These dogs will continue to be used as donors for several years until they are retired. The dogs receive full clinical health checks by a veterinarian and experienced animal technicians. They will be retired if there are signs that their, normal health state is affected by the project, their age or health issues. Training of donors is however not always possible and in the case of mice and Guinea pigs, to avoid the need for restraint of the donor and for handler safety, sedation prior to bleeding is performed. Adverse effects from repeated blood collection are not expected under this project but could (rarely) include slight bruising, anaemia or uncontrolled bleeding. Any animal with anaemia or poor clotting mechanisms will be removed from the bio-products donor pool. Adverse effects of the sedation are not expected. Any adversely affected animals will cease to be used and will be referred to the responsible veterinary surgeon who will determine the need for any treatment, consider its suitability for rehoming or if the animal should 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

Drug research programmes rely, in part, on biological materials obtained from human or animal sources to validate and confirm disease-associated drug targets and the mechanisms of action for potential new medicines. This programme of work supports the replacement of using living animals by enabling the supply of high quality blood and tissue samples where living cells are needed for experiments due to the lack of appropriate cells from existing alternative sources or instances where it is not possible to use cell culture techniques.

There are a number of promising technologies in development which aim to utilise human cells to recreate the physiological functions of organs without using animals. However, these *in vitro* approaches do not yet offer an alternative to totally replace the need for research animals and authentic blood, blood products, body fluids and tissues to enable their use in all the investigations required to support the research and development of new medicines.

Reduction

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

Reduction

The number of animals used is minimised by using proven collection techniques including taking blood under non-recovery anaesthesia to ensure that large volume, non-clotted samples can be obtained.

Tissue requests will be co-ordinated in order to supply multiple samples from one animal (e.g. whole blood, pancreas, femurs and liver) to a number of requesters for their individual purposes. This reduces the total number used.

The project aims to provide blood components and tissues of the highest quality as this improves the significance of test results in studies involving animals and can often lead to improved scientific knowledge and a reduction in the overall number of animals.

The project can reuse animals and this means that multiple samples can be obtained from a smaller number of donors thereby reducing the need to kill animals for the purpose of taking each sample.

The use of blood products, tissues and organs that are obtained from animals that are not suitable for direct use in research reduces the total numbers of live animals required for research

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 there is scientific need to preserve tissue integrity/architecture or obtain high volume and quality blood samples, then taking samples under appropriate and well maintained non-recovery anaesthesia is considered the most refined approach and we have refined the technique so that we will cause the minimum amount of discomfort and distress to the animal when we anaesthetise it.

Persons taking samples are well trained in the techniques involved to ensure high quality samples are obtained quickly & effectively with minimal impact on animal welfare.

The choice of donor species is driven by the scientific needs of research scientists.

When it is prudent to sedate the donor prior to sampling (if the donor cannot be readily trained or if it would be hazardous for the person taking the sample), then a second drug is used to reverse the sedative and thereby speed up recovery from sedation.

By only using donor dogs from the colonies we house, we ensure they are kept in appropriate long-term housing. The reuse of animals in a donor pool means they are used for a minimum 1 year in ferrets and several years for dogs. The animals benefit in the long term by being housed in appropriate socially enriched housing, cared for by trained staff. This housing is in the holding rooms of the general population and they therefore benefit from being in busy, familiar surroundings with social contacts of other dogs.

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	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 Rats, 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 to carry on the experimental plan with rats 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 rat 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.

Rats 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 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	Diet Induced Obesity model in rodents
Key Words	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;
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 licence in which the programme of work is designed to produce prepared rodents and/or use of rodents to conduct basic early stage research. The rodents form the basis of several different animal models of diseases such as such as type 2 diabetes, high blood pressure, high cholesterol and other diet induced circulatory problems.

Essentially animals are fed a high fat diet or high fat/high sugar diet for a number of weeks. As a result, they become obese, and usually have high blood sugar, and develop impaired sugar tolerance. These animals are then used to study the adverse clinical and genetic effects of obesity and type 2 diabetes and to test drug candidates that are expected to affect these conditions in humans.

This is a demand-led service supported by a scientifically justified need. We will never exceed the moderate severity limit.

The scientific background for each individual use is specific to each researcher but broadly addresses scientific and clinical needs in the following areas of research: obesity and metabolic related 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 the service program are therefore to provide facility for the scientific community to complete these programs of work where resources may otherwise have been limited. The availability of appropriate facility and expertise then ensures the procedures will be performed to a consistently high standard allowing greater reproducibility and ensuring the highest standards of rodent care. The benefits of this project are principally (i) the control of the number of animals being bred and developed, due to the ability to plan better due to greater demand (ii) good understanding of the model by the staff looking after them, potentially resulting in better animal welfare.

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

The numbers of animals used is not expected to exceed 5000 mice and 2000 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?

There are several potential outcomes at the end of the protocol: shipped to the client for further study; prepared and kept at our Establishment to continue onto approved early stage experimental studies: or placed directly on early stage experimental studies. Most of the animals are expected to experience no more than mild clinical signs associated with obesity. A small number are expected to develop clinical signs due to the development of diseases such as diabetes or dermatitis and these animals may experience moderately severe adverse effects, which may be controlled by special care, veterinary treatment or by humanely killing the animal if it appears to be developing adverse effects which are worse than predicted. Where live animals are not required by the researchers, collection of bloods and tissues following immediate humane killing or after induction of terminal anaesthesia will be performed.

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 research into human and animal diseases, as behavioural models and full system responses cannot be replicated using non-animal methods.

Our Establishment will make consideration to the use of alternatives for every study and if unable to replace the use of live animals, will identify the most appropriate reduction strategies for the research work.

Our AWERB (Animal Welfare and Ethical Review Body) will assess the project proposal internally for all new diet induced obesity (DIO) clients/protocols to ensure all projects have good scientific and ethical justification and the study design is sound and meets the 3R's and scientific outcome required by the customer. No new projects will begin without the full approval of the AWERB in line with the Home Office licence.

Reduction

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

Reduction

Consideration to identifying the most appropriate ways to reduce the numbers of animals used for their research work will be discussed with every new client project enquiry.

Our AWERB will also assess the project proposal internally for all new DIO clients/protocols.

Recommendation will be made to reduce the number of animals used to the minimum required to safely deliver a valid result, use of pilot studies to define the optimum schedules for induction/treatment will be considered.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 Establishment will ensure that the client has taken consideration into identifying the most appropriate refinement strategies for their research work. Where we have greater experience than the client we will provide advice on refinements to the procedure.

Anaesthetics and analgesics will be implemented on advice of the vet to minimise cost to the animals.

Our AWERB will also assess the project proposal internally for all new DIO clients/protocols.

Animals will be housed in bio-secure enclosures/rooms to ensure a high health status and routine health testing will be in place; both to ensure their wellbeing and to ensure that experimental outcomes are consistent and therefore use a minimal number of animals.

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	Cancer Drug Discovery
Key Words	Cancer, Immunology, 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:
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):

Over 150,000 people die from cancer in the UK each year. Although there are have been significant advances in recent years it is clear early there remains an urgent need for more effective treatments as well as for treatments with fewer negative side effects.

Recent high profile successes have come from “immunotherapy”, which seeks to harness the body’s immune cells to better fight and hopefully eradicate the cancerous cells. Despite these successes, the new drugs are far from 100% effective and have considerable side effects. Nevertheless they have prompted an intense global focus of pharmaceutical companies on designing new and better immunotherapies for cancer.

Our main objective is to contribute to this field of knowledge by identifying novel substances that can be used in immunotherapy against a range of types of 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)?

There are many aspects of the anti-cancer immune response that can be potentially boosted to develop new immune based treatments for various types of cancer, for example pancreatic or breast cancer. This requires an expert basic knowledge of the immune system which is our area of expertise. We provide a wide range of sophisticated cell and animal based models which can monitor the effects of new drugs on the immune system and identify those with potentially anti-cancer activity. Work with cells in the laboratory makes up a significant proportion of what we do and the first steps in identifying potential new treatments do not involve live animals. This is making the process of developing new drugs faster and more efficient. So the overall aim is to more rapidly and effectively identify anti-cancer drugs that can progress into human trials. These studies will benefit the scientific and pharmaceutical community but ultimately the goal is to improve treatments for patients with a range of cancer diagnoses where treatments are currently

unavailable or not effective. It is likely in the longer term that these treatments may also benefit the veterinary field in providing novel treatments for animal cancers too.

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

We will use mouse immune and cancer models that are already established and used widely in cancer studies. We anticipate that we will use up to 5,000 mice in cancer studies and up to a further 3,000 in more simple immunology studies, 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?

Broadly, the cancer models we will use come in two forms. Some mice are modified to have gene mutations that are known to drive different forms of human cancer. In these models the cancer can therefore arise spontaneously (or after administration of an inducing chemical). In the other type of model, cancer cells are implanted or injected and grow, either in the organ from which they were derived, or under the skin. In general, the mice do not show ill health as a result of the cancers themselves, because our experiments do not need run to that stage. In the simpler immunology experiments, the mice receive one or more injections, which may cause some localised inflammation. Some studies do require that the cancer model is allowed to develop for a longer period of time in order to mimic what would happen in human disease. In this scenario animals are very closely monitored and will be killed if clinical signs are seen beyond that allowed by the project licence.

Application of the 3Rs

Replacement

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

Replacement

Importantly, many of our assays are performed in the laboratory, rather than in animals. These often use human immune cells, including from cancer patients. This allows us to test how different immune cells respond to potential drugs. However, once we have defined the most likely candidate drugs, we must understand whether these can in fact help the immune system to fight the growth of cancers in the body, which is a much more complex requirement than can be modelled in the laboratory.

Reduction

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

Reduction

Our advanced immunology assays allow us to advise our clients on which of their test compounds are most likely to be effective in cancer studies. This saves time and money and, most importantly, reduces the numbers of animals required for cancer 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 have the best characterized immune system, with many reagents available, allowing us to perform detailed immunological studies. There is also a wide range of different cancer models, that mimic human cancers, available in mice. This is important because certain drugs might be expected to target particular forms of human cancer more effectively than others.

We have well defined end-points (size of tumours) in place, which determine when an experiment will end. The mice a carefully monitored for tumour growth and for their general health. In models that involve surgery, appropriate anaesthesia is given, as well as pain relief as required.

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	Evaluation of new treatments for polycystic kidney disease
Key Words	ADPKD, polycystic
Expected duration of the project	5 year(s) 0 months

Purpose of 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 the project is to identify novel therapeutic strategies to treat Polycystic kidney disease. Compounds are tested for their ability to reverse or slow down kidney cyst formation and improve kidney function and for adverse events to be minimal in order to provide significant improvement in the quality of life compared to current 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)?

Polycystic kidney disease is a debilitating disorder characterised by progressive kidney cyst formation leading to end stage renal failure. It affects about 70000 adults and children in the UK and accounts for 1 in 8 people in need for a kidney transplant. This work is expected to provide new information on mechanisms regulating kidney cyst formation, which will be used to identify new therapies with better efficacy and fewer side effects.

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

18000 mice 2000 rats both 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?

Kidney cyst growth and progressive related perturbations in function will be induced in rodents by subcutaneous or orthotopic implantation of cystic human cells or by genetic deletion of proteins known to cause the disease in humans. The majority of studies described in this license are well tolerated by rodents and those models are well described in the literature. It is not expected that serious adverse effects will occur but any side effects are likely to involve bodyweight loss and deterioration in clinical signs. Any animals exhibiting such signs will be removed humanely 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

Polycystic kidney animal models are required to assess the effect of a test compound on kidney cyst formation and global renal function (efficacy)

Cell and ex-vivo assays can give a good indication of the potential ability of a compound to modulate the formation of cysts but they cannot fully predict in vivo efficacy on global renal function or disease progression. In vivo models are therefore an absolute necessity to relate in vitro data to efficacy in order to predict a potential clinical benefit. In addition, the PK/PD relationship, driven by distribution, metabolism and elimination, cannot be accurately modelled in vitro.

Finally, proven in vivo efficacy data is a prerequisite of the regulatory bodies who have the authority to approve or reject a new drug application

Reduction

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

Reduction

Protocols covered by this project licence application are designed to use the minimum number of animals possible.
Tolerability studies are performed with small groups of animals in order to establish the maximum tolerated dose and suitability of dosing regimen prior to larger efficacy studies. Only then, can the more complex in vivo efficacy studies commence in the knowledge that the animals are likely to tolerate the compound.

Minimum group sizes for efficacy studies will be calculated using power analysis and will incorporate consultation with a statistician.

The use of non invasive techniques to repetitively record both cyst/kidney volume and glomerular filtration rate will avoid unnecessary sacrifice and enhance the amount of mechanistic data obtained in a single animal, therefore decreasing the number of animals necessary for each particular 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

It is well documented that deletion of either polycystin-1 or polycystin-2 proteins in mice leads to progressive development of kidney cysts and loss of kidney function, which reflect the pathology and biochemical changes associated with the human disease. These models are based on the gene mutations that have been shown to cause ADPKD in human patients, therefore improving the likelihood of translation from efficacy observed in these models to a clinical benefit. Studies in rodents deliver robust, reproducible data and so it is often unnecessary to evaluate efficacy of new compounds in higher species.

The project uses techniques that can also be used in patients during clinical trials such as total kidney volume and glomerular filtration rate. These techniques will provide efficacy data on the key symptoms of the disease and key information on the mechanism of action of the compound tested that will be directly translatable to the clinical situation.

In addition to tolerability studies, pilot studies may be conducted in a small number of animals in order to refine the parameters and methodology for ensuing studies. These are intended to define the risk/benefit ratio of each procedure to generate statistically significant data whilst causing the least adverse effects.

Finally, longitudinal non-invasive imaging and/or measure of specific urine markers will be used to assess disease progression and will be correlated to the general physical condition of the animal in order to define accurate, quantitative humane endpoints.

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	The Production of Antibodies
Key Words	Antibody, Polyclonal, Monoclonal, Immunogen, Antigen
Expected duration of the project	2 year(s) 3 months

Purpose of 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 provide a service for the production of antibodies for both the medical research community and diagnostics manufacturing industry within the UK and Europe. 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 disease and their use as critical tools in the areas of research and medicine, including basic research of cells and their function in disease, diagnostic technology and therapeutic medicine 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)?

Antibodies routinely help scientists to research the function of both healthy and abnormal cells in disease, by the detection of proteins within the cell 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 a clinical environment. This can allow for the rapid diagnosis of life threatening disease and assist in providing clinicians (clinical Scientists and Doctors) with specific information in terms of the most appropriate course of treatment to follow, thus preventing death. The use of antibodies in therapeutics is a fast developing area of medicine, with the use of antibodies as constituents of direct medicine in order to treat various diseases including cancer, auto-immune disorders and infection.

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

Rat 3,500 (5 Years) Mouse 6,500 (5 Years) Guinea Pig 2,500 (5 Years) Rabbit 12,500 (5 Years) Chicken 750 (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. When raising antibodies against DNA, special technology has been developed to do so. This technology involves the use of DNA coated gold particles which are introduced to the animal via bombardment of the skin with pressurised gas. This procedure is carried out under general anaesthesia and has minimal associated effects, which can include slight redness of the skin at the site of inoculation. The production of antibodies against bacteria requires the inoculation of a bacterial liquid(without adjuvant) direct into the blood stream. This methodology can result in the loss of animal body weight and (rarely) the onset of symptoms that appear similar to that of an allergic reaction e.g. laboured breathing, reduced mobility and redness of the eyes with associated light sensitivity. 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. All animals used for the production of antibodies 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 (monoclonal) antibody secreting cells.

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 and Chicken will be made available for the production of antibodies. Selection of a specific species , from one of the above 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 the production of antibodies. 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 the field of antibody production.

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	Characterisation of vaccine candidates against viral diseases
Key Words	vaccine, immune correlates, antibodies, cytotoxic T cells, virus
Expected duration of the project	5 year(s) 0 months

Purpose of 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 project is to develop novel vaccine candidates for protection against viral diseases. Those we are immediately interested in working towards are Ebola, Lassa Fever, Marburg, pandemic flu as well as seasonal flu and noroviruses.

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

Vaccines have saved millions of lives around the world and continue to offer enormous benefits in lowering health care costs globally by reducing the morbidity and mortality associated with infectious disease. However, new and improved vaccines are needed against emerging diseases, as well as existing infectious diseases for which existing vaccines do not offer 100% protection or where the protection is only short lived. We expect to be able to identify 1-2 vaccine candidates against severe viral haemorrhagic fevers that will be taken into clinical trials in collaboration with pharmaceutical companies as well as 1-2 vaccine candidates against influenza.

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

Adult mice (2000 over 5 years) and guinea pigs (750 over 5 years)

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

The animals are expected to show no adverse effects after vaccination (either by injection or delivery of naked DNA) and testing for immune responses by taking blood samples. However animals infected with disease causing agents may show

signs of disease. Vaccinated animals are predicted to be protected by their immune response to the vaccine (this is why they will have been taken infected) but this may prove to be incorrect. The control animals to which we compare vaccinated animals are more likely to show disease signs. Clinical signs may include raised fur and hunched posture, weakness, inactivity, light aversion, and weight loss. These will only be allowed for a maximum of 24 hours before euthanasia. Some animals will be transferred to another project licence for infection should the containment facilities for the disease causing agent used require this and transfers will be in their established groups using climate controlled vehicles. Eventually, all animals with be humanely killed at the end of experiments.

Application of the 3Rs

Replacement

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

Replacement

The induction of the immune responses is complex and has never been recapitulated fully in cells in a laboratory setting , thus animals are required to provide the complex interactions of a whole body system. Animal challenge models are required to establish proof of protection delivered by a potential vaccine before decisions can be made to advance vaccine candidates towards clinical trials in people.

Reduction

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

Reduction

Group sizes are designed to give statistically significant results. Groups are designed to enable comparison between immunised experimental animal groups as well as with 'mock' immunised control animals. The mock immunised control groups may be used to compare against more than one experimental group at a time and this 'sharing' of control groups means animal numbers can be reduced accordingly. Animals that show appropriate levels of immunity after immunisation can be taken into 'challenge' studies. We have linked vaccination studies to challenge studies, to reduce the need for a second set of animals for challenge experiments

For studies where large volumes of blood are needed to detect antibodies the guinea pig is used rather than the mouse.This is because far fewer animals will be used in consequence to obtain the volume of blood needed for further analysis.

Refinement

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

Refinement

Mice will be used in some immunisation experiments where we want to look at how immune responses to the vaccines are generated. This is because mice have many available reagents to interrogate responding immune cells. This includes both early responses and later adaptive immune responses that may produce antibody. Guinea pigs are infectable with several viruses causing disease and provide a larger animal model for testing possible vaccines in which larger volumes of blood can be sampled during immunization schedules allowing different tests to be run with the same sample to measure antibody responses throughout the course of induction of possible protective immunity. This reduces the number of animals used compared to mice.

Vaccine constructs will be tested in the lab before use in animals. Adverse effects will be minimised by only allowing experimental groups with detectable immune responses to be taken into challenge studies. These animals should therefore be protected from disease or show little/lesser effect from exposure to the disease causing agent . Mock immunised animals may show clinical disease such as raised fur, soft faeces, hunching and weight loss) but as stated above this will be monitored closely and animals humanely killed within 24 hours if the signs continue.

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	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 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	Collagen (I) homotrimer and cellular stress in ageing and disease
Key Words	brittle bone, collagen, osteogenesis imperfecta, cellular stress
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

Collagen (type I) is the most abundant structural protein in the body and a major component of bone and joint tissues. It forms fibres that surround cells and make tissues resilient to mechanical loading. It is degraded and reformed by cells using biological enzymes and this process allows skeletal tissues to adapt to changes in mechanical loading. When the tissue structure is inadequate to resist external loads, tissue injury including fractures and ruptures can occur. This leads to diseases including osteoporosis (weak bone), osteoarthritis (cartilage loss and bone overgrowth) and soft tissue injuries. Over-production of type I collagen (termed fibrosis) furthermore restricts tissue function leading to disability and increased morbidity and mortality.

An abnormal form of type I collagen, termed collagen (I) homotrimer, is present in both degenerative and fibrotic diseases. This abnormal collagen alters the properties of collagen fibrils and is resistant to breakdown. Collagen (I) homotrimer may therefore affect the ability of tissues to respond to changing mechanical loads and to counteract fibrosis.

The effect of abnormal type I collagen on tissues has been well-studied in a spontaneously occurring mouse model (oim) of the brittle bone disease ‘osteogenesis imperfecta’. The genetic mutation in these mice appears to have side-effects that detrimentally affect the bones, but is very similar to a mutation causing brittle bones in humans. The side-effects are related to cellular stress, which has been previously implicated in osteogenesis imperfecta. A mouse line engineered to produce collagen (I) homotrimer alone, without the genetic side-effects does not have brittle-bones.

The project aim is to characterise the side-effects that cause brittle-bones in the oim model and determine how abnormal collagen itself affects collagenous tissues such as bone and tendon. To do this the oim and engineered mouse lines will be compared for the objectives below.

Objectives:

1 – to monitor type I collagen production and degradation, and assess collagen fibre structure and organisation

2 – to measure the mechanical strength of bone and tendon

3 – to analyse the fine structure and mineral content of bone

4 – to monitor the development of osteoarthritis in joints

5 – to identify and classify the genetic side-effects of the oim mutation.

In parallel, in vitro cell culture studies will be carried out to identify molecular regulators of abnormal collagen synthesis and identify potential new therapeutic targets. In a separate project a computational model of collagen synthesis is being developed to predict conditions driving abnormal collagen production.

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

The potential benefits of this project relate to both identifying the genetic side-effects (cellular stress) in the mouse model of brittle bone disease and in understanding the effect of abnormal collagen (I) homotrimer on tissues. Identifying and classifying the genetic side-effects would be expected to make a substantial contribution to the evidence supporting future pre-clinical and clinical trials of pharmaceuticals known to target cellular stress. Human brittle bone disease occurs in 1 in every 10,000-20,000 live births with 3,000-4,000 affected people in the UK. Patients have brittle bones but joint laxity, hearing loss and other connective tissue problems also occur. An effective treatment for osteogenesis imperfecta could transform the lives of up to 500,000 people worldwide. If abnormal collagen (I) homotrimer itself is found to cause cellular stress this would also support trials of pharmaceuticals known to target cellular stress in age-related human musculoskeletal, cardiovascular and fibrotic diseases. These common conditions affect quality of life, cause pain and disability and are costly to the NHS. If abnormal collagen (I) homotrimer solely has adverse effects on tissue structure and strength, the parallel non-animal studies should identify drugs to block its production, which could be new pharmaceuticals or repurposed existing drugs. The research could ultimately benefit the ageing population, in particular those suffering from osteoporosis and osteoarthritis, as well as the patients' families and supporting healthcare systems.

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

It is expected that a total of 1,500 mice would be bred over a period of 5 years, of which 250 would have brittle bones. Of these up to 72 could be injected with a dye to monitor bone production and degradation, up to 72 fasted for up to 18 hours before being put down. and up to 48 placed in a metabolic cage with a wire base for up to

24 hours (a maximum of three times less than once per week) to collect urine to measure collagen degradation. A quarter of those injected, fasted or metabolically caged could have brittle bones. Similar numbers of mice that produce collagen (I) homotrimer alone, without the genetic side-effects, may be injected (up to 72), fasted (up to 72) or metabolically caged (up to 48).

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

Expected adverse effects are spontaneous fractures, bone deformities, brittle teeth and a smaller size. Mice with brittle bones are expected to sustain at least one long-bone fracture with associated limping and abnormal gait. Mice with extensive fractures preventing movement or standing will be put down (using a legally-defined appropriate humane method). The maximum expected level of severity is severe for those with fractures. Mice will be bred and maintained until after weaning (8 weeks of age) and up to adulthood (18 weeks) at which point they will be put down. As well as obtaining tissues for this study, other tissues will be preserved so that future projects can be carried out without breeding more mice. Injection is expected to produce mild adverse effects such as mild transient pain. If localised irritation or infection occurs, those affected will be put down (using a legally-defined appropriate humane method). Fasting may potentially increase aggression in which case animals would be separated. Mice prone to fractures will be monitored in metabolic cages and removed if mobility is affected.

Application of the 3Rs

Replacement

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

Replacement

The effect of abnormal collagen on tissue cannot be evaluated in other systems due to the complex structure of collagenous tissues. Non-protected animal alternatives, 3D culture systems and computational models are not sufficiently developed for this purpose. However the project will also encompass cell culture experiments to investigate the early stages in collagen production and a separate project is generating a computational model.

Reduction

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

Reduction

Sample size calculations were carried out by a qualified chartered statistician. Tissues from the hind limbs of each animal will be used for different analyses to halve the numbers involved. Procedures to measure bone degradation are incorporated to reduce future breeding.

Refinement

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

Refinement

The ‘oim’ mouse is the only model of human brittle bone disease where abnormal collagen is present and all models of the disease have brittle bones. The comparison to the engineered mouse line, in which genetic side effects are not present, is the only means to pinpoint the source of the bone fragility. To reduce pain and suffering, non-steroidal anti-inflammatory pain relief will be provided in drinking water or as a self-medicating gel. Mice will be checked regularly and those with extensive fractures preventing movement or standing will be put down. Otherwise stronger pain relief (e.g. morphine) will be given as required with veterinary advice. Handling will be minimised and gentle capture methods will be used. Easily accessible soft food and water, soft bedding and non-tangling nesting material will be provided. Mice will be housed socially where possible to provide distraction and given floor-level tunnels and refuges. For bone degradation assays, injection will be carried out with gentle handling and/or anaesthetic. Mice will be closely monitored during any fasting or metabolic caging and relocated as appropriate.

PROJECT 190. 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 and outcomes of systemic inflammation and cancer in relation to cellular metabolism
Key Words	Acute pancreatitis, treatment, kynurenine, 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.
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 think that breakdown products of essential components of the diet influence the development of serious diseases such as acute pancreatitis, inflammatory bowel disease, sepsis and cancer, and also in the natural energy balance involved in cell aging. We aim to understand specifically how metabolism of a part of the diet called tryptophan and kynurenines is involved in those processes, to allow us to continue to understand and develop new medicines.

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

Increased understanding of kynurenine metabolism in serious diseases such as acute pancreatitis, inflammatory bowel disease, sepsis and cancer, and in cell aging, will help us to develop better ways of treating these conditions in humans and animals, for example by making new medicines, that act by altering that metabolism.

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

We will use rats – approximately 1700, mice – approximately 7300, over a period of 5 years of which breeding and maintenance accounts for: rats – approximately 200, mice – approximately 3000 over 5 years.

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

A large proportion of the animals (approx. 60% of the total) will be used for breeding and maintenance of the colonies, and for generating new genetically-altered strains. The expected severity for these animals is mild. The majority of the animals to be used (35% of the total) will have a moderate level of adverse effects, for example, being anaesthetised in a scanner, and having repeated injections, or having a moderate level of colitis. Because the diseases we are studying are very serious in patients – for example in severe acute pancreatitis, the risk of death to people who

have it is around 1 in 5 ¬– in order for our research to be meaningful, some of the animal models we use have to reflect that level of severity. Therefore, in some animals (fewer than 5% of the total), the level of severity is expected to be severe, with a risk of death and complications approximating that seen in human disease. At the end of each experiment, the animals used in each experiment will be humanely killed by experienced staff and blood samples and tissues taken for analysis, and analysed to help answer our research questions.

Application of the 3Rs

Replacement

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

Replacement

The diseases we are studying and complex, and although we can investigate lots of aspects using computer models, and cells in dishes in the lab, there are some complex interactions, for example between body tissues and the immune system, that can't be modelled in a petri dish. In addition, some of the technologies that we will use – for example genetic alterations – are at the moment only practically possible using mice and rats

Reduction

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

Reduction

We always plan our experiments carefully, and work closely with statistics experts to come up with the experimental design that uses the fewest animals to get the right answer

Refinement

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

Refinement

Our patients in the clinic are humans and therefore mammals, and we try to use the species that has similar general features – for example a cell-based and non-cell based immune system working together – to understand the disease processes that we study. Therefore we use mammals, and rats and mice are the most appropriate species for this work. There are certain technical factors, for example the size of a rat being much more suitable for experiments that require drip lines into a vein for

repeated injections, which makes rats more suitable for some experiments, and other factors for example the way we breed and develop genetically-altered mice that makes mice the most appropriate for other experiments. We always try to avoid using animals where we can get an equivalent or satisfactory answer without using animals, but when we do use animals, we minimise distress by careful and experienced handling, use of anaesthetics wherever appropriate or necessary, use of pain relief wherever pain might be expected, and careful monitoring by a team of experienced staff. In particular for some of our experiments, we will use implantable telemetry devices that give a constant readout of temperature and activity and can alert us early to any animals that are getting unexpectedly sick.

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	A service to enable the cryopreservation and rederivation of genetically modified mice
Key Words	Cryopreservation, Rederivation, Genetically Altered
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:
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):

A genetically altered mouse (GA mouse) is a mouse that has had its genome altered through the use of genetic engineering techniques in the laboratory or by a naturally-occurring event. A genome is an organism's complete set of genetic instructions. Each genome contains all of the information needed to build an organism and allow it to grow and develop. The instructions in our genome are made up of DNA. Single strands of DNA are coiled up into structures called chromosomes. Within our chromosomes, sections of DNA are "read" together to form genes. Sometimes genes get damaged, or the building blocks of DNA called nucleotides get mixed up, and this is known as a mutation. Mutations may cause illnesses and disease.

Genetically altered mice are commonly used for research as animal models of human diseases as we can't use humans themselves, and they are also used for research on genes and how they contribute to
disease. With this, in time, will come a better understanding of diseases and potentia l treatments. In order to make sure that any data that is gained from these animal models is interpreted correctly, they must be "clean". That is, they should not have been infected with contaminants known as pathogens (viruses, bacteria) which may cause disease. In this project we aim to take around 700 'lines' of mice from another establishment and "clean" them by a process called rederivation. This process will use fewer animals than if we were to remake all the GA lines from the beginning again. In addition, mice with pathogens are more likely to die or develop disease, whi ch in turn may infect other mice. We want to avoid this so we can use as few animals as possible to answer the scientific questions these mice will ultimately be used for. In addition, we will cryopreserve (freeze) the GA lines to safeguard them for future use.

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

When mice have pathogens it is possible that you might interpret some results you see from an infected mouse as being due to the genetic alteration you have

engineered, when in fact it is caused by these pathogens. Additionally, mice may unexpectedly die or develop disease, or infect other mice within a colony. We therefore aim to 'clean up' some specific mouse 'lines'. These clean mice can then be used to answer scientific questions about genes and their role in disease, without the potentially confounding presence of pathogens.

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

We will use adult mice for this project, as well as sperm and eggs, and early embryos. We estimate to use a maximum of 27,080 adult mice over a 3 year period.

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

We will use established and refined techniques which have the least adverse effects. This licence is being used to provide a service to "clean" animals so they can be moved to a brand new animal facility. This will mean initially cryopreserving (freezing down) sperm and embryos from the genetically altered mice. This will use a technique called superovulation to generate a large number of eggs in the female mouse, which can then be fertilised by mating to males, or in a dish with sperm called in vitro fertilisation (IVF). Superovulation involves two injections of hormone into the mouse's abdomen and will cause only momentary pain. We do not anticipate any adverse effects from this technique. The females will then be humanely killed, and embryos or eggs harvested. The next stage in the process will be to re-animate the mice at a planned time. We will thaw the sperm and embryos, and perform an IVF using the sperm with eggs from superovulated females. Resulting embryos which have been thawed and those produced by IVF are placed into a pseudopregnant female mouse, either surgically or non-surgically, and allowed to develop to term. The mice are made pseudopregnant by mating the female to a male that has previously been surgically vasectomised. This means the female male mouse is producing all the correct hormones to maintain a pregnancy without actually being pregnant. Whilst in this condition, any embryos transferred to the female will be taken to term and birth. All surgeries use a general anaesthesia and pain-killers, followed by post-operative monitoring from trained and skilled technicians. After mice have been born they will be identified individually within a cage by taking a small ear biopsy on a particular location of the ear, causing momentary pain. This 'ear clip' as a bi-product of identification, will also be used for genotyping to ensure the gene of interest is present. Selected mice are then bred to generate more mice for moving to their new accommodation. The vast majority of mice will show no adverse effects, with less than 5% showing some harmful effects caused by the genetic change. The harmful effects caused will be addressed where possible with husbandry and veterinary support. Where this will not help, the mice will be humanely killed. During the course of the project, if at any stage an animal experiences adverse effects that cannot be ameliorated, it will be killed humanely

and in a timely manner. All animals that have reached the end of their study will be humanely killed.

Application of the 3Rs

Replacement

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

Replacement

This project will involve taking genetically altered
mice that are already established and their use justified in other project licences, in o rder to 'clean' them up for future research. We therefore cannot replace them with no n-protected alternatives.

Reduction

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

Reduction

The other establishment's genetically altered mice have a number of pathogens associated with them. In order for the other establishment to gain the most meaningful scientific data from these animals, the mice need to 'cleaned' and freed of these pathogens before being sent to a new facility. One way to do this, where materials are available, would be to re-make the genetically altered mice from the beginning. Another way would be to 're-derive' the existing lines. This is done by transferring embryos obtained from the original 'dirty' mice and washing them to free them of any pathogens, and subsequently transferring them into 'clean' female mice to establish them as clean 'lines'. By adopting the rederivation method, we can save a substantial number of animals by avoiding the initial production and breeding phase, as well as, in cases where mice carry genes with multiple changes, avoid the long and complex breeding required to generate such mice. The saving by adopting the rederivation route is likely to be several thousand animals.

The type of cryopreservation used will be chosen on the criteria which results in the use of the least possible animals to secure the genetically altered animals. To this end, we will use sperm cryopreservation after the animal is killed wherever feasible. If the strain is amenable, superovulation may be used to maximise the number of eggs obtained for IVF. IVF will also be exploited as frequently as possible to produce large numbers of embryos, which can then be frozen in order to archive stocks. This procedure can lead to a substantial reduction in the number of superovulated females required to archive stocks.

Monitoring of efficiency rates will be performed and any technological advances embraced to ensure that the minimum number of mice are used in this process. For superovulation, doses and ages will be optimal to reduce the number of females required for embryo generation.

At all times the minimum numbers of embryo recipient females will be used to re-establish a mouse line to match the anticipated number of genetically altered animals required. We will keep the number of females used as egg donors required in each IVF session to a minimum number to avoid wastage.

We will aim to breed only when the numbers of animals recovered directly from embryo transfers fail to give the number required for re-housing. Well-established breeding calculations will be used.

Where a mouse colony is to be used by more than one group of researchers, stock will be shared making the most efficient use of the mice bred.

A genetic 'contaminant' in the in-bred stocks could have far-reaching effects on the integrity of genetic research, potentially leading to the culling of mice. Routine genetic testing of mouse colonies can go some way to avoiding this by providing defined genetic backgrounds.

Refinement

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

Refinement

Husbandry and health monitoring of all animals under this licence is performed by a team of highly competent Animal and Scientific Technicians that are assessed under the Institutes competency assessment program. Cleaning regimes are minimised to ensure stress and disturbance to breeding and stock animals is reduced. Environmental enrichment is provided to account for the individual needs of the animals e.g. nestlets for nest making by pregnant or lactating females. All animals will be group housed where possible.

All surgical techniques will look to adopt the principles of aseptic techniques as described in the LASA Guiding Principles for Preparing and Undertaking Aseptic Surgery
http://www.lasa.co.uk/pdf/lasa_guiding_principles_aseptic_surgery_2010.2.pdf

All animals may experience some post-operative pain or discomfort following surgery. Pain-killers will be given and maintained after surgery for as long as is necessary to alleviate pain. We will monitor for pain by observation of the mouse’s

behaviour and general appearance, and this will guide the administration of appropriate levels of pain-killers.

For genotyping, we will use tissue from ear-clips taken primarily for husbandry purposes to provide tissue for genotyping. If a second tissue sample by tail-tipping is required for technical reasons, authority from the Project licence holder and Head of the animal facility will be required.

We will ensure that when we have to transport any mice to another establishment, we will use ways which meet the highest welfare standard, for laboratory animal transport. Mice will be contained within secure and appropriate containers. The containers will allow adequate ventilation, be escape-proof, leak-proof, and capable of being handled without the animals posing a risk to handlers, and be of such a design and finish that an animals will not damage themselves during loading, transport or removal from the container. Where possible, animals will be grouped as socially harmonious pairs or groups.

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	Determining important regulatory pathways that control immune responses to infection
Key Words	immune system, infection, parasite, bacteria, virus
Expected duration of the project	5 year(s) 0 months

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

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

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

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

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

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

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

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

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

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

Application of the 3Rs

Replacement

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

Replacement

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

Reduction

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

Reduction

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

All data analysis will be conducted according to a pre-specified plan drawn up with the statisticians, with statistical tests performed with their input.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

Choice of species, models and methods.

As stated above, given the complex organisation of the mammalian immune system, it is unfortunately not possible to recreate these conditions using lower organism models (which do not contain a complex immune system) or *in vitro* cell models. Thus, the use of mice is crucial in the study of mammalian immunity. There are numerous examples of discoveries made in mice that have led to the direct identification of similar systems in the human immune system, with such cells and molecules now being clinically targeted in disease.

There may be opportunities to perform more focussed *in vitro* experiments if, during the course of mouse experiments, we identify types of immune cell that are directly affected during infection. We would then isolate these cells and determine their responses to different parasite/bacterial/viral products, and how different molecules/pathways affect their responses to pathogens.

Minimisation of animal suffering

Intestinal parasite infection models

All intestinal parasite infections are well established models of infection used by researchers over many years, with the majority causing no detectable suffering or distress to the animal. In a minority of cases (e.g. infection of mice with *Toxoplasma gondii*) mice will develop short-lived illness during infection. Here, animals will be closely monitored (with frequency increased leading up to time points where illness has previously been shown to occur) and should any unreasonable loss in condition be observed, the animals humanely killed.

Viral infection models

The viral models used will result in short-lived infection with moderate weight loss and illness, but mice expel the infection and fully recover from symptoms. However, all mice will be monitored closely in peak times of infection, and if any unreasonable loss of condition is observed the animals humanely killed.

Bacterial infection models

Some bacterial infection models (e.g. *Francisella tularensis* LVS) cause a severe infection. It is important for this level of infection to be reached, to determine cells/molecules and interventions that reduce the harm of infection. Thus, we need to reach a point in control animals where infection is established to determine whether any benefit has been achieved from gene/cell knockout. Other models (e.g. *Citrobacter rodentium*) cause modest weight loss and diarrhoea but resolve within 3-4 weeks with mice fully recovering.

In all experiments, we will closely monitor mice (with increased frequency of monitoring at time points close to when illness is known to occur), and have a detailed scoring system in which to assess the health of the mice during severe acute infection. If an agreed level of discomfort is reached (based on a robust clinical scoring system), the mouse will be immediately humanely killed.

PROJECT 193. 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	Patterns and mechanisms of avian timing
Key Words	Bird, Biological Rhythm, Season, Year, Tracking
Expected duration of the project	0 year(s) 8 months

Purpose of 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) higher education or training for the acquisition, maintenance or improvement of vocational 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 thrive and survive, all organisms, including humans and birds, have to align their activities with the daily and annual cycles of their environments. Birds achieve this through biological rhythms that prepare them for the changes in the environment. These biological rhythms need to be alligned with environmental factors. Despite detailed molecular understanding of biological clocks we know surprisingly little about their functioning in wild animals. Therefore, the work will address daily and annual cycles of birds in the wild, where we study timing and movement patterns, and under captive conditions, where we investigate underlying mechanisms. Specifically, we use miniature, wireless glue-on radio-transmitters which allow for remote tracking of free-ranging animals. With these, we monitor distinct events of individuals. We examine to which extent timing is genetically and developmentally determined, and which a species are relevant for fitness and survival. We will also collect information on health of the birds and ectoparasites to monitor diseases. For daily and annual clocks, we investigate the role of hormones in timing by sampling but also manipulating of hormone levels. I will also test an alternative method for measuring daily clocks in the laboratory from small skin samples to replace animal experimentation. Some wild birds will be brought into captivity to validate these methods and to investigate their temporal behaviour in response to changes in the surrounding environment. To understand the mechanisms that underlie annual cycles, we examine annual activities and specifically moult in captive birds. From wild and captive birds we collect small samples of blood, tissue and feathers to assess clocks, health, physiology and genetic background.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Knowing how birds cope with environmental change, and likely to derive from this how their bodies meet the challenges of correct timing, can inform us about consequences of disrupting body clocks. This includes a better understanding about the risks we face in modern societies that are active around the clock, for example

involving shift-work and bright illumination at night. In addition to gains in science, conservation concerns can also be addressed by this basic research on avian clocks. One example are the ways current climate change affects the progress of the growing season. Bird and other organisms already pay a cost for such changes because they time many of their activities by biological clocks that had evolved under natural condjtons prior to major, man-made change. In animal welfare and also in conservation, there is need for remedies against disruption of biological clocks. For example we advise on conservation projects towards breeding and reintroducing threatened bird species, and we collect information about seasonal changes in habitat use of birds that need protection.

What types and approximate numbers of animals do you expect to use and over what period of time?

We use wild birds, mostly songbirds, and also domesticated fowl. The great majority of birds will be released directly after animals capture and brief sampling. The total number of birds that we will use over 5 years will not exceed 2,500.

In the context of what you propose to do to 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 my best of our knowledge, all proposed procedures are of mild severity. The overwhelming majority of wild birds will be released at the site of capture. We expect birds to recover completely and, based on our earlier studies, to successfully breed again in their original territories. Very few wild birds will be humanely killed to collect information about underlying mechanisms and about physical stage. Domestic birds will be humanely killed after procedures.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The project strives to understand clocks under natural conditions. This cannot be achieved without studying animals. Birds are an ideal study group, to the best of our knowledge, because of their well-known ecology, and because they are large enough to carry miniature, wireless biologgers. With these data can be collected without a major burden to the birds, and recapture is not necessary.

For the studies that are carried out in captivity, I will try to the greatest extent possible to conduct research on cells that are in culture *(in vitro),* so that only small samples would need to be taken from the birds. While I pursue this possibility actively, it still first requires additional experiments for validation. For other captivity

studies, in the context of annual-cycles, I use domesticated fowl (from the chicken family) which have been bred to fare well in human establishments.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To understand how biological clocks help birds reproduce and survive, we need to collect data from a relatively large group of birds. These experiments will however only involve mild severity (blood sampling, either skin or feather sampling, or brief captivity). In addition, we will make the most efficient use of samples that I collect from the birds. For example, we will keep a stock of DNA for future investigations, which will allow us to answer upcoming questions without a need to resample the birds. For blood-sampling, we will reduce the amount taken to the minimum that is necessary to acquire reliable data. Furthermore, we will work closely with colleagues who carry out related research, so that information will be shared between our licences.

For our captive experiments I will use as few animals as possible, while making sure that the research questions can be answered satisfactorily. For several project parts I can · determine this number using calculations that are based on previous findings. For the project parts that touch new ground we use the closest comparable data from the literature to inform the study design. We will conduct power calculations to establish that with a calculated error probability of 5%, the power for detecting expected differences will be in excess of 67%. We will get support from colleagues with great strengths in analytical and computational biology.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 proposed project involves birds because we know so much about their behaviour and ecology. Therefore, I can test well thought-out hypotheses on a relatively low number of subjects. Whenever possible, I will work on birds in the wild where I can perform procedures on site. This will greatly decrease the stress, and birds can be quickly returned to their natural environment. I will use the mildest possible procedures to collect tracking data. By using PIT tags (commonly used for pets) attached to rings and wireless radiotelemetry, I can monitor birds remotely. I deploy an antenna in the nest cavity or on a tree, which then records identity, activity, body temperature or visiting rates of birds. Without the need to recapture

and handle a bird, I will minimise subsequent disturbance and get large sample sizes from relatively few birds.

Some objectives require relatively long-term keeping and application of substance. Because of the commercial interet and their particular breeding for human use, the most suitable group of birds for this project is the chicken family. For the chicken, analytical tools are well developed. Also, the relatively large size of these birds makes them more robust with respect to repeated sampling of blood or feathers, and will allow for the collection of sufficiently large samples for further analyses. In all cases, we strive to reduce any animal suffering caused by the project. REDACTED

PROJECT 194. 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	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 195. 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	Neurobehavioural Mechanisms of Mental Health
Key Words	mental health, psychiatric disorders, cognition, 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):

Our research aims to understand how and why mental health disorders occur, and to develop new treatments for people suffering from these disorders. Our work focuses on the psychological processes (e.g. attention, memory, compulsion) that are dysfunctional in a number of different mental health disorders, meaning that our work goes beyond individual disorders (i.e. it is “transdiagnostic”). Some aspects of our work, however, have greatest relevance to specific mental health disorders, including drug addiction, obsessive-compulsive disorder, schizophrenia and post-traumatic stress disorder. We use animal models that allow us to investigate dysfunctional psychological processing – developing new models if necessary – to understand the neurobiological causes and consequences of mental health disorders. Building on our previous work, our research aims to identify new drug targets and new forms of behavioural therapy that could treat mental health disorders. Some of our previous work is now beginning to be translated 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)?

Mental health problems cost the UK an estimated £105 billion per year, with 50% of these costs reflecting decreased quality of life for those affected. Thus, mental health disorders place a considerable burden on not only the affected individual, but also social and economic burdens on society. The case for new treatments is strong, as currently available therapies are not effective for all patients; for example, only 50% of those with post-traumatic stress disorder show a reduction in fear with cue exposure therapy. Our research aims to understand the bases of these disorders, and develop new treatments for them. We aim to develop new rodent models for mental health disorders that give us a better understanding into why certain behavioural or drug therapies work, to investigate why certain subpopulations are vulnerable to mental health disorders, and why they respond differently to treatment. We also aim to use these models to develop new and better treatments for mental health disorders.

What types and approximate numbers of animals do you expect to use and over what period of time?

We use the minimum numbers of animals possible to achieve biologically and statistically meaningful data. We anticipate that we will use approximately 8700 rats in 5 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The specific research questions that each experiment aims to address will determine the types of procedures that are experienced by the animals. Many of our research questions can be addressed by testing animals' memory and decision-making in sophisticated behavioural tasks rewarded with palatable food. Where we are attempting to modulate the psychological processes we are studying, we may give injections of specific types of drugs that affect activity in the brain; sometimes, we will give multiple injections, either with different doses of the same drug, or with drugs having different effects on the same chemical system (e.g. increasing or decreasing activity in that system). We only give multiple injections to the same animal where we need to be able to compare an individual animal's behaviour across these different conditions. Some of our research questions investigate the parts of the brain that are involved in these psychological processes. We target the parts of the brain that constitute the 'limbic corticostriatal circuitry'. For these experiments, we have to manipulate the brain directly by intracranial surgery (by e.g. surgically damaging specific parts or implanting recording devices). For our research into drug addiction, we have to implant the animals with intravenous catheters so that they can later self-administer drugs of abuse. This is critical for our experiments, as our addiction research studies the psychological processes that allows drug use to become compulsive (rather than dependence, which could be induced by experimenter-delivered injections of drugs). We need animals to be able to initiate their drug use in order to address our scientific questions, and to produce translational models that will be of maximum benefit to addicted patients. Whenever our animals undergo surgical procedures, they receive appropriate anaesthetics and painkillers around the time of the operation, and are very carefully monitored at the time of surgery and throughout the experiments for any signs of pain or distress. If the animals show signs of suffering and we are not able to ameliorate these in consultation with the Named Veterinary Surgeon, then we euthanise the animal. Fortunately, such instances are very rare. Some aspects of our research address disorders in which aversive learning plays a major role (e.g. phobia, or post-traumatic stress disorder). These disorders can only be studied by exposing animals to inescapable (uncontrollable) stressors, and in our experiments we use mild electric shocks as the aversive outcome. Many of our animals experience no more than three mild inescapable electric shocks in their lifetimes, and this is sufficient to allow us to study the psychological processes that underlie learning about stressful events. Electric

shock is the most useful aversive outcome for our scientific purposes, because it allows us to precisely control the timing of cues predictive of an aversive outcome and the outcome itself (unlike more general stressors, such as exposure to the scent of predators) and because it engages the same brain circuitry as the mental health disorders that we are studying (unlike, for example, air puffs to the eye, which engages reflexive circuitry with has little relevance to our scientific questions). Animals that experience these inescapable stress conditions do not show changes in behaviour outside the environment in which the shock is delivered (e.g. changes in body weight or interaction with other animals). For some of our research addressing post-traumatic stress disorder, the animals are required to experience stronger stressors, and in these experiments they are exposed to up to 15 mild inescapable (uncontrollable) electric shocks in a single training session, which acts as a trauma analogue. This procedure leads to changes in the brains of animals that are relevant to post-traumatic stress disorder, and are necessary for us to understand changes in psychological processing that are relevant to the development of the disorder, and to developing new treatments. This procedure is also not effective for all animals (approximately 13% do not show changes in behaviour or brain changes), which allows us to study this 'resilient' population with the hope of identifying why they are effectively protected against post-traumatic stress disorder following stressful conditions. This is a well-established model of post-traumatic stress disorder and represents a refinement over some other models, but we will investigate further refinements to this procedure in parallel with our behavioural studies. Animals may experience more than one of these broad types of procedure in the course of an experiment, depending on the specific question that the research is addressing. Where we are specifically investigating vulnerability to mental health disorders, animals may undergo early life stressors (e.g. repeated intermittent maternal separation or social isolation) in addition to the procedures listed above. For questions relating to comorbidity of mental health disorders (e.g. between post-traumatic stress and addiction, for which comorbidity is estimated to be as high as 60% of patients) animals may undergo the more stressful aversive conditioning and later be implanted with catheters so that they can self-administer drugs of abuse. Thus, although animals may experience more than one type of procedure, for each animal we perform the minimum number of procedures that will allow us to address the specific scientific question we are investigating with that experiment. At the end of experiments, all animals are humanely killed, and wherever possible and appropriate we collect brain tissue for further 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

This research is only possible with the use of animals. Human studies (e.g. brain imaging studies) are useful, but can only provide correlative data that do not address causation. Furthermore, it is not ethically possible to study the genetic and/or environmental factors that underlie predisposition to, and the development of, neuropsychiatric disorders in humans. Similarly, it would not be possible to develop new treatments for brain disorders without testing them in animal models first. In vitro models (e.g. brain slice preparations) or computer simulations cannot be used because the modelling of behaviour in these systems is not yet sufficiently advanced.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We are fully committed to using the minimum number of animals required to obtain data that are statistically and biologically meaningful. We carefully design our experiments to maximise the behavioural data collected from each animal, and to minimise distress. We take replicability of our data very seriously, and routinely calculate effect sizes from pilot studies or previous literature to determine the minimum number of animals required for reliable data. We randomly allocate rats to experimental groups wherever possible, though sometimes rats are 'pseudorandomly' allocated (e.g. if we testing the effects of a particular treatment on a specific behaviour, rats are assigned to groups to ensure that their pre-treatment behaviour is the same). We also make use of automated software to collect behavioural data wherever possible, and where this is not the case (e.g. when behaviour has to be quantified by a person) we take great care to ensure that the person scoring is unaware of the experimental group allocations. We design our statistical analyses of the data in advance, and have extensive experience of this; additionally, when we are designing new studies that might require new analysis methods, we can refer to experts within our establishment to advise on this.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 because they are the least sentient species that can model neuropsychiatric disorders. The brain circuitry implicated in many mental health disorders is very similar between rodents and humans, and the behavioural tasks

that we use are widely recognised as modelling specific aspects of these disorders. (Some of these tasks can be used in both humans and animals.) As stated above, one aspect of our work involves developing animal models of mental health disorders, and this includes refinement work where necessary. We take the welfare of the animals very seriously. Most of our animals are trained to perform sophisticated behavioural tasks, and any type of illness or pain would compromise their behaviour. Thus, we have scientific as well as ethical reasons to ensure high standards of welfare. When an experiment requires that animals undergo surgery, we conduct surgery to aseptic standards and provide pain relief during and after surgery. Animals are monitored frequently (often undergoing daily testing) and any adverse effects are observed by scientific and animal care staff, recorded and discussed with the Named Veterinary Surgeon. If these cannot be quickly ameliorated then animals are euthanised to prevent suffering. We have extensive experience of working with rats and we are well-trained in the clinical signs that mean an animal is unwell. If any animals show any signs consistent with brain damage following surgery, they are immediately killed to avoid suffering. If animals show other clinical signs such as subdued behaviour, piloerection or hunching, they are monitored closely and the Named Veterinary Surgeon will be consulted. If no improvement was shown within 24 hours, then the animal would be humanely killed.

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	Non-rodent/Large Animal Models of Surgery, Toxicity and Safety
Key Words	Safety, Efficacy, Surgical models, Livestock species
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 enables a range of studies to evaluate efficacy and safety/toxicity in domestic animals for the following purposes:

- To support the development of safe and effective veterinary medicines and other animal health products by generating data to determine their efficacy, safety, tolerance and toxicity in the target species.
- To support the development of safe and effective human pharmaceutical products by generating data to determine their safety, tolerance and toxicity in relevant animal models.
- To assess the efficacy, safety and tolerance of medical devices (or other treatments) used in connection with human or veterinary surgery or disease treatment, including surgically induced models of human or animal disorders.
- To use animal models of skin and mucosal wounding and wound healing to assess the safety, tolerance and efficacy of medical devices, medicines and surgical treatments on wound healing.
- To determine the metabolism and residue characteristics of veterinary medicinal products in the target species
- To determine the metabolism and residue characteristics of agrochemicals or other chemicals to which food producing animals may be incidentally or accidentally exposed
- To assess the potential of relevant chemicals to induce delayed neurotoxicity in humans using the chicken model.
- To obtain biological samples from live animals for use in ex vivo work and quality control, where this is directly related to the other purposes in the licence.
- To develop and or validate new, alternative or refined procedures/techniques in order to determine new scientific endpoints, or to improve/refine data quality, or to improve/refine animal 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)?

Governments require, and the public expects, that substances/articles to which humans or domestic animals may be exposed are effective and safe and/or well-characterised. Therefore, new substances or treatments must be evaluated before they are made widely available for use; this is a mandatory legal requirement which requires the use of animals in studies to evaluate systemic exposure, efficacy and toxicity. The principal benefit of the project is the provision of data to facilitate sound decisions on safe/effective product development and appropriate regulatory decisions on clinical trial approval or marketing authorisation for new medicines or other substances or articles to which humans or domestic animals will be exposed, thus contributing to their protection and safety.

What types and approximate numbers of animals do you expect to use and over what period of time?

This project uses domestic animal species, which for the purpose of this project are defined as those species generally regarded as farm livestock, i.e. cattle, pigs, sheep, goats and poultry (chickens, turkeys); or as companion animals, i.e. REDCATED, dogs; or species which may fall into either category, i.e. horses, rabbits. The evaluation of safety/efficacy of veterinary medicines and other animal health products, and safety of other substances to which they may be exposed, is self-evidently best achieved through testing in the target species. For studies in which non-rodent species are used as models for the assessment of human safety, species selection is made on a case-by-case basis according to various criteria including physiological, morphological and anatomical similarities with humans. In cases where conventional non-rodent models (pigs or, where justified, dogs or non-human primates) are unsuitable an alternative is needed, and large ruminants, particularly sheep, may often fulfil the necessary validity criteria for use as a toxicological model. Pigs, sheep and goats are all well-established models for surgical studies of various types, again based on suitability/validity criteria (for example, sheep and goats are used extensively in orthopaedic research because of similarities in their bone architecture and bone regeneration processes to those of humans). There is one case in this licence where chickens are used as an animal model for humans, in the evaluation of delayed neurotoxicity; this is because they share a specific biochemical characteristic with humans that makes both susceptible to this type of toxicity. Dogs, REDACTED and equidae (horses) will be used only where the purpose of the study/programme of work cannot be achieved using any other species. In nearly every case, the justification for their use is that they are target species for veterinary/animal health products, where evaluation in the target species is mandatory. REDACTED and horses will not be used for any other reason under this licence. In rare instances, it may be necessary to use dogs in supporting studies such as pharmacokinetic and metabolism studies, where they have been selected – with appropriate justification – as the non-rodent species for safety/toxicity

or pharmacology assessment studies that will be carried out under other project licences. Over the five-year duration of the project licence, it is estimated that approximate maximum numbers of animals used will be as follows: cattle 330, pigs 1080, sheep 610, goats 210, horses 160, REDACTED 150, dogs 380, rabbits 230, chickens 3520, turkeys 1600. 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 and efficacy data in the subject species. The estimates actually represent total numbers of experimental uses rather than the total numbers of individual animals used, which may be lower due to re-use of animals in circumstances where such re-use does not add significantly to the overall harms experienced by the animals or confound the scientific objectives.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

In general, animals are dosed/treated by the intended/likely route of human (or target animal) exposure, and observed regularly to monitor appearance, behaviour and clinical health. The main study types that are performed under this licence for each class of test item are: Veterinary medicines/animal health products: Efficacy – animals are dosed at clinically relevant doses and observations on expected parameters of efficacy are made. In some cases it may be necessary to administer a challenge treatment to elicit a condition against which efficacy can be assessed – for example an experimental infection with intestinal nematodes in sheep to test a veterinary worming medicine. Target animal safety - Animals are dosed at clinical doses and low multiples thereof, and observed regularly. Typical investigative procedures are similar to diagnostic procedures that might be used medically to monitor progress of a human patient (e.g. collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function). Terminal investigations will involve sampling and processing tissues for pathological assessment. Pharmacokinetic, metabolism and residue studies – Animals are dosed at clinically relevant doses and observed regularly. The metabolism, fate and distribution of the test item is investigated by analysing samples of blood, excreta, expired air, milk, eggs and tissues taken post mortem, as appropriate. Agrochemicals/chemicals: Pharmacokinetic, metabolism and residue studies – Animals are dosed at clinically relevant doses and observed regularly. The metabolism, fate and distribution of the test item is investigated by analysing samples of blood, excreta, expired air, milk, eggs and tissues taken post mortem, as appropriate. Human pharmaceuticals: Safety/Toxicity studies – Dose levels for definitive toxicity studies in animal models are determined in preliminary studies and are selected to investigate mechanisms of toxicity and a safe exposure level (no-effect level) that can be related to expected clinical exposure. Typical investigative procedures are similar to diagnostic procedures that might be used medically to monitor progress of a human patient (e.g. collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function). Terminal

investigations will involve sampling and processing tissues for pathological assessment. Pharmacokinetic, metabolism and biodistribution studies – Animals are dosed at clinically relevant doses and observed regularly. The metabolism, fate and distribution of the test item is investigated by analysing samples of blood, excreta, expired air and tissues taken post mortem, as appropriate. Medical devices/surgical models: Surgical models included in this licence are designed to evaluate devices used in the treatment/correction of cardiovascular diseases (grafts, stents, cardiac pacemakers); orthopaedic treatments for bone and cartilage disease; techniques to improve kidney transplant technology; treatments for the ablation/destruction of cancerous tumours; devices for treatment of diabetes; neural devices for treatment of chronic pain and seizures; stem cell/device combinations for surgical repair of lung and bile system defects; treatments for lymph node regeneration; treatments intended to reduce operative or traumatic blood loss; and robotic surgery devices designed to improve surgical outcomes (reduced trauma/pain/blood loss, reduced surgical complication, improved outcomes and faster recovery times). The surgical procedures are performed under general anaesthesia with full monitoring of vital signs and pre-/post-operative preventive analgesia and antibiotic treatment. Animals are monitored closely during surgical recovery and appropriate investigations are carried out similar to those used in safety/toxicity studies. Terminal investigations will involve assessment of healing at the surgical sites and sampling/processing of tissues for pathological assessment. The protocols for all of the above studies have a moderate severity classification. However, most animals are expected to experience no adverse effects, or only mild effects such as slight weight loss or transient discomfort due to dose injection or blood sampling. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss/reduced activity. A very small number of animals may experience severe adverse effects without intervention, but humane end-points are applied to avoid this and to prevent unnecessary suffering. Animals in surgical studies are normally regarded as experiencing moderate adverse effects (though they are given appropriate pain relief medication) and may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients; for example, in the case of renal studies, animals may experience some degree of impaired renal function which could potentially lead to kidney failure without appropriate interventions. However, supportive treatments are given to eliminate or minimise these adverse effects, and humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with full peri- and post-operative analgesic cover to reduce/eliminate as far as possible any pain or discomfort during surgical recovery, as would be the case for a human patient. In addition, there is one protocol in this licence with a severe severity classification: the assessment of delayed neurotoxicity in the chicken. Most birds used under this protocol will nevertheless experience no adverse effects or only mild to moderate effects; however, it is necessary to determine (and test for delayed neurotoxicity at) the highest non-lethal dose possible in order to provide clinically

relevant data (because humans most at risk of delayed neurotoxicity include those who have been exposed to very high doses of organophosphorus compounds but have survived the acute effects due to medical intervention); this means that the occurrence of severe effects and potentially death in a few birds is likely. However, these potential effects are minimised as far as possible by sequential dosing and the use of antidotes where they are effective to protect birds from acute toxic effects.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Although non-animal (*in vitro, in silico*) studies can provide useful supporting data to refine and reduce animal studies, definitive assessments of systemic exposure, efficacy and toxicity can only be achieved in studies using intact animals, and this remains a mandatory legal requirement; currently, there are no scientifically, ethically or legally acceptable non-animal alternatives available.

However, no studies in animals are conducted under this licence until an assessment has been made to determine that the specific study is necessary and justified, i.e. the study aims and objectives are consistent with the scope and purpose of the licence and cannot be achieved by any other means not involving the use of animals. This assessment will involve consideration of any potential non-animal alternatives, review of existing data on the test item and reference to any other relevant information (including literature review, in-house data, information on similar items).

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The numbers of animals used within each study are kept to the minimum commensurate with meeting study objectives, through careful assessment of results at each stage of testing, reference to all available sources of information on the test article under evaluation, compliance with guideline recommendations on minimum group sizes where applicable, and the appropriate use of statistical principles in study design.

In some cases, numbers of animals used may be minimised by appropriate re-use of animals in more than one unrelated procedure; however, a rigorous harm-benefit

assessment is made to ensure that the overall harms experienced by the animals are not significantly increased.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 used under this project licence, and the reasons for their use, are as indicated in the first section of this summary.

Sequential testing, with review of findings at each stage and modification of subsequent stages as necessary, maximises opportunities for refinement to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals.

Where appropriate, positive reinforcement training (treat rewards) is used to encourage co-operation in (and minimise any stress of) handling/procedures. Environmental enrichments appropriate to the species are used within the animal facilities.

Animals are monitored for clinical signs of toxicity or other effects on their health and wellbeing, and in order to prevent unnecessary suffering, humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).

In cases where study objectives do not require that animals are killed for terminal investigation, they may be kept alive after completion of the procedures and considered for re-use in further procedures, or release from the control of ASPA for rehoming as companion animals (dogs, REDACTED and horses only) or for return to commercial livestock use (farm livestock species and horses only). The criteria for keeping alive, re-use and rehoming/return to livestock use are applied in accordance with the legislative requirements of ASPA.

Where re-use of animals is considered as a strategy to reduce the numbers of animals used, this is assessed against the potential overall welfare harms to the animals, taking into account their overall lifetime experience. After each use, animals are assessed for suitability for keeping for re-use, and any animals showing significant adverse effects will not be re-used.

The rehoming of animals as companion animals is subject to careful assessment and confirmation of health, suitability for rehoming including appropriate socialization procedures, and confirmation that the animals do not pose any risk to human health,

animal health or the environment. In addition for animals released back to commercial livestock use, checks are made to ensure that any other applicable legislative requirements (e.g. DEFRA requirements on use of animal health products and withdrawal periods for food producing animals) are met.

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	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. Based on comparable work from the last years, 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 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	Regulation of energy metabolism and immunity in health and disease
Key Words	Aryl hydrocarbon receptor, thermogenesis, obesity, diet, 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;
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):

Two types of fat cells exist in mice and humans. White fat cells store energy and brown fat cells burn energy to generate heat. Brown fat cells utilize fat and sugar as an energy source and are usually activated when individuals are exposed to environmental cold. Many of the health problems associated with obesity and type 2 diabetes are caused by accumulation of excess fat and sugar and it is thought that activation of brown fat cells in obese individuals will help to improve their health by reducing the amounts of fat and sugar in their body. However, exposing a large proportion of the population to cold is not a feasible therapeutic strategy and thus alternative ways to activate brown fat cells are urgently needed. The aim of this project is to gain a deeper understanding of how brown fat cells are activated and to identify compounds that can stimulate brown fat cell activation. In particular, our studies will focus on the aryl hydrocarbon receptor (AHR), which is present in fat tissue and in cells of the gastrointestinal (GI) tract. Receptors are proteins that are activated by specific factors termed ligands. Ligands for the AHR are found in the diet such as certain types of green vegetables including broccoli and kale. We will generate and analyse mice with genetic modifications of AHR and test whether AHR ligands derived from the diet can activate brown fat cells and reduce obesity in mice. By studying both the gut and fat tissue we can gain a better understanding of how AHR contributes to function of these two organs in healthy and obese 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)?

Obesity represents one of the most challenging public health problems in the developed world. The research output from this project will lead to a better understanding of how brown fat cells are activated and has the potential to identify novel dietary components capable of stimulating brown fat cell activation. This will be applicable to human health as it could lead to better therapies for the treatment of obesity and associated diseases.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use approximately 12200 mice over a period of 5 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The majority of mice used in this project will experience mild adverse effects such as cold exposure, weight loss from dietary manipulation and transient pain after systemic injection of substances. 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. 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). 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

Brown fat cells are part of a complex tissue environment referred to as adipose tissue which is composed of fat cells, blood vessels, nerve fibres and various types of immune cells. Proper function of brown adipose tissue involves collaboration between these different cell types and also requires communication of adipose tissue with other parts of the body, such as the brain and the gut, in order to sense nutrient availability and react to varying environmental conditions. It is currently impossible to faithfully reconstitute the complexity of adipose tissue biology in a culture dish and thus the use of mice is necessary to decipher the intricate processes involved in brown fat cell activation. Furthermore, characterisation of the physiological consequence of brown fat cell activation e.g. changes in body temperature and measurement of fat and sugar metabolism requires the use of intact animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use the minimum number of animals necessary to achieve statistically meaningful results. Experiments are carefully designed and executed in order to

maximise the biological information obtained from a single animal. We will employ breeding strategies that maximise the use of offspring derived from each cross and carefully adjust breeding output with demand for experimental animals. 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. In some cases, we can reduce the number of experimental mice by using imaging and telemetry technology that allows studying a cohort of mice over several time points without the need of killing them for analysis.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

We propose to use the mouse as model organism for several reasons. Firstly, mice represent the lowest mammalian species available in terms of displaying physiological and disease states with extensive similarities to humans. Secondly, the use of genetically modified mouse models is well established and has proved to be a powerful tool for studying brown fat cells and obesity. Finally, a vast array of available reagents and experimental methods facilitate the study of brown adipose tissue and obesity in mice in contrast to other organisms. All the procedures in this project 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. To maximise their well-being, mice will be housed in cages with environmental enrichment which encourages instinctive behaviour such as burying or hiding and reduces the risk of over-grooming. Experimental protocols, including dosing and sampling volumes and frequencies, will be in accordance with current best practice.

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	RoboChick: an autonomous platform for data-collection in poultry sheds
Key Words	animal welfare, chickens, precision livestock farming, animal behaviour
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;
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):

Poultry meat birds (broilers) are bred for high muscle gain but require very careful husbandry. Monitoring technologies have been developed to automatically collect flock level data to help manage these delicate birds. However, current technologies are limited as they are unable to dynamically record environmental data across a poultry shed and at the height of the birds. The overall aim of this project is to develop and trial a multi-functional robotic system that can autonomously collect data (such as climatic or atmospheric conditions or bird condition) within a poultry shed, to fulfil this need in the industry. At this preliminary stage in the project the aim is to trial the robotic platform in a small flock of broilers whilst it is under manual control to monitor the behavioural responses of the birds and to ensure that the robot does not negatively affect bird welfare.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Within the intensive chicken sector, tens of thousands of birds are housed together and often across multiple buildings with small numbers of stockmen to monitor them. Monitoring technologies can be used to aid the stockmen on large farms, ensuring that any issues in the flock are detected quickly to maximise bird welfare, health and productivity. As the robotic platform being trialled will move among the animals it is important to ensure that they react appropriately and that their welfare is not compromised. Broiler chickens should benefit through improved health and welfare, and humans through improved bird productivity.

What types and approximate numbers of animals do you expect to use and over what period of time?

Approximately 1500 Ross 308 broiler chickens will be housed together to recreate a small-scale commercial environment. They will be housed at 1 day old and will be kept until they are approximately 39 days of age in line with industry standards.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The animals will be raised as they would be on a commercial farm. There are no expected adverse effects beyond industry standards and the animals will be sent to a commercial slaughterhouse at the end of the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The purpose of this study is to observe commercial broiler chicken behaviour in response to the robotic platform. It is therefore essential that a common commercial strain (Ross 308) is used.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The room used for this study is the smallest possible to ensure that the robotic platform can move through it as it would in a commercial house, whilst ensuring that the birds can freely move away from the robot.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

The Ross 308 broiler chicken is the most popular meat chicken in the UK. It is therefore a good example of the type of bird that this robotic platform will eventually be implemented among. Welfare of the birds is of utmost importance during this feasibility study. The robot will be manually controlled to ensure that it can be stopped if birds do not behave favourably and birds will be treated/culled as appropriate if they are found to have developed any health issues (as a result of the breed, rather than the robot).

PROJECT 200. NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Murine models of cancer progression and therapy
Key Words	Cancer, Melanoma, Precision Medicine, Metastasis, 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, prvention, 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):

Our aim over the next five years is to continue to develop both our basic and translational research programmes in order to improve our understanding of cancer biology and implement precision medicine to improve outcomes for melanoma and other cancer patients.

Our specific aims are:

To understand the relative contributions of ultraviolet radiation, genetics, pigment and inflammation to the development and course of melanoma

To improve knowledge of, and develop new treatments for uveal (eye) melanoma, a form of melanoma with particularly poor prognosis upon metastasis, and for which notreatments are available.

To implement precision medicine for melanoma and other cancers, tailored to patients whose tumours carry particular genetic changes.

To improve knowledge of, and develop new treatments for different types of metastatic disease, such as metastatic melanoma, breast, pancreatic, and prostate cancers.

To evaluate and understand the biological mechanisms of new cancer drugs and assess their efficacy in faithful cancer models.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

This project will contribute to the understanding of tumour progression and metastasis mechanisms, and potentially to novel therapeutic approaches for cancer care; the mouse models characterised in this project will provide more powerful methods to identify the underlying mechanisms of tumour progression and spread, and to introduce drugs targeted to individual cancer patients. In addition, by

assessing standard-of-care and new treatment approaches we aim to support therapeutic decisions in the clinic, based on the results from our studies. Finally, we expect to publish our work in peer reviewed journals thus sharing our findings with the scientific community.

What types and approximate numbers of animals do you expect to use and over what period of time?

Mice; 17,600; 5 years

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The vast majority of mice in the project will carry some form of tumour – mostly superficial tumours that develop or are implanted in the skin, but in some studies internal tumours developing or implanted in such organs as the eye, breast, prostate and pancreas, or in organs where metastatic tumour cells spread to naturally. In our melanoma studies, animals will be exposed to short doses of UV radiation, and across our studies, animals will be treated with therapeutics (typically orally, or by injection) appropriate to the cancer types under study. Where possible, non-invasive imaging will be used to maximise our understanding of tumour progression and spread and to accurately monitor tumour growth. In approximately 75-80% of cases, mice would be expected to experience a “Moderate” or lower level of discomfort, as the tumours they carry would not make a significant impact on their general health and wellbeing, and the majority of other procedures (UV exposure, non-invasive imaging, injection of therapeutics), will generally result in no more than transient discomfort and no lasting harm. However it is sometimes difficult to predict the growth and behaviour of internal tumours and of metastatic spread, and thus in some models the tumours may have a more significant impact on the animal’s well-being. All the mice will be killed by humane methods at the end point of the experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The development of effective cancer therapeutics is an important goal of modern biomedical sciences. To identify potential cancer therapeutic targets, the processes involved in tumorigenesis must be understood at all levels, which requires the development of model systems accurately mimicking tumour progression.

Cancer development is dependent not only on the changes occurring within the cancerous cells, but also on the interactions of the cells with their environment. and

surrounding cells The majority of our current understanding of carcinogenesis comes from the laboratory analysis of late-stage tumour tissue removed from cancer patients. In our lab, we perform a collection of laboratory assays to understand important points of tumour biology. While this has revealed many changes experienced by cancer cells, it provides little information about the factors influencing early-stage cancer development in their tissue environment.

Also certain hallmarks of cancer, such as metastatic spread and blood supply changes, are impossible to study in the laboratory. Therefore, mouse models are important for studying the physiological aspects of human cancer development. Transgenic mouse models have been engineered to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. This cannot be replaced by laboratory studies or different live models such as zebrafish or insects.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Our use of laboratory-based methods limits the number of animals required for the live models investigation stage.

For our transgenic (genetically modified) models we will use standardised experimental approaches, so that control groups can be used across experiments.

We are also reducing the number of transgenic animals bred for our studies by propagating tumour cells obtained from these models and re-implanting them into unmodified mice.

By standardising our experimental methods, we are often able to compare data from new experiments to data from historical experiments, rather than set up new similar experimental groups to those used in previous experiments.

The proposed experimental designs and methods of analysis of the results are always in agreement with statistical guidelines and advice from our bioinformatics team to provide meaningful data from a minimum number of animals used per experiment. The design of individual experiments will generally involve factorial designs, which maximise the information obtained from the minimum resource.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

We strive to review and improve husbandry and procedures, which minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable.

We ensure we provide the appropriate anaesthetic and analgesic regimes as well as appropriate humane methods of culling within the animal facility. We ensure procedures are undertaken out of view from other mice. Transport of live mice between facilities will be in appropriate containers.

By propagating tumour cells obtained from transgenic models and re-implanting them into unmodified mice, we are more easily able to control, and minimise tumour burden.

Introduction of viruses carrying the genetic material necessary to induce tumour growth also allows us to minimise transgenic mouse breeding and better control tumour development.

Our imaging capabilities are continually improving, allowing us to use imaging not only to acquire valuable scientific data, but better monitor tumour growth and spread.

PROJECT 201. 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	Collateralisation of blood vessels
Key Words	Blood vessel, Diabetes, Vascular disease, Endothelial collateral
Expected duration of the project	5 year(s) 0 months

Purpose of 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):

People with diabetes are more likely to suffer from peripheral or coronary vascular disease (ischemia). These diseases occur because blood vessels get blocked. In people without diabetes new blood vessels can grow around the blockage, but diabetics are less able to grow new vessels around areas of blockage. We don't know why this happens in diabetes but there is evidence that is due to changes in the way that white blood cells make growth factors that stimulate vessel growth, and changes in the extracellular matrix proteins that help these vessel grow. After identifying some of the molecules that control this, and potential ways to reverse this in cultured cells, we aim to find out whether this can reverse this in mouse models of ischemia and diabetes. We will give agents (molecules, cells, antibodies) that may improve blood flow to the animals after they have undergone a surgical procedure to limit blood flow to their hindlimb.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Finding out what the changes are in diabetic patients that stop blood vessels growing in ischemia would help us develop new treatments for this, the major cause of amputations, and of heart attacks in diabetics. While we can get some of the data from studies on human cells and cells in culture, to test these ideas we need to show in an animal model where all the factors controlling vessel growth are present. If we can identify ways of increasing vessel growth then this could lead to new treatments for patients resulting in better quality and length of life.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use approximately 8 mice per week over five years (2000 mice).

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

We will use mice that have diabetes, pre-diabetes (metabolic syndrome) as well as non diabetics. Diabetes can be either spontaneously diabetic due to genetic mutation, or we can induce diabetes chemically. This will make the animals hyperuraemic (they urinate a lot), so bedding is frequently changed. We will give them tests that will tell us whether the diabetes is affecting their blood vessels - this includes measuring protein in their urine, and measuring their ability to detect a stimulus that they would try and avoid (e.g. a heat spot or pressure point). Both the urine test and the behaviour test are an indication of diabetic vascular disease. They will all undergo a surgical procedure where one of the arteries supplying the hindpaw is closed off, reducing blood flow to that paw, which makes them lame for a few days. As blood vessels grow round the arterial closure the blood flow to the paw recovers, and they start to walk normally again. We will measure blood flow to the paw five times over the following four weeks using a laser based camera. Most of the animals will be treated with compounds, cells or other agents that we have identified from non-animal experiments to have the potential to restore blood vessel growth and blood flow to the hindlimb. Some will be given placebo type agents or inactive molecules so that we can compare the effects with the active ones. They will have a general anaesthetic for this. Animals will be killed at the end of the experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Growth of blood vessels occurs in a complex environment depending on blood flow, the tissue through which it is growing and the immune system. These are not fully formed in non protected animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Experiments are designed to use sufficient animals to answer the questions we are posing and no more. We can reduce numbers by using non invasive imaging, so that each blood flow measurement is paired with its previous one, and we compare one paw to the other, non-ischemic paw.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

The vascular disease model in mice mimics human disease, both diabetic and ischemia. Blood vessels in mammals grow differently from non mammals due to the differences in the DNA. The mouse is used as it can be genetically or chemically manipulated or bred to be diabetic, or have molecules that will help or hinder blood vessel growth more easily than rats, rabbits or other animals. The minor blockage means there is low likelihood of damage to the paw, and we check the animals carefully for any adverse effects. Animals are given anaesthetics for surgery and blood flow measurement, and are given painkillers after surgery.

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	Functional and Genomic Studies of Limbal Stem Cells
Key Words	Stem Cells; Cornea; Gene Expression; Label-retaining 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):

1/ We will establish the gene expression profile of corneal stem cells compared to non-stem cells (basal and differentiated cells). To achieve this, slow-cycling stem cells and actively dividing cells are isolated from transgenic mice and the RNA is extracted from each population for sequencing to map their gene expression profiles. Elucidating the gene expression profile of slow-cycling corneal stem cells could reveal new stem cell markers to isolate and study in human stem cells for transplantation.

2/ Secondly, we will reveal the distribution of slow-cycling stem cells and their cell cycle rates after they are activated to renew differentiated cells removed by corneal scrape. This will help us to understand how slow-cycling stem cells repair corneal injury, as well as determining whether there is a hierarchical mode of turnover in the corneal epithelium.

3/ Our final aim is to create a new mouse model of limbal stem cell deficiency (LSCD), which is a form of corneal blindness that is currently difficult to treat, however, it can potentially be treated by stem cell therapies. This LSCD model is developed by physically removing the stem cells from a mouse’s eye. It is a more refined model than previous studies, where the entire limbus or cornea tissue are removed as opposed to specifically targeting stem cells.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

This work will improve our understanding of stem cell gene expression and the mechanisms by which they renew epithelial tissues over a lifetime. We also hope to develop a mouse model of corneal blindness for use in stem cell transplantation studies. This would allow us to move to cadaver human tissue to study stem cells and their efficacy as a stem cell therapy.

What types and approximate numbers of animals do you expect to use and over what period of time?

We expect to use approximately 650 mice over 5 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Mice will be anaesthetised before the surface of their eye is scraped with a scalpel to remove corneal stem cells through non-invasive surgery, and the clinical effects will be imaged under a microscope. We anticipate that removing these cells will lead to corneal opacity and neo-vascularization that can be treated, in the future, by stem cell engraftment. The most severe outcome will be partial corneal blindness in one eye of the mouse, however these mice are already blind from weaning age as they have an inherited mutation causing retinal degeneration. Because they are nocturnal, vision is not vital for mice as they primarily rely on hearing, olfaction and their whiskers to sense their environment, so there are not expected to be any lifestyle changes. Any ocular pain will be alleviated at the injury site by the use of general anaesthesia (i.e., isoflurane) and systemic analgesics will be used to prevent post-operative pain. Administration of DNA-labels such as EdU and BrdU will be limited to 7 injections over 7 days so that their toxicity limits are not exceeded. At the end of the experiment, mice will be killed by a schedule 1 method to study the limbus after stem cell removal.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

There is currently a complete absence of an antibody that is able to mark epithelial stem cells in mammalian tissue. H2B-GFP/K5tTA transgenic mice are the only available model to label epithelial stem cells based on the functional marker of stem cell quiescence. In this study, we aim to develop new markers that can be used to isolate stem cells from cadaver human tissue.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The gene expression and surgical experiments have been optimised at the REDACTED, which allowed us to determine the number of mice used in this project. To obtain enough RNA (approx. 1µg) to perform gene expression analysis of

epithelial stem cells, approximately 30 mice are required for each time-point of 28 days chase. An *a priori* power analysis based on corneal opacities up to 20% corneal opacity indicates that a minimum of 12 mice are required for surgical experimentation to statistically confirm the induction of LSCD.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

These transgenic mice are bred from the FVB strain, which are homozygous for the retinal degeneration 1 allele of Pde6brd1, which causes blindness by weaning age (21 days) but does not affect the corneal stem cells in this study. In general, mice are not considered visual animals as they are nocturnal and primarily rely on hearing, olfaction and their whiskers to sense their environment, therefore, corneal injury is not expected to significantly impact the lifestyle of the experimental mice.

Previous LSCD mammalian models have relied on complete excision of the peripheral corneal region or chemical burn of the entire cornea, which does not specifically target stem cells and damages the entire ocular surface. We aim to more specifically target stem cells through *in-vivo* fluorescence microscopy, so we can attempt to recapitulate LSCD and avoid any injury to important underlying or adjacent structures, such as the corneal stroma and endothelium, trabecular meshwork, or the conjunctiva, which are not implicated in LSCD.

All surgery will be performed on mice under general anaesthesia (e.g. isoflurane). Systemic analgesic and topical antibiotics recommended by the named veterinary surgeon will be used to prevent any discomfort or infection to the mouse ocular surface.

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	The biology and ecology of UK mammals
Key Words	mammals, Health, Physiology, Population 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);

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 overall aim of the programme is to investigate how environmental change impacts on the ecology, physiology and population dynamics of some large UK mammals. Information on their abundance, distribution, behaviour, fitness and health will be used to provide advice to government departments and conservation agencies on the management of populations.

There is a fundamental need to know how predators are likely to respond to rapid changes and the sort of ecosystem shifts that are now being observed. The dynamics and abundance of the two study species have changed dramatically in the last 15 years. Some have increased or begun to stabilise whereas others have experienced dramatic declines of up to 90% in some regions. The reasons for these changes remain unknown.

The work will thus focus on determining the mechanisms and processes that lead to the observed dynamics of the study populations around the UK. It is based on having a fundamental understanding of their biology and ecology and requires basic scientific underpinning studies which can provide data on how animals respond to change.

The work will comprise studies at different scales, of individual animals comprising colonies and populations. By combining information collected from different approaches at the individual level, population models will allow us to predict responses to environmental change and increased human activity (for example, investigating the effect of power installations, increases in noise, changes in contaminant and toxin inputs, changes in weather and climate patterns and interactions with industry).

One hypothesis for the decline in one species is an increase in competition for limited resources with another. Knowledge of how these two species use space is crucial to addressing this question. Sophisticated tracking devices will allow us to

investigate fine scale use of the ecosystem by both species. Detailed studies of how the two species use their breeding and resting sites, the factors affecting their choice of breeding colony and individual reproductive success will continue to be of critical importance to predict the impact of future environmental change.

Defining habitat requirements is a key component necessary for the conservation and management of the species. Both are species are listed under EU protection legislation where 'favourable conservation status' should be ensured by member states. Defining the requirements that allow animals to successfully feed, breed and rest will be used by policy makers to fulfil this conservation objective.

We also require detailed knowledge of the foraging behaviour of the animals including measurements of the effects of activity levels, season and development on energy requirements and foraging capabilities, and on how diet can vary.

The work focuses on the two species that are found in the UK. The results from these studies will be used to inform policy makers about the management and conservation of these species.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

This project is a response to the needs of government and conservation agencies to maintain a high level of expert knowledge about UK mammals. This need is driven by three main processes: (i) mammals are now being seen as increasingly important indicators/symbols of the state of our environment. Many are at the top of food chain and therefore experience outcomes and pollutant flows resulting from anthropogenic activities; (ii) some species are viewed by some important pressure groups as a pest species within the UK but, on a larger European scale, they are seen as rare species that require protection to conserve their critical habitat. This leads to conflicting interests in the debate about how best to manage populations and this debate needs to be informed by high quality information about the impact of the particular species upon the environment and the vulnerability of the animals to changes in that environment within the UK; (iii) mammals are potentially important parts of the structure of the marine and terrestrial ecosystems in the UK. They are clearly vulnerable to natural environmental and prey fluctuations and one species is declining rapidly in some parts of its range for as yet unknown reasons. The work in this study will allow us to assess the effects of disturbance and help to inform the development of management decisions aimed at improving animal welfare and conservation.

What types and approximate numbers of animals do you expect to use and over what period of time?

Typically a few hundred mammals be involved in this research each year.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Animals may be temporarily restrained in nets, anaesthetised if required then weighed and measured. Blood and tissue samples may be collected and tracking devices may be glued to their fur (these last only until the next moult when the devices fall off). Some animals will be exposed to noise or other stimuli to assess their effects. All animals are released to the wild.

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 study the behaviour and physiology of the animals directly because there are no alternative approaches that will allow us to answer questions about human impacts on populations and how they are likely to be affected by increasing pressure on the ecosystem. These studies will be carried out in conjunction with sophisticated statistical and mathematical modelling of populations, dynamics and movements so we can predict how their distribution and abundance may change in future. We will also continue to develop appropriate *in vitro* approaches so that, for example, the effects of particular contaminants and toxins on cells may be assessed.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The use of robust statistical calculations for each stage of the project will ensure the minimum number of animals is used whilst making sure the scientific questions can be answered with enough power to detect any change.

Refinement

Explain the choice of animals and why the animal model(s) you will use are 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 mammals are chosen due to UK and European conservation legislative drivers. All procedures carried out constitute current best practice, improved with veterinary advice. This process continues by review and refinement among licence

holders, ensuring standards continue to improve. Animals may be anaesthetised to reduce stress and handling times are kept to a minimum.