Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 27

Projects with a primary purpose of: Basic Research – Multisystemic

Project Titles and keywords

1. **Molecular basis of vertebrate development**
    - Mouse, DNA binding proteins, signalling pathway, knockout, transgenic

2. **Genetics of behaviour and stress response in fish**
    - Genetics, stress, evolution, fish, behaviour

3. **Brain mechanisms regulating cardiovascular homeostasis in health and disease**
    - Brain, mechanisms, regulating, cardiovascular homeostasis, health, disease

4. **Breeding, freezing and recovering genetically modified mice**
    - Breeding, freezing, genetically modified, mice

5. **Mechanisms of stem cell differentiation in fish**
    - Transgenic fish, embryo development, gene function

6. **Pre-treatment and monitoring of the organ donor to improve graft function for transplantation**
    - Donation, Transplant, Kidney, Liver, Ischemia

7. **In vivo studies of ADP-ribosylation signalling**
    - ADP-ribosylation, post-translational modification (PTM)

8. **Health of aged cloned sheep**
    - Health, aged, cloned, sheep

9. **Functional analysis of factors implicated in X chromosome inactivation**
    - Gene regulation, X chromosome, chromatin

10. **Pathways of gut health and inflammation**
    - Gut, ageing, inflammation, microorganisms

11. **Organ dysfunction following acute illness**
    - Intensive Care, Sepsis, Organ Failure

12. **DNA Strand Breaks, DNA damage responses, and Disease**
    - DNA damage, Neurodegeneration, Cancer

13. **A study of changes which occur during ageing**
    - healthy ageing connective tissue crosslinks

14. **Models of viral hepatitis**
    - Hepatitis; virus; treatment

15. **Oxidative stress and aging**
    - Oxidative stress; degenerative disease; aging

16. **Mouse membrane traffic mutants**
    - Basic science, human disease

17. **Genetic Analysis of Stem Cells Using the Mouse**
    - Stem cells, mouse, genetics

18. **Breeding and Production of Genetically Altered Mice**
    - Genetic alteration, mouse, breeding

19. **Investigation of the in vivo action of G protein coupled receptors**
    - Neurodegeneration, physiology, drugs, cancer, diabetes

20. **Cell signalling in neural and cardiovascular development and regeneration**
    - blood vessel growth, nerve regeneration

21. **Study of Infection and Antibiotic Resistance**
    - Antibiotics, bacteria, resistance

22. **Regulation of mammalian Hox genes**
    - Mouse, Hox, gene regulation, enhancer elements

23. **DNA damage response involvement in genome stability and cancer**
    - DNA damage, genome stability, cancer

24. **Molecular regulation of development**
    - Development, zebrafish, embryo, mechanism

25. **Investigating the pharmacology of traditional and nanoformulated medicines**
    - Pharmacokinetics, nanoformulation, bioavailability, tissue distribution

26. **Utilising MRI to probe physiological state**
    - MRI, contrast agent, diagnosis, disease

27. **Podoplanin-CLEC-2 in Sepsis and haemolytic uremic syndrome**
    - CLEC-2, Podoplanin, sepsis, HUS

28. **Fungal infection, diagnosis and therapy**
    - Fungus, infection, antifungal, therapy, diagnosis

29. **Nuclear reprogramming by Xenopus eggs and oocytes**
    - nucleus, reprogramming, Xenopus

30. **Complex mammalian responses to alkylating agents**
    - DNA damage, DNA repair, metabolism, alkylation

31. **Polyclonal antibody development**
    - Polyclonal, Antibody, Immunisation, Rabbit, Sheep

32. **Mechanism underlying diet induced metabolic disorders**
    - High Fructose, high fat, sucrose, ketohexokinase, NAFLD

33. **Pathophysiology of the unfolded protein response**
    - Protein misfolding, Endoplasmic reticulum, Unfolded protein response Protein Chaperones, Diseases of aging

34. **The immunobiology of mycobacterial infection**
    - Tuberculosis, Mycobacterium, vaccines, chemotherapy

35. **Understanding how parasitic worms (roundworms) have evolved and developing new drugs to treat diseases caused by them**
    - Parasite, Trichinella, novel drugs, anthelmintics

36. **Immune regulation of tissue repair in ageing**
    - Immunity, senescence, ageing

37. **Control of Central Nervous System Autoimmunity**
    - Multiple sclerosis; neuroprotection; repair, autoimmunity; spasticity

38. **Studying macrophage plasticity in inflammatory disease**
    - Macrophages, inflammatory disease, genomics/genetics

39. **Regulation of cell polarity in vertebrate development**
    - Embryo, development, cells, regenerative medicine

40. **Treatment of abnormal retinal development**
    - Mice, Albinism, L-DOPA, Treatment, Vision

41. **Blocking senescence in ageing**
    - Ageing

Project 1	**Molecular basis of vertebrate development**	
Key Words (max. 5 words)	Mouse, DNA binding proteins, signalling pathway, knockout, transgenic	
Expected duration of the project (yrs)	Five Years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	During embryonic development in mammals including mice and humans, a single cell, the fertilized egg undergoes an ordered series of changes to give rise to a complete functional animal. The aim of the research in my laboratory is to understand how these processes are controlled and regulated so precisely and how stem cells and their specialised progeny are kept in balance to maintain a functional embryo and then an adult organism. Our main objectives in this PPL are the generation, breeding and analysis of genetically altered mice in which the above processes are perturbed. We wish to understand how stem cells are maintained and regulated in the context of embryonic development and organ formation as well as in adult organisms. These mice will help us understand how genes regulate cell division, shape and function of developing and adult organs	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The findings from our research will provide information on how stem cells are maintained in organs such as brain and intestine. Also this information will have applications in developing better methods of cell reprogramming, tissue engineering and repair as well as increase our understanding of	

	cancers.
What species and approximate numbers of animals do you expect to use over what period of time?	We use mice for our regulated procedures. Over the five year period, the projected use of mice based on power calculations is 20,175. This also includes normal mice produced as a consequence of breeding mouse lines many of which are conditional genetically altered mice as these minimise welfare costs. We believe given our approaches for reduction and refinement, we will work below these numbers.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the procedures we use are of mild to moderate severity. The knockout of most of the genes produce effects that are also mild to moderate. We have two KO mouse strains which show embryonic lethality in the homozygous state and we circumvent this in one case by a conditional KO. For our double mutants and newer KOs we are unable to predict the phenotypes at this moment in time as these experiments will commence only when grants are in place for this work. The level of severity will be assessed in consultation with the Home Office Inspector as and when the mouse KO strains are derived.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Organs are not homogenous populations of identical cells but are heterogeneous and have a 3-D organization which depends on complex cell interactions. Hence, any analysis of the co-ordinated regulation of proliferation, differentiation and patterning in any organ needs to be carried out in developing embryos. This is because such processes occur within a defined anatomical three dimensional tissue architecture containing the multiple cell types. Similarly, in order to study stem cells in situ and tissue homeostasis, an animal model is most appropriate. For the above reasons, tissue cultures although excellent to carry out biochemical and molecular biological characterisations of the functions of genes and genetic pathways are not entirely appropriate for our studies. Wherever possible, for evaluation of vectors and preliminary studies, we already make use of tissue culture models. We have also been developing 3-D organoid models for carrying out biochemical studies using human induced pluripotent stem cells or mutant mouse ES cells (funded by the NC3Rs and BRACE). I recently held a project grant (2009-2012) from the NC3R's for developing patient specific induced pluripotent stem

	cells as a disease model to reduce and replace animal models of motor neuron disease. At present there are no appropriate alternatives to mice in order to answer questions related to stem cell biology and mammalian development, but we use early zebrafish embryos to evaluate gene constructs to reduce mouse numbers.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Initial evaluation of vectors and constructs will be carried out in ES cell cultures. Wherever possible for biochemical studies we will establish cell lines and use these. Breeding colony size is also generally kept small following very good colony management strategies. Generally the breeding nucleus consists of one to two breeding pairs.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the animal of choice since it is a mammal and its genetic makeup is very similar to humans. Sequencing of the entire mouse and human genome has been done and the robust genetic information shows shared pathways and regulatory mechanisms of development and stem cell biology. The other advantages are (1) the mouse has a relatively short lifespan (2) short gestation time, (3) produces sufficient number of embryos/offspring per litter (3) can be maintained in the laboratory with relative ease (4) genetically well characterised strains of mice are available for experimentation which help in generating data that are not affected by genetic variations (5) efficient and reproducible technologies (which are constantly being refined) are available for producing genetically altered mice. Many of the genetically altered mouse strains we have generated, are based on refined approaches i.e. conditionally ablating/altering the function of a gene which can be specifically induced in a particular cell type or organ and /or at a specific developmental/adult stage, thereby leading to less severe effects. This is because the effects are not manifested until the genetic alteration is induced at a particular time in embryonic development/ in the adult animal or only in a specific target tissue rather that in every cell in the body. Thus, the phenotypes are moderate thereby minimising welfare costs.

<table>
<tr><td>Project 2</td><td>Genetics of behaviour and stress response in fish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genetics, stress, evolution, fish, behaviour</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
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<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>In biology, stress is often a poorly defined concept, and one that is negatively associated with health in humans and animals. However, stress responses actually play an important positive role in maintaining viability and health. When challenged by a threat in the environment – perhaps a predator, disturbance, or adverse conditions – a set of neuroendocrine pathways trigger physiological and behavioural responses (e.g. fight or flight behaviours) that have evolved under natural selection to counter the threat. Nonetheless, while these acute stress responses are thought to be adaptive, it is also well documented that chronic stress exposure can reduce the health of individuals and sometimes – particularly when mothers are exposed - their offspring. Because chronic stress responses, also called “tertiary stress responses” (TSR) are typically bad for fitness (survival and/or reproduction), natural selection should act over evolutionary time to get rid of them. The fact that the TSR is widespread, being found in vertebrates ranging from fish to humans, therefore poses an important question– what constrains evolution of the stress response towards a state</td></tr>
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	where these harmful, or maladaptive, effects do not occur? The goal of the proposed work is to answer this question by conducting genetic studies. We will use small fishes, and primarily the guppy (*Poecilia reticulata*) as a model system. Practically, the work will involve experimentally manipulating stressors in the environment, determining how different individuals and genotypes respond through behavioural and hormonal processes, and determining the long term consequences of this variation for fitness. In particular we will test two hypotheses about where the evolutionary constraint comes from that maintains the TSR. The first possibility is a trade-off between the effects of acute and chronic stress on fitness. In simple terms, genes that cause the TSR may persist in a population precisely because they are the ones that lead to the most appropriate acute stress responses. A second possibility is that, where mothers experience chronic stress, a trade-off occurs across the generations. Here, some maternal genotypes are better able than others to maintain the mother's own health, but do so at a cost to offspring (e.g. by reducing the amount of care she provides).
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In the short term the principal outputs of the research will be academic, benefiting the broad set of biologists working from an evolutionary perspective across disciplines and levels of biological organisation. This is because the project will tackle fundamental scientific questions about the nature of evolutionary constraint, a phenomenon that is far wider in scope than the specific context of understanding maladaptive stress responses. However, the work will also provide us with a better understanding of the genetics of chronic stress response. In turn this should yield tangible benefits for improving welfare in captive animals. The presence of genetic variance for the tertiary (chronic) stress response would open up the possibility of using artificial selection as a tool to improve animal welfare in captive populations (e.g. livestock, aquaculture, scientific research). Understanding the genetic architecture of the stress response, its performance consequences under

	chronic stress exposure, and identifying biomarkers for its efficient characterisation is a necessary first step towards this.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years we expect to use approximately: -4000 guppies -1000 swordtails -2000 zebrafish -1000 sticklebacks
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Any adverse effects are likely to be mild. Fish will be subject to tagging for identification (and in some cases fin clipping to obtain samples for DNA analysis). These procedures, which will be conducted under anaesthetic, are routine in fish studies and complications (e.g., unsuccessful recovery from anaesthesia) are rare. Data on behaviour, size/growth, maturation time, hormone levels, metabolic rate and longevity will all be collected using completely non-invasive methods the severity of which is sub-threshold. A subset of animals (up to approximately 2000) will be subjected to a chronic stress treatment that is expected to induce reduced growth (or weight loss) and reduced reproductive output. Since longevity and reproductive performance are traits of interest in this study we expect most animals subject to licensed procedures (e.g. tagging under anaesthetic) to remain “on license” for the duration of their lives. Euthanasia criteria (agreed with the named veterinary surgeon) are in place to deal with any diseased, injured or senescent fish. Where fish are not required for further breeding or data collection they will be euthanized by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no non-sentient alternatives that could be used and in vitro approaches are not applicable to organism level questions. The study aims to test evolutionary theory about the pathways linking animal behaviour, physiology, health and the environment (the source of stress) in vertebrates. It is only possible to do this using a vertebrate model.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Studying the evolutionary genetics of traits in wild-type populations generally requires large sample sizes and our study is no exception. Experiments have been designed using computer simulations to determine the minimum sample sizes that will allow powerful testing of our hypotheses. In doing this we have also ensured that the statistical methods we will use to analyse the data are the most powerful ones available. To reduce animal numbers further we will not address all objectives in all of the study species. Rather guppies will be used to test all our key hypotheses, while other species will be used for smaller parallel studies where they add particular relevance. For example, by verifying our findings in zebrafish we can assess the potential for genetic improvement to improve welfare in captive populations of this widely used scientific model.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The fish models to be used are exceptionally robust, easy to care for, and highly fecund under laboratory conditions. In all these regards they are more suitable than other possible vertebrate models (e.g., mice, rats). All procedures to be applied are mild or sub-threshold. There are no alternative procedures of lower severity. General welfare will be ensured by maintaining housing conditions and husbandry standards (e.g. daily inspections, frequent water changes, a robust program of water quality testing) that meet or exceed all HO requirements.

Project 3	**Brain mechanisms regulating cardiovascular homeostasis in health and disease**	
Key Words (max. 5 words)	Brain, mechanisms, regulating, cardiovascular homeostasis, health, disease	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Despite the prevalence and menace of high blood pressure, little is known about the fundamental mechanisms of its aetiology, although it is well known that certain environmental conditions, nutritional choices, life events and lifestyles, acting on a genetic substrate, can contribute to the development of hypertension. In order to address the gaps in knowledge revealed by this unmet medical need, we have studied the role of the brain in the regulation of the cardiovascular system. We hypothesise that the development and/or maintenance of cardiovascular disease is associated with changes in the expression of a network of a large number of genes in the brain that, through their activity, form a functional network and that are influenced by lifestyle choices, environmental stimuli as well as age and sex. We will identify these genes, and determine their 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)?	In the short-term, the proposed studies will generate important basic knowledge about the way that the brain regulates the cardiovascular system, and how these mechanisms can go wrong in high blood pressure. In the long term, we anticipate that these studies will provide essential information necessary for the development of innovative therapeutic approaches (drugs and gene therapy) for cardiovascular diseases (particularly hypertension).	

What species and approximate numbers of animals do you expect to use over what period of time?	Rats. Maximum 5500 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects will be at most moderate, and mainly related to possible, but rare, side effects of surgery. Typically this will involve post-operative pain, which will be controlled by analgesics. Very rarely, an animal will experience more severe consequences, such as haemorrhage or infection. Whilst the latter can be controlled using antibiotics, if any animal deteriorates as a consequence of a procedure (eg. failure of normal eating, drinking or grooming, signs of pain, obvious weight loss, hunched posture, behavioural changes such as lethargy or aggression) it will be humanely killed using a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are necessary because we propose to investigate the workings of the whole animal, specifically how the brain controls the cardiovascular system, and how this goes wrong in high blood pressure. Non-animal alternatives do not exist. Cell lines corresponding to adult neurons do not exist and currently available in vitro techniques for the study of the brain, and its interactions with the body, are deficient and are of dubious physiological relevance. Further, these techniques usually require the use of use cells and tissues taken from juvenile animals, thus demanding the sacrifice of large numbers of rats.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Strenuous efforts will made to reduce the total number of animals used and to reduce the number of *in vivo* experiments. The use of highly specific techniques that allow genetic changes to be introduced into the brains of adult animals, instead of traditional techniques in which the genetic change is present in every cell of the developing animal, is more precise, less detrimental and reduces animal use. *Note that our strategy allows an animal to serve as its own control.* For example an animal can be physiologically assessed to obtain basal data, then subjected to manipulation of gene activity *in vivo*, followed by further assessment to obtain the experimental data. We will use the minimum number of animals required to give statistically valid data.
3. Refinement Explain the choice of species	We will use rats in our studies. The rat is the species of choice for studies in neuroscience and physiology. The anatomy of the rat brain is well mapped, and the

and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	structure, function and regulation of the rat brain have been the subject of detailed study for many years. The large size of the rat makes it easily accessible for a whole range of physiological measurement and intervention, but its reproductive capacity and gestation time are equivalent to the mouse. The availability of the Spontaneously Hypertensive Rat is extremely important to us as an accepted and well-studies model of human hypertension. Further, we have adopted modern methods of measuring blood pressure using radio-transmitters. This avoids the need for arterial catheters, which present a potential for discomfort and infection to the animal.

Project 4	**Breeding, freezing and recovering genetically modified mice**	
Key Words (max. 5 words)	Breeding, freezing, genetically modified, mice	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The publication of the human and mouse DNA (a molecule the makes up genes) sequences, coupled with the availability of an array sophisticate tools make it possible to alter any specific gene of interest. This in turn has led to the potential to understand the function of every gene in the body. Understandably, investigators want to tap into this potential and they like to use the mouse as a model for understanding gene function in man. Investigators also like to be able to exchange the mouse lines they work on with other colleagues. Freezing embryos and sperm ensure unique mouse strains can be made available to future generations of scientists This project has been designed to provide an efficient service for the breeding, freezing (embryos/sperm) and recovery of genetically altered strains of mice. These strains will be transferred to projects that have been set up to investigate specific aspects of mammalian biology. By providing a central service we will be able to invest in the most up to date equipment/techniques and get unique mouse models of human disease to investigators quickly. By employing highly trained staff who can maintain their proficiency in breeding and cryopreservation techniques and we will be able to provide an efficient service to the scientific community. In doing so we	

	will help to reduce animal numbers and minimise the impact on 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)?	This project will benefit science through the rearing and distribution of genetically altered mouse strains that can be used to better understand how genes control all aspects of mammalian biology. In particular, this project will help scientist around the world study new models of human (and animal) disease which will lead to better treatments and reduced human and animal suffering.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will last for 5 years and will only use mice. It is expected that up to 225,000 animals will be used to support the scientific projects that are serviced by this licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The techniques used in this project have been designed to minimise suffering and animal numbers. The techniques are all well established and the incidence of adverse effects is known to be low. Each animal line is examined comprehensively throughout its life-span for indications of ill health. If a mouse is exhibiting detrimental signs (such as weight loss) then the mouse will be put down. No animal will be kept in a prolonged state of suffering. Embryos will be transferred using an operation that will be performed under general anaesthetic and pain relief will be given. Ear clips will be taken in order to confirm which mice carry the gene of interest. This procedure is only associated with momentary discomfort. Similarly, embryo production will be facilitated by injecting hormones which is a technique that also only induces momentary discomfort. When mice are mated, care will be taken to ensure that the mice are mature enough to mate. Any over vigorous males will be removed from the mating. The overall severity limit of this project is expected to be moderate. Mice used in this project will either be transferred to another project for further study or humanely culled
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Model organisms are the key to working out the function of genes and proteins. We are now able to manipulate genes using genetic engineering and investigate the consequences for the whole animal. Animal models, such as the mouse, present scientists

	with a unique opportunity to uncover the function of genes and the genetics of disease. Although we will be able to cross reference existing databases and cell culture systems, non-animal models cannot be used for this project because we need to know how particular genes affect complex organs like the heart, liver & brain. At the present time there are no cell culture systems available that can provide these results. However, it is hoped that this project may offer the material that will support future developments in research by providing tissue for the development of cell lines.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before starting any study in this project, data will be collected from any previous relevant studies and statistical analysis used to make accurate predictions of how many animals we will need to produce a decisive scientific result. In order to keep the number of animals to a minimum, only mice required for such studies will be bred. The efficiencies of all techniques used in this project will be subjected to regular audits to ensure consistently good results whilst striving for improvements. The idea behind providing a breeding, cryopreservation and distribution service is to establish a pool of skilled people with expertise in core activities so that they remain proficient. Thus keeping animal usage to a minimum at all times. New mouse strains used within this project will be cryopreserved. This eliminates the need to continually breed genetically altered mice when they are not needed in active research programs. Freezing embryos and sperm also preserves these unique strains of mice for future research.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the most appropriate animal model for this project because our intended aim is to work out the function of all mammalian genes and proteins. Animal models are important because we are able to manipulate their genes using genetic engineering and investigate the consequences for the organism. The mouse occupies a unique position in determining gene function and the genetics of disease for a number of reasons. Firstly, as a mammal it demonstrates a remarkably similar development,

	physiology and biochemistry to the human. Secondly, mouse geneticists have developed a very extensive genetic toolkit that enables very specific alteration of genes in the mouse. Thirdly, we now know the complete sequence of all the DNA the mouse carries. We will minimise the welfare costs to the animals by using the minimum number of animals at all times. We will constantly reviewing the techniques we use and introduce new refinements at the earliest opportunity.

Project 5	**Mechanisms of stem cell differentiation in fish**	
Key Words (max. 5 words)	Transgenic fish, embryo development, gene function	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	After fertilisation of eggs and sperms, single fertilised cells will divide and generate many cells in the new life (embryo). These cells are called stem cells. The stem cells eventually change their character and differentiate into all tissues forming the adult body. This event is regulated by many genes. However, genes sometimes mutate and become malfunctional, causing genetic diseases in humans as well as in fish. Since fish and humans are both vertebrates and have very similar gene functions, studying gene function in fish is very useful for understanding gene function in the human embryo. We aim to understand the gene functions that regulate stem cell development in the embryo. By knowing more about the gene function, we will be able to diagnose diseases in fish as well as in humans. The diseases that we study in this project include a variety of birth defects caused by abnormal development of embryos such as microcephaly, cyclopia, spinal bifida, limb defects and dwarfism. In future, finding obtained from this project and similar projects from other laboratories would contribute in developing drugs to cure diseases, and in generating healthy cells and tissues in the laboratory for therapeutic purposes.	
What are the potential benefits likely to derive from this	Our project would discover novel genes and function of these genes that are crucial for normal embryonic	

project (how science could be advanced or humans or animals could benefit from the project)?	development and regeneration of tissues. Mutations in these genes cause abnormalities in the embryo development in fish and are predicted to cause birth defects in human. By leaning such gene sequence and function, we will be able to diagnose cause of the defects. The fish embryo is also an excellent tool for testing novel drugs and supplemental nutrients. We can screen and test novel drugs that can reduce the abnormality of embryonic development in fish. Such drugs would be useful for treating birth defects.
What species and approximate numbers of animals do you expect to use over what period of time?	The species used will be zebrafish, medaka, mangrove killifish, Arabian killifish, brown knifefish, brown ghost knifefish; 2000 fish including larvae, juveniles and adults may be used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The fish larvae will be injected with genetically modified spermatogonia cells (stem cells which will develop to sperm). Recovery of larvae from injection is rapid (within 5 min) and no obvious adverse effects are known. The larvae will be raised to adult and genetically modified eggs. These eggs develop to embryos which exhibit some abnormalities (e.g. short tail, short fin, small tail). These embryos can be used as a model for studying human genetic diseases. When the fish become old and fecundity is reduced, the fish will be euthanized. Most of embryos showing abnormalities will be terminated at embryonic period and therefore causes no adverse effects. However in some occasion, when the abnormality is mild (e.g. short tail, short fin, small tail), we will carefully raise the fish to adult. Such variation of the body pattern is often seen in different strains of god fish. Though their body pattern is different, they are healthy fish and can feed, swim and mate to raise the new generation. Therefore we consider the adverse effect is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Genetically modified fish are a very important system to monitor tissue responses to stress, disease, injury and environmental changes. Such live animal analyses are essential for studying gene function, diagnosing disease and testing therapeutic methods because live organisms are very complicated systems and tissue responses are very different from non-animal based experimental systems. Fish organs are very similar in most of tissues (e.g. spinal cord, skeletal muscle, blood vessel, central and peripheral nervous systems) to their human counterparts and

	therefore the fish provides an excellent model for learning about human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We aim to develop a novel method to modify gene function in the fish (called “transgenic technology”). Using this method, we will use testis cells called spermatogonia (SG) cells for making genetically modified fish instead of using the live embryos that are currently used as conventional method. By using SG cells instead of embryos/larvae our method could substantially reduce the usage of animals in future for (type of) research. For instance, in the conventional transgenic technology, about 400 fertilised eggs will be injected with DNA. These eggs will be raised to adults and tested if some of them became genetically modified fish. However in our novel method using SG cells, we will create genetically modified SG cells in a culture dish and inject the cells into less than 100 larvae which would become a genetically modified fish. This means we would reduce the usage of fish up to 4 fold and still be able to create genetically modified animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	To develop a new method to create genetically modified fish species, different fish species are required to test the technique. Firstly, we need to apply the technique to the most popular model fish including zebrafish and medaka. Genetic information and manipulation techniques available for these species, therefore the development of a new technique would be most easily achievable with these animals. Mangrove killifish will be used because they are a self-fertilising animal, and therefore the maintenance and generation of genetically modified animals would become even easier. Arabian killifish will be used because the embryo is extremely transparent and suitable for microscopic analyses. Using these fish larvae, SG cells will be injected into the body cavity to create genetically modified fish. This process is highly established technique. However it is certainly necessary for researchers to develop highly sophisticated skills of injection using microscope. To train the researchers who are involved in the project, they will practice the injection and microscopic manipulation using fish eggs first to develop skilled researchers before conducting the experiments. Brown and brown ghost knifefish will be used because the knifefish is well known as an excellent

	model fish for studying the tail regeneration process. The previous researchers have been using tail for studying regeneration because it is a relatively small tissue in the body and therefore small resection does not cause damage in the health of the body. In the knifefish, the tip of tail is very thin and does not contain any fin, therefore resection of the tail does not create large scar. It even does not breed. With such small resection, we can still study the whole process of tissue regeneration without compromising health of the animal.

Project 6	**Pre-treatment and monitoring of the organ donor to improve graft function for transplantation**	
Key Words (max. 5 words)	Donation, Transplant, Kidney, Liver, Ischemia	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this project is to: - Determine how organs (the kidney and liver) become injured during the donation process. - How we can intervene in the donor or during organ storage to protect and preserve these donor organs By achieving these aims we will increase the number of organs available 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 main benefits will be for patients of end stage kidney or liver disease who require a transplant. The major barrier to transplantation is the lack of good quality organs. This project aims to increase our knowledge about how organs become injured during donation and evaluate what can be done to improve the outcomes. We focus on trying to manipulate pathways concerning hypoxia and metabolism in the donor and during organ preservation once the organs are explanted.	
What species and approximate numbers of animals do you expect to use	We will be using mice and rats for the proposed experiments, mimicking donation scenarios and transplanting organs. We estimate that we will use	

over what period of time?	under 100 rats/mice per year over a 5-year period. In our previous licence (over a 5 year period) we used less than 100 rats/mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Some of the experiments will require altering the diet of the rodents before they may be subject to procedures. We expect this to have minimal impact on the rodent, but they will be monitored closely for any adverse effects. The donation experiments (living (LD), brain dead (DBD) and donation after circulatory death (DCD)). are performed under terminal anaesthesia, we do not anticipate any adverse events based on our previous knowledge and work carried out using these models. The kidney ischaemia and transplantation models are recovery experiments, pain and organ failure are possible adverse events. With the liver ischaemia reperfusion model fulminant liver failure is not expected as part of the liver is clamped (not the whole organ). We will monitor the rats and mice closely for these adverse events and where unnecessary pain or adverse events are detected, contact vets and when deemed appropriate, experiments will be terminated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The donation and transplantation processes are physiological responses to disturbances in a number of different systems including immunological, hormonal, blood clotting etc. Mimicking these complex physiological processes in non-animal alternatives is not possible. For example after brain death we see activation of systemic inflammation and in addition alterations in metabolism and also a pro-coagulatory state. Producing this in cell culture models is simply not possible. The use of animal models allows us to observe the effects in a highly controlled in-vivo model. Where it is possible to infer data from cell culture models e.g. dose finding experiments, we will perform this.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have involved statisticians in the development and design of studies to ensure that minimal numbers of animals will be used. We discuss experimental plans with the department and other researchers to ensure the experimental design is well thought

	through. Transplantation experiments will only be performed with interventions having shown promise in the ischemic or donation models.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats and mice have previously been used as models of organ donation. Rodents also allow for the genetic determination of the importance of a pathway (e.g. knock out mice). We have previously experience with using these models of donation, ischemic models and transplantation. We have developed highly refined protocols for the use of rodents in experiments which have been developed in Oxford in conjunction with external collaborators from the University of Groningen. Animals will be not kept for extended periods. Following arrival to the animal facility, animals will be allowed to acclimatise for 1-2 weeks prior to experimentation. It is estimated that all experiments will be completed within 1 month of the arrival of the rodents to the animal facility. After exposure to ischemia reperfusion injury or transplantation rodents will be kept alive for a maximum of 5 days. Animals will receive analgesia to control pain following surgery. Animals will be housed in cages with free access to food and water in accordance with Home Office regulations. Humane end points for the experiments have been described as in the protocols.

Project 7	**In vivo studies of ADP-ribosylation signalling**	
Key Words (max. 5 words)	ADP-ribosylation, post-translational modification (PTM)	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	No	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	Yes	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Proteins perform the vast majority of biological functions within a cell and indeed in a living organism. They can act as enzymes to catalyse reactions, as scaffolds to attract and bind other proteins, as transporters and can replicate DNA. Proteins are what genes in DNA code for and their disruption and/or deregulation is implicated in a whole plethora of human disease, including diabetes, cancer, neurodegenerative and autoimmune disorders. Importantly the control of proteins occurs at many levels, including before (transcriptional regulation) and after (post-translational modification – PTM) a protein has been made. We are primarily concerned with the PTM ADP-ribosylation and its role in controlling cellular processes in living animals (*in vivo*). ADP-ribosylation is the process whereby one or more ADP-ribose molecules are added to a protein. ADP-ribosylation is a fully reversible PTM and addition/removal of ADP-ribose chains or single modules is speculated to regulate the activity and/or subcellular localisation of proteins. Indeed proteins involved in ADP-ribosylation metabolism already have a well characterised role in response to DNA damage. However recent evidence suggests the	

	roles for ADP-ribosylation proteins are far more diverse, playing a role in neurobiology, metabolism, cell replication, intracellular transport and transcriptional regulation. In addition, the recent identification of novel proteins in these pathways with as of yet undefined roles *in vivo* represents a substantial gap in our knowledge which requires further investigation. Therefore, we aim to characterise novel genetically modified (GM) animals (both mice and zebrafish) with mutations in important genes involved in protein ADP-ribosylation metabolism in order to deepen our understanding of this important PTM. We plan do this by analysing GM animal behaviour, metabolism and response to DNA damaging 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)?	This research will contribute to our understanding of the PTM ADP-ribosylation and address fundamental gaps in our knowledge with regards to *in vivo* functionality of these proteins. For example; it is known that deficiency of some ADP-ribose proteins causes the development of rare progressive and ultimately fatal neurodegenerative diseases – a mouse or zebrafish model to help study the mechanism of pathogenesis will help those families affected. Additionally, ADP-ribosylation is known to play a role in energy metabolism and therefore study of the GM animals in this project may also shed light on metabolic disorders such as diabetes and obesity. Also, targeting the proteins involved in pathways regulated by ADP-ribosylation by small molecule inhibitors represents a very promising strategy in cancer treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: 4,000 per year Zebrafish: 6,400 per year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Due to the unpredictable nature of the genetic knockout studies we aim to carry out (i.e. deletion of genes) we may produce animals which are inherently ill/abnormal in some way. The most likely severe side effect might be the development of a progressive neurodegenerative disease from which the animal may not recover. In order to safeguard against unnecessary suffering incurred by such animals the

	breeding of these animals is a regulated procedure with humane end points outlined within the project licence therefore any animal which shows any deviation from normal health and wellbeing will immediately be examined by a vet the same day, given treatment if appropriate or euthanised. Another moderate source of harms within this project is anxiety created by the adversely motivated behavioural tests. In this project the most aversive stimuli is loud noise and bright light which should not cause more than a transient moderate unease in mice. We also plan to examine the DNA repair pathways in animals by administrating DNA damaging agents (for example by radiation exposure and chemical induced damage). The commonest side effects of which are leukopenia (loss of white blood cells) and gastrointestinal syndrome (causes anorexia, diarrhoea and loss of fluid). However since these adverse effects occur several days after the administration of agents we will avoid these syndromes by keeping the time points short (typically 4 hours). In rare cases where we might want to follow up treatment for longer periods animals will be monitored closely and any animal showing the predictable signs of gastrointestinal syndrome will be humanely killed – in strict adherence of the humane end-points. Following a procedure, animals may enter into another protocol under continued use or will be humanely killed using an appropriate method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible we always use non-animal alternatives, however some important, basic biology questions can only be answered using whole organisms – such as, is gene X *essential* for life? Does gene X have a role in behaviour? Does gene X affect metabolism and/or play a role in DNA repair pathways? These questions can only be answered using animals since no cell culture nor computer technology is currently sophisticated enough to replace the complexity of a whole living organism.
2. Reduction	

Explain how you will assure the use of minimum numbers of animals	When it is not possible to replace the animals in our research we will still rely extensively on non-animal alternatives to reduce the total animals required and to guide our research. Prior to conducting an experiment, the minimum number of animals will be calculated based on a combination of the latest published methods and in consultation with a statistician. We will continually analyse our data using appropriate statistical methods (such as power analysis) to ensure we always maintain a rigorous approach to experimental planning.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice share similar genetics, metabolism and physiology with humans and this combined with their short generation time make them the animal model of choice for a number of different areas of biomedical research, including the genetic studies proposed here. In addition zebrafish, although non-mammalian, are more closely related to humans than invertebrate models (such as flies or worms). Importantly; during their embryonic phase of development zebrafish are transparent therefore allowing greater opportunity to study early development (compared with mice). We will constantly check the latest methods for further refinements/improvements as well be in close communication with other researchers using the same techniques to be aware of any potential adverse effects. Regardless of whether under a procedure or not all our animals will be examined daily by trained animal technicians (possibly by use of welfare scoring sheets if appropriate) and any animal displaying signs of stress or suffering such as lethargy or hunched appearance etc. will require immediate veterinary attention. Humane end points will be adhered to strictly and any animal which is in pain or discomfort that cannot be alleviated by simple treatment will be euthanised.

Project 8	**Health of aged cloned sheep**	
Key Words (max. 5 words)	Health, aged, cloned, sheep	
Expected duration of the project (yrs)	3	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	No	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to continue to monitor the health of 13 cloned sheep up to but not exceeding the age of 10 years. The purpose is to generate sufficient detailed and quantitative information on the health of aged cloned offspring as to inform policy makers and the general public of the safety of cloning.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We will be able to describe in detail the nature, extent and progression of aged related non-communicable diseases such as metabolic disease, heart disease and arthritis in cloned sheep, and determine if this differs from how these diseases develop with age in non-cloned sheep.	
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep (13)	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	These animals will be maintained and used in moderate health assessment procedures for up to three years. No adverse effects are anticipated. Any animal showing adverse clinical signs (most likely associated with arthritis and their advanced age) will be given appropriate veterinary and husbandry treatment. If the animal fails to respond	

	promptly and effectively, it will be humanely euthanized by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project is specifically interested in the long-term health of the 13 cloned animals in question. We have access to similar detailed health records for a contemporary group of aged non-cloned sheep, which we will use for reference.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are constrained to work with the number (i.e. 13) of animals available, but we have access to data representing similar measurements in a contemporary group of aged, non-cloned sheep at our institute. Health assessments conducted with these clones to date indicate that we are able to generate statistically meaningful data where differences exist.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The work will be conducted in cloned sheep. Working with such animals provides the best means of investigating long-term health implications of cloning as firstly they extend the observations made on the original cloned sheep (i.e. Dolly), and secondly they complement similar measurements made previously on a contemporary group of aged sheep. Methods selected to monitor animal health during the lifetime of this license are refined in both their invasiveness and frequency. Sheep are the primary species of clinical interest in this study – hence why these assessments need to be conducted in this species. Assessments are for the most part non-invasive, but include blood sampling, which will occur at monthly intervals. Formal assessments of lameness, associated with osteoarthritis, will be conducted monthly using a formalised and published seven-point locomotion scale. Daily observations will be undertaken and any concerns will be discussed with the named veterinary surgeon. Further detailed assessments of joint health will be conducted by x-ray post mortem, following euthanasia using approved humane methods when it is judged that animals are nearing the end of their natural lives.

Project 9	**Functional analysis of factors implicated in X chromosome inactivation**	
Key Words (max. 5 words)	Gene regulation, X chromosome, chromatin	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	√	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our overall goal is to understand mechanisms of gene regulation in mammals. More specifically we are interested in how genes are switched off or inactivated in different cell types and different stages of development. It has been shown that specific RNA molecules can play a key role in gene inactivation, but the molecular mechanisms underlying this are poorly understood. We have identified several protein factors that are implicated in RNA mediated gene silencing. Some of these have features associated with proteins that bind RNA. This project is directed at using genetic analysis tools to determine the functional importance of these proteins in RNA mediated gene inactivation.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work is expected to advance our fundamental understanding of how genes are switched on and off during development in mammals. The information we gain will be published in peer reviewed scientific journals to inform our scientific community or field and also other researchers or lay persons interested in the work. The project is aimed at advancing basic scientific knowledge which is essential for the development of new technologies and approaches for tackling disease. Notably understanding gene silencing in mammals is widely recognised as being central to harnessing stem cells and somatic cell reprogramming for regenerative medicine or tissue engineering that has the potential to improve treatment for a myriad of common diseases.	

	Moreover, new drugs that affect activity of key factors involved in gene silencing have recently been found to act as potent anti-cancer agents and major pharmaceutical companies are increasingly active in this field of research.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate that we will use up to 21,000 mice over the 5 year span of this project licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	95% of animals are expected to be returned under a mild severity as these are to be used for breeding and schedule 1 with no adverse phenotype. We may use 500 mice for superovulation and these will be either bought in normal mice or GAA mice from protocol 1 or 2. These will typically be given two intraperitoneal injections, mated and killed by schedule 1 within 48 hours. 1% of the mice on protocol 1 or 2 includes a number of mouse lines that may develop a harmful phenotype and are recognisable and controllable. 150 mice may be used for implantation surgical procedures and these may be commercially bought mice that will be killed by schedule 1 soon after litter is weaned. Analgesic agents will be administered as required for surgical procedures. There may be a possibility of wound dehiscence or tumorigenesis (<1%) in some mice and these will be monitored. If a tumour is found the mice will be killed. The majority of animals may be killed by a Schedule 1 method at the end of the protocol.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We study developmental gene regulation mechanisms that are specific to mammals and our experiments are therefore carried out using the laboratory mouse, the most well understood laboratory model for mammalian development. Where possible we use tissue culture models to address specific questions and we are continually trying to improve the potential of these strategies. However, genetic regulation during embryonic development is a highly complex process involving interactions of multiple genes in multiple cell types and tissues. Generally speaking it is not possible to

	model all aspects of this process using a single cell type grown under artificial conditions in a tissue culture dish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible we obtain genetically modified/mutant lines from colleagues or internationally accredited repositories/suppliers, obviating the need to produce lines in house. We share vasectomised sterile male studs with other users, reducing the need to create and maintain duplicate animals. Where appropriate we will use cryopreservation of embryos and sperm to archive genetically modified strains that we have produced or imported, obviating the need to continually breed animals to preserve the lines. Stock levels of mouse strains will be set to minimise animal breeding whilst at the same time ensuring that given strains are not lost. Trained animal house staff will carry out the majority of breeding and maintenance, constant contact and instruction will ensure the colonies are maintained at the correct levels.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The systems and many of the factors that we are studying are specific for mammalian species and the laboratory mouse is the only appropriate animal model for studying their function. We will minimise animal suffering in the following ways; We will make full use of primary scientific literature, locally available advice and NC3Rs literature to continually update our measures to minimise harm to animal. All genotyping assays will be optimised to use minimal amounts of material such that we can use ear clip rather than tail tip sampling. It is no longer necessary to collect tail samples from our standard mouse lines, although it may be necessary for defining founder animals. Where applicable we will apply non-invasive genotyping of genetically modified strains based on detecting expression of fluorescent proteins. All mouse crosses are designed and maintained to produce the least amount of wastage as possible. We have recently successfully genoytyped mice by PCR from plucked hair samples offering the possibility to reduce the number of ear biopsies, our

	preferred method to date. We will endeavour to improve/extend this technique to get reliable results from fewer hairs. Vasectomised males will be bought in from a commercial supplier obviating the need for a protocol to surgically prepare them ourselves. We also share these studs with other groups so reducing the need to duplicate. Transgene inducing or deleting agents will be administered in diet or drinking water when necessary, avoiding the need to carry out intra-peritoneal injections. Analgesia will be administered to animals recovering from embryo transfer, this can be given 30 minutes pre-surgery by injection or supplied in a diet form. The Smchd1^{mommeD1} (FVB) mouse line that we use has a known background phenotype, specifically stereotypical behaviour of running in circles. This is alleviated by avoiding singly housed mice and by environmental enrichment. Non-surgical embryo transfer is available and will be used where appropriate, specifically ES cell/ CRISPR microinjection and culture until blastocyst stage. The method is not appropriate for the transfer of earlier stage embryos necessitating continuation of surgical transfers in parallel. Mice undergoing surgical embryo transfer will be administered an appropriate dose of an analgesic agent.

<table>
<tr><td>Project 10</td><td>Pathways of gut health and inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Gut, ageing, inflammation, microorganisms</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Disease affecting the gut are the third most common cause of death, the leading cause of cancer death and the most common cause of hospital admission in the UK; 40% of people have at least one digestive symptom at any one time and 1 in every 74 citizens of the UK have a serious bowel disease. Treatment options for the majority of gut-related diseases are limited and constrained by the lack of understanding of gut function and health and in particular of how the vast population of microbes resident in the gut (the microbiota) contribute to health and/or disease. This project aims to address two questions that are fundamental to our understanding of the importance of resident populations of microbes in the GI-tract (the microbiota) to human health:

1. How are populations of gut microbes established and maintained throughout life, and how do they communicate cells of the body?
2. Can gut microbes be manipulated to improve or restore GI-tract health?</td></tr>
<tr><td>What are the potential benefits</td><td>The proposed studies will increase our</td></tr>
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likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	understanding of not only how gut health is preserved but also the impact of natural processes such as changes in diet (metabolic stress) and ageing. This could lead to the identification of the sources of the factors and molecules responsible for triggering and perpetuating diseases such as inflammatory bowel disease that arise from mis- or altered-communication between gut microbes and the immune system. The ability to manipulate gut microbes using various strategies involving the use of single bacteria, their products or, complex mixtures of bacteria offers exciting new possibilities for intervening in at risk individuals for which there are currently no or relatively ineffective treatment regimens for disease states such as inflammatory bowel disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, ~6,150 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will administer substances that include harmful and harmless microorganisms, chemicals, and drugs such as antibiotics and steroids to induce inflammation and colitis that replicates key features of intestinal inflammation and inflammatory bowel disease in patients. These substances will result in no lasting harm in the majority of animals (>90%) using the minimum dose consistent with the scientific objectives. Doses will be no more than the maximum tolerated dose (MTD - defined as that associated with weight loss of no more than 20% of the animal's initial bodyweight). All mice receiving these substances will be inspected and weighed daily. Mice will be promptly and humanely killed if they are approaching 20% weight loss and/or display signs of pain, distress or of significant ill health such as abnormal posture/positioning, abnormal breathing, decreased food/water consumption, dehydration, muscle rigidity, twitching/trembling or pilo-erection All animals anaesthetised for imaging purposes are expected to make a full recovery. The interval of repeated general anaesthesia (a minimum of 24 hours) enables animals to make a full recovery as determined by exhibiting normal behaviour, eating and drinking normally and socialising with cage-mates. Any animals failing to fully recovery or that develop signs of significant ill-health such as

	breathing difficulties, inappetance, hunching, crouching or pilo-erection, will be promptly killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The reasons why the study cannot be undertaken without using protected animals are: 1. Laboratory-based assays cannot model the complex interactions involving microbes, epithelial cells and immune system important in gut disease. 2. Many different types of cells in the gut cannot be kept alive outside of the body and cannot be kept alive in the laboratory. 3. The vast majority of microbes in the gut cannot be kept alive outside the body and in the laboratory. 4. Microbe interactions with cells of the gut cannot be studied in laboratory-based cell culture systems because of the multiplicity of cell types involved. 5. Intervention studies cannot be performed in humans due to ethical barriers.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing experiments we rely on 30 years past experience, literature searches, consulting the institute statistician and statistical analyses to ensure the minimum number of mice per group that will be informative are used. As a general principle, for quantitative experiments, sample sizes will be set using power analysis. Generally, the significance level will be 5% and the power 80%. The exact numbers of animals required will vary with the particular experimental design, the estimate of variation in response, etc. For qualitative experiments, the amount of material required will be the minimum necessary to provide an adequate description. To reduce the number of breeding pairs, genetically altered (GA)-mice expressing the mutant gene will be kept wherever possible, provided they remain healthy and able to breed. To maximize the information from a single animal and to minimize suffering, we will aim to collect samples post mortem; we will share tissues with other appropriate scientific colleagues for use in their work.
3. Refinement Explain the choice of species	The mouse is an appropriate and validated model for gut biology and disease and is the species in

and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	which reliable genetic engineering technology and germfree experiments is best established. Also, mice are our model species of choice based upon our 30-year personal experience of undertaking these experiments. They are also the standard species for producing GA-animals and are the de facto standard for this work globally. Prior to embarking on animal experiments we will collect as much evidence as possible to determine whether a candidate gene or microorganism is likely to regulate intestinal epithelial cell barrier function or immune response. This will involve collaborating with colleagues that have expertise in gut microorganisms and intestinal organ, and isolated epithelial cell culture. While these techniques hold considerable promise they cannot reproduce the interactions of more than two cell types. Wherever necessary local and general anaesthesia will be used to minimise animal suffering. In all long-term experiments, animals will be promptly killed if they are approaching 20% weight loss and/or if they show any signs of ill health during experiments such as hunching, lack of group behaviour or breathing difficulties. Microorganisms will be administered at doses known to induce an appropriate inflammatory response. Where the outcome of infection is unknown, dosage will be carefully titrated using small-scale trial studies to minimise any adverse unknown effect.

Project 11	**Organ dysfunction following acute illness**	
Key Words (max. 5 words)	Intensive Care, Sepsis, Organ Failure	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall objectives of the project are to (i) examine the response to disease processes encountered in an intensive care setting, (ii) to assess the impact of potential therapies and to (iii) continue the development of novel therapies/diagnostics. Many patients in intensive care die of multiple organ failure. However, the way the disease progresses from the initial insult (e.g., infection, trauma) to organ failure is poorly understood. Sepsis is defined as a 'whole body' response to infection and is the leading cause of death in critically ill patients. It (and other insults such as trauma and bleeding) is often accompanied by shock, where the circulation cannot deliver enough oxygen to the body's tissues, or when oxygen is available but cells are unable to use it due to poisoning-type mechanisms. We have been working to better understand the biology of acute illness, shock and sepsis for over 15 years. We have found that metabolism by cells may be implicated, particularly the specialized apparatus of the cell that generates energy. We propose to continue our current line of investigation into the effects of shock and sepsis in critical illness to better understand how the progression to organ failure occurs. We will also attempt to use drugs that can potentially lead to protection,	

	amelioration or faster resolution of organ failure. In addition to affecting cell metabolism, shock and sepsis affect other body systems such as the hormonal and nervous systems. The way that these systems are altered by disease are fairly well recognised but the interaction between these systems remains poorly understood. We wish to expand our studies to assess changes in immune, hormonal and metabolic pathways, and the brain's control mechanisms. We can do this by, for example, measuring 'biomarkers' in the blood – these chemical warning signs will allow a better understanding of the interactions between these systems and their significance in terms of outcome and disease progression (including resolution). This will hopefully lead to better targetted therapies. As these can often be measured in both animals and humans this will facilitate translation of our scientific findings into patients. It could also be extremely useful for the refinement of animal models. We also wish to explore the link between multiple organ/whole body diseases such as sepsis, to single organ diseases such as heart attack and stroke. It is becoming increasingly clear that the biology behind these diseases is similar e.g. inflammation, free radical production. Again, this could be very useful for the better understanding of disease processes, to test novel diagnostics for early identification, and to trial therapies that can potentially improve outcomes.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work will assist in better understanding of the biology of acute illness, shock and sepsis states. The evaluation of new therapies that may either offer protection from organ failure or enhance recovery will also prove to be of great benefit. This could both reduce the severity of illness, the number of people that die and the high financial cost of caring for these very sick patients in intensive care and thereafter.
What species and approximate numbers of animals do you expect to use over what period of time?	We will predominantly use rats as their metabolic response to infection and other insults is similar to that seen in humans. We have projected the use of up to 6,525 rats over 5 years. Mice show a dissimilar metabolic response as they tend to hibernate, dropping their body temperature and metabolism, in response to some insults. However, we may use them for immune studies and other specific studies (such as mice that are born with an altered genetic background) to test specific pathways. We have projected the use of up to 1,875 mice over 5 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects during the pre-treatment phases of experiments (e.g. drug administration) are not generally anticipated. Pain relief will be given to animals recovering from operations. Animals are always observed until full recovery from anaesthesia, and regularly monitored throughout the day and early evening. Using clean surgical techniques reduces the risk of infection. Our considerable experience with these experiments reveals no clear evidence of surgically related infection or other complications. Following the administration of bacteria, animals may show characteristic 'shock' symptoms within a few hours. These are documented regularly (minimum 4 times daily) with clear end points offered by a clinical scoring system that we use. Any animal that is deemed to be suffering unduly will be promptly culled. We also use basic biological measurements (for example heart rate) to predict outcomes. Those predicted to be sicker will be monitored more closely and additional support provided (e.g. mashed food, extra bedding, pain relief) as needed. Our protocols have moderate and severe severity limits. The majority of experiments fall under the moderate category where severity limits are strictly imposed using our scoring system. A limited number of experiments will be performed under a severe severity limit. For example, the effectiveness of a treatment for a shock condition is measured by improved survival. Although we generally aim to terminate the study at pre-specified times, we feel there is an important place for some protocols to provide firm verification of benefit. At experiment end all animals are culled humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexity of shock, involving multiple bodily systems (as described above), mandates use of whole animal experiments. While we will also use cell cultures to address specific questions, these are unable to replicate the complexity of disease and differing organ responses. This work will complement parallel studies in patients where limitations in the ability to sample vital organs such as liver and kidney mandate the use of comparable animal models. This will allow better understanding of these disease conditions. Furthermore, potential future therapies need to be assessed in whole animals before attempting to use these drugs in patients.
2. Reduction Explain how you will assure the use of minimum numbers of	Careful experimental design can help to minimise the number of animals used. For example, control studies will be performed, where possible, on the same day to account for environmental factors such as temperature. All animals will be matched in terms of age, weight and gender, again

animals	to minimise variation, and thus the number of animals used. We also take samples for future study to learn as much from each animal as we can.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have found that rats show a similar metabolic response to shock and sepsis as compared with humans. We have recently modified our clinical scoring system following consultation with biological services staff and our named veterinary surgeon. It now includes more clinically relevant measurements. Our scoring system dictates that monitoring be performed more frequently in line with the severity of illness. This means the animals are being monitored more effectively, allowing better care as required by each individual animal. As indicated above, we will provide additional support (e.g. mashed food, extra bedding, pain relief) when they need it.

<table>
<tr><td>Project 12</td><td colspan="3">DNA Strand Breaks, DNA damage responses, and Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">DNA damage, Neurodegeneration, Cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td><u>Yes</u></td><td></td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td><u>No</u></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td><u>No</u></td></tr>
<tr><td>Preservation of species</td><td></td><td><u>No</u></td></tr>
<tr><td>Higher education or training</td><td></td><td><u>No</u></td></tr>
<tr><td>Forensic enquiries</td><td></td><td><u>No</u></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><u>Yes</u></td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Understanding how cells, tissues, and organs respond to DNA damage is crucial to our understanding of how DNA damage causes disease. DNA damage arises both during normal growth and from exposure to chemical toxins in our environment. The threat posed by DNA damage is illustrated by the existence of human genetic diseases in which our ability to properly repair damaged DNA is abnormal or absent. However, our understanding of how DNA damage causes disease is limited. Only by using mouse models can we address this deficit and thereby contribute to better diagnosis, management, and treatment of human diseases associated with defects in the response to DNA damage.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">This project will enhance our understanding of how mammals respond to DNA strand breaks/DNA damage and how these DNA lesions impact on tissue development and maintenance. In particular this work will address the mechanistic cause of diseases that arise because of genetic defects in DNA repair and DNA damage responses. This work will also enhance diagnosis of DNA damage-related</td></tr>
</table>

	pathologies and, in the longer term, provide new approaches towards clinical intervention.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using a maximum of 38,000 mice over the 5-yr period of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects arising from new genetically altered strains are difficult to predict. However, we anticipate that most mice will exhibit little or no phenotype, with only a small fraction (<10%) exhibiting mild or moderate phenotypes associated with developmental dysfunction and/or tissue degeneration. The mice will be assessed daily or twice daily as appropriate throughout their lives for adverse effects, e.g. signs of pain or distress, weight loss, reduced motility, neurologic symptoms. Animals exhibiting any severe phenotypes will be killed, or in the case of individual animals of particular scientific interest, advice sought from the local Home Office Inspector and the Animal Care Staff and Veterinarian. If severe phenotypes are observed i.e. causing substantial discomfort such as major reductions in body weight (20%), severe reductions in motility (e.g. difficulty to feed/drink or remain upright), mice will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible, we use microorganisms or immortalised human cell cultures for our studies, since these do not require animal models. However, mice are required for some experiments where the impact of unrepaired DNA damage on developmental and degenerative pathologies is under investigation. These questions are critical to our understanding of a plethora of human DNA repair-defective human diseases that are associated with neurodevelopmental dysfunction, neurodegeneration, and/or cancer predisposition. Finally, where mouse cell lines can replace whole animal experiments, we employ these to further reduce the number of animals we require.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always ensure our breeding colonies are no larger than minimally required for stock maintenance, and we use cryopreservation to avoid housing animals in the laboratory in the long term. Where appropriate, group sizes will be determined by standard power analysis (SISA), and we ensure

	these are as small as possible by appropriate experimental design (e.g. by addressing multiple related questions simultaneously, with single test and control groups).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the best available mammalian model for understanding how DNA damage casues diseases such as cancer and nervous system dysfunction in humans. Only in those cases where critical questions cannot be addressed by other methodology do we utilise mice. Under all circumstances we minimise animal suffering by daily/twice daily health and behaviour monitoring, including the use of health score sheets, and by humane killing of animals if the appropriate humane end points (see specific protocols) are exceeded. Where in doubt, advice will be sought from the Animal Care Staff, Veterinarian, and the Home Office Inspector.

Project 13	**A study of changes which occur during ageing**	
Key Words (max. 5 words)	healthy ageing connective tissue crosslinks	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	As our bodies age changes occur which make the connective tissues (skin, tendons, blood vessels etc.) less elastic. The changes which occur are known to be due to joining together of the fibres in these tissues, but the detail as to which changes are most important are not fully understood. The aim of this project licence is to provide materials for the study into what chemical changes are occurring as we get older and identify which of these changes are most significant in the process of getting old.	
What 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 befter understanding of the processes involved in ageing could ultimately help in both treatment and prophylaxis of diseases associated with normal ageing. It is expected that this project will lead to the development of methods to study ageing tissues and identify the important changes which occur as we age. Ultimately we aim to transfer the methods developed here to study the ageing of tissues in humans, The tissues generated in this project will be stored and made available to other researchers interested in ageing, providing a valuable and unique resource of isotopically labelled tissues of different ages. It is reasonable to expect that other researchers studying normal ageing processes would find this resource	

	valuable.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use mice up to an age of 24 months. Up to 620 mice in total over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We are studying normal healthy ageing processes and so the mice are not expected to experience any harm during their life span. At various ages mice will be killed humanly and the tissues studied to determine changes that are occurring as the population of mice age. All animals will be killed before they experience age related disease, or when the first signs of age related disease are observed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The ageing of tissues is a complex process involving a multitude of cell types and factors. For example, a blood vessel like the aorta is a complex highly organised structure of cells, collagen, elastin and other connective tissue components. The blood vessel wall is not believed to have a fixed composition at a molecular level, changing with age and in response to factors such as blood pressure. Similarly, the structure of other tissues such as tendons and bone respond to stresses and age related factors which are impossible to reproduce in culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A small group of 30 animals will be used initially and the tissue from these extensively studied to generate a hypothesis for which factors are significant in the ageing process of tissues. A statistician will then be consulted to determine the minimum number of animals required to generate statistical significance in a larger experiment for the hypothesis.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse has a life span compatible with the study of aging and is expected to show changes which are likely to occur in humans. Normal healthy ageing is being studied so no harm is expected to occur to the animals.

Project 14	**Models of viral hepatitis**	
Key Words (max. 5 words)	Hepatitis; virus; treatment	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Globally, there is approximately 5 times the number of people living with hepatitis C virus (HCV) infection than with HIV. Chronic infection can cause severe complications including cirrhosis and a liver cancer, and is a leading reason for the need of liver transplantation. Unlike HIV, some people can eliminate the virus naturally. While there are drug therapies for HCV there is no licenced vaccine, which is the most cost effective approach to combatting infection and disease, particularly in developing countries. The project aims to understand the nature of the immune response an infected individual would need to raise in order to eliminate the virus and minimise liver damage. It will also study how viruses change during infection and how responsive they are to novel 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 information could be used to design vaccines against HCV as well as providing a system in which novel drugs could be assessed to see how well they work against HCV.	
What species and approximate numbers of animals do you expect to use over what period of time?	Tamarin; approximately 10 per annum over 5 years; mouse (10), guinea pig (10), rabbit (2) in the 5 year period.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The severity is no more than moderate. Adverse effects may be moderate but are managed by the NVS to ensure human end points are not exceeded. Refinements to the procedures result in only transient effects experienced by the animals. At the end of a study animals are euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To best understand an immune response it must be studied in an animal system, such that its development in the context of the whole body is assessed. In this way, the best approximation to infection of humans is achieved.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Analysis of studies ensures that the minimum number of animals is used to achieve statistically significant data. The use of biopsies allows the study of stages of infection in one animal, thus reducing the number of animals required should individual stages be assessed.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	HCV doesn't infect small animals; the use of the tamarin system is well-documented. It is established at the site and the local experience has enabled significant refinement in animal welfare on study. These include refinement in anaesthetic, tissue sample removal and animal groupings.

Project 15	**Oxidative stress and aging**	
Key Words (max. 5 words)	Oxidative stress; degenerative disease; aging	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Just as iron implements becomes rusty over time and stop performing correctly, the molecules that comprise our bodies (biomolecules) also degrade over time and fail to function as they should. This molecular failure ramifies upwards: cells that are built from these molecules start to misbehave, and the organs that are built from these cells fail as a consequence of this. For example, muscle cells (myoblasts) lose flexibility and you lose grip and strength. Brain cells (neurons) fail to propagate electrical currents and Parkinson's disease results. Cardiac cells start to pulse out of sync with each other and you suffer a heart attack. Scientists use the formal term 'oxidative stress' to refer to degradation of biomolecules. As the frequency of death due to infectious diseases diminishes through improved hygiene and use of antibiotics, so diseases of old age have emerged as the major cause of mortality in the Western world. These diseases, which include Parkinson's- and Alzheimer's diseases, diabetes, cardiovascular disease and cancers, and indeed aging itself, are believed to be the direct consequence of oxidative stress. At first glance, this might appear to be a grim diagnosis as oxidative stress is irreversible and	

	accumulates over time at an ever accelerating rate. However, the situation is less bleak than might at first appear; while oxidative stress is indeed irreversible, the rate at which it accumulates might be decelerated by, for example, treating the population with anti-oxidants that prevent biomolecular damage. Unfortunately, the 'holy-grail' of aging research – the identification of effective anti-oxidants that slow down the aging process – has been hindered by the lack of appropriate models for measuring oxidative stress in living organisms. For, if we cannot measure oxidative stress, how can we tell whether an anti-oxidant has been effective? The work we are performing is aimed at generating novel mouse models that will allow us for the first time to evaluate anti-oxidants and assess whether the retard the aging process. We refer to these models as reporter models as they measure ('report' on) oxidative stress.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In addition to the moral imperative to alleviate the suffering of patients and their families, age-related diseases represent an increasing and unsustainable economic burden on society; it is estimated that these diseases will cost the NHS £13bn a year by 2022. The animal models that we are establishing will be instrumental in identifying drugs and behaviours that can reduce oxidative stress and so the extend the healthy lifespan of the population. A healthier population will allow scarce NHS resources to be targeted to other pressing health problems, such as the increasing problem of resistance to antibiotics.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using mice. We expect to use approximately 8000animals over a period of five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In most cases, the interventions being trialled in the animals are actually expected to be beneficial and to improve their health and lifespan of the mice. Thus, in most cases the expected level of severity is zero (formally mild). We are however proposing to use one strain of mouse that suffers accelerated aging. We intend to use these ice before they become symptomatic; however, we cannot rule out that in symptoms of moderate severity might become manifest in rare and unexpected cases. We will also use a limited number of environmental toxins, and other cytotoxic agents,

	that can cause overt toxicity at certain doses. It is our intention only to use doses of such chemicals that lie below the known toxic thresholds. Mice will be killed in a humane manner at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Aging affects multiple organs and can only be explored in an intact organism. Although in an ideal world, some of these studies would be performed *ex vivo* using human cell lines, these lines are invariably cancerous in origin. Unfortunately, once cells have become cancerous, they suffer oxidative stress in a different manner to normal cells. This makes them unacceptable for our purposes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our laboratory has been at the forefront of devising clever experimental approaches that reduces the numbers of animals required for any one experiment. The details are necessarily technical and here we provide but a single example to exemplify our approach: we have engineered our reporter mice to emit visible light in amounts that rise-and-fall with oxidative stress. The amount of light can be measured with a sensitive external camera. For this reason, we do not have to kill the mice but can re-use them. This will lead to a significant reduction in the number of mice used in our experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Of the model organisms commonly accepted as surrogates for humans, the mouse is the closest in evolutionary terms to humans. Simpler, more socially-acceptable alternatives, such as the worm species *C. elegans,* have well-documented differences with humans in how they handle oxidative stress and this makes them unacceptable for this project.

Project 16	**Mouse membrane traffic mutants**	
Key Words (max. 5 words)	Basic science, human disease	
Expected duration of the project (yrs)	5 yrs	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There is a lot that we don't know about how our cells work, including how all the thousands of different proteins get to the right place. We have found some of the machinery used by the cell to traffic proteins, but we still don't know what it all does, so the cell is like a jigsaw puzzle with pieces waiting to be put in the right place. What we do know, however, is that if certain pieces of the puzzle are missing, as in genetic disorders affecting the trafficking machinery, the consequences can be devastating. We also know that viruses can be very clever at exploiting the trafficking machinery and using it for their own devices, such as to get inside the cell, or to prevent an infected cell from signalling to the immune system that it has an infection - both of which are strategies used by the Aids virus.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Although this project is mainly basic science, with the aim of increasing our understanding of how our cells work, it has medical implications as well. In particular, mutations in some of the machinery we will be studying lead to hereditary spastic paraplegia; and we know that some of the other machinery we will be studying gets hijacked by the Aids virus.	
What species and approximate numbers of	We intend to use mice for our research: not more than 4000 animals over a period of five years.	

animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use both naturally occurring mutants and genetically engineered mice to try to understand how various components of the trafficking machinery work. For some of our research, the mice will be completely healthy. For other studies, the mice will have similar genetic disorders to certain human patients, and thus they are expected to show some mild clinical signs of neurodegeneration, such as a slightly altered way of walking.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although for much of our work, we will be using cultured cells, these cells never entirely recapitulate what goes on in an intact animal. For instance, to understand why certain mutations cause neurodegeneration, it is essential to look at the nervous system in the context of the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We intend to keep our breeding colonies of mice as small as possible. In addition, we will allow other research groups to use any surplus mice, cells and tissues. This will minimise the use of live animals, because we will be replacing animals with non-animal alternatives.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	To understand certain human genetic disorders (e.g., hereditary spastic paraplegia) and infectious diseases (e.g., Aids), it is essential to use mammals, because other animals are just too different from ourselves. Out of all the available mammalian systems, mice have the lowest sentience, and they are also the most suitable for genetic studies. The mice to be used in this project will be housed in state-of-the-art facilities, and siblings will always be housed together, to minimise boredom and to encourage natural behaviour.

Project 17	**Genetic Analysis of Stem Cells Using the Mouse**	
Key Words (max. 5 words)	Stem cells, mouse, genetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Stem cells are essential components of the human body. Abnormal stem cells are causes of many diseases including cancer. We aim to use the mouse as the in vivo (in body) model to study stem cells in normal development (self-renewal and differentiation) and in disease.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Although stem cells are essential in normal development, many aspects of stem cells are unknown. We propose to study several stem cells including a new type of stem cells with features similar to early embryos, which can be useful for producing similar human stem cells for regenerative medicine. Better understanding stem cells in specific tissues/organs such as blood and the mammary gland will potentially facilitate therapies for human disorders such as blood disease and cancer.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. Approximately 97,100 in five years	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	The procedures proposed in this project are all well used in the scientific community. The severity limit is mild to Moderate. Most procedures do not cause any adverse effects, but animals will be humanely killed if	

level of severity? What will happen to the animals at the end?	they show any unexpected signs of distress. For mice expected to develop tumours, NCRI Guidelines for the Welfare and Use of Animals in Cancer Research will be followed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Stem cells require proper microenvironments to survive, to produce more stem cells and to differentiate into proper functional cell types. This microenvironment involves cell- cell and cell-matrix interactions, and immune system surveillance. Therefore, we need to use mice to study stem cells in vivo (in the body). However, we have been developing protocols to maintain stem cells in an undifferentiated state and to try to differentiate stem cells in vitro (in culture).
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use high quality reagents in experiments and to carry out carefully designed experiments to minimise repeated experiments. We will use a powerful database system for animal experiment management. We have developed new technologies and will continue to do so to minimise mouse usage. We will refine our experiment designs and procedures so that experimental results could be conclusively obtained using minimal number of mice.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have developed and used technologies that represent refinement of the procedures, for example, we use technologies that enable us to study gene functions in specific stem cell types in the mouse. We will use tissue obtained from routine husbandry procedures for genotyping as much as possible so that extra samples need not be taken. We have a powerful mouse database that allows us to monitor animal welfare in detail.

Project 18	**Breeding and Production of Genetically Altered Mice**	
Key Words (max. 5 words)	Genetic alteration, mouse, breeding	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will breed transgenic mice for use in biomedical research. These animals are needed to investigate a range of human diseases and also to help understand the influence of different genetic factors in human development. The mice bred on this project will be used on a range of different projects that only require small numbers of animals. Centralising our breeding of small groups of mice ensures they are cared for by experienced technicians, who are also trained in assessing possible adverse effects of the genetic alterations. We also ensure that the minimum number of mice are bred by carefully monitoring colony size.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The types of research project undertaken using these animals include investigations of the mechanisms of several inherited human diseases, development of novel methods of treating cancer, the mechanisms underlying some forms of male infertility, and a range of studies investigating early human development.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used, and it is expected that up to 5000 mice will be used over 5 years.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	A few animals (<100) will be used to establish new breeding colonies at our facility. These animals will undergo surgical implantation of embryos that have been genetically altered, and these animals will give birth to transgenic offspring that are used to start a breeding colony. In order to prepare the mice for implantation of the eggs, they are mated with male mice who have been made sterile by vasectomising them (a procedure also undertaken as part of this project, to approximately 100 mice). Alternatively, adult mice are imported, and these are bred to produce more transgenic mice. To check their genetic make-up, small tissue samples may be taken, for example a small piece of ear or tail. Pain-killing drugs or anaesthetics are given whenever these are likely to be needed, so that the minimum of pain and distress is caused. Although all of the mice bred for these projects have genetic alterations, most of them show very minor or no adverse effects. Other types of mice are normal when they carry only one copy of the altered gene, but would be abnormal if they have two copies of the altered gene. To reduce the number of animals that are produced with adverse effects (such as abnormal development of particular tissues or organs), many studies are carried out on tissues obtained from animals humanely killed at an early stage of development.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of the research groups working on mice bred on this project also undertake studies in humans, in human and mouse tissue culture, and in other animal models such as fish, and only use mice when it is absolutely necessary for the particular research that they are undertaking. Some work, especially that which involved studying the development of multiple organ systems requires some work in whole organisms. This is because the complex interactions between different organ systems cannot yet be studies fully in isolated cells and tissues grown in a dish in the laboratory.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise animal use by only performing work in whole animals when alternative are unsuitable, or when work cannot be conducted directly in people. We will minimise the numbers of mice bred by careful colony management. We will aim to make tissues available from any animals bred to other research

	groups either directly, or by participating in national and international schemes for sharing such resources. Where specific types of mice are readily available from academic or commercial sources, mice will be acquired for each study, to avoid maintaining a breeding colony.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are used for these projects, because the genetic make-up of the mouse has been successfully decoded, and all of its genes identified. This information is now being used to determine the function of these genes, and in particular how they interact in a whole animal. The welfare costs of the work are minimized in two main ways. When animals undergo surgery (for vasectomy or embryo transfer) pain is prevented by use of analgesics, and distress is minimized by high standards of perioperative care. When genetically altered mice are bred, then they are monitored for any adverse effects of the genetic alteration. If there is any harmful effect, then carefully defined criteria are established to limit these effects. This usually involves humanely killing the animals before their health is compromised, but most of the animals bred on the project remain clinically normal throughout their life-span. When a new type of genetically altered mouse is to be imported, details of the anticipated effects of the genetic modification will be obtained from the supplier and this will inform the initial decisions in relation to breeding and care of the mice. When possible, “mouse passport” data that contains more specific husbandry advice will be sought. During establishment of the initial breeding colony, litter size, number successfully weaned, and any specific adverse effects will be documented by regular (daily) examination of the animals. Husbandry modifications (eg use of soft diet, later weaning dates for smaller juveniles, additional bedding etc) will be adopted as required. If the genetic alteration could lead to a reduced resistance to infections, we would change the way we house the mice to reduce the risk of them being exposed to disease agents.

Project 19	**Investigation of the in vivo action of G protein coupled receptors**	
Key Words (max. 5 words)	Neurodegeneration, physiology, drugs, cancer, diabetes	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The human body is made up of many trillions of cells. These cells make up organs such as the heart, liver, kidneys and brain. The cells in our body need to communicate with each other. They do so by releasing messenger molecules that attach to specific receptor proteins on the outside of cells. This project is interested in understanding these receptor proteins and working out how the messenger molecules activate of the receptor proteins and how this process changes the way the target cells (or organs) behave. The project will also aim to understand how we might make drugs that activate these receptor proteins and how these drugs might be designed so that they change the response of cells/organs in a way that help combat a number of human disease conditions such as memory loss in neurodegenerative disease, dysregulation of glucose levels in the blood in diabetes and in 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	This project will have the following benefits: • Scientifically – by studying the receptor proteins described in this proposal we will better understand one of the key mechanisms employed by our bodies to control numerous	

project)?	biological processes such as heart rate, learning and memory, hormone responses and even vision. • Medically – our study will provide a framework from which the next generation of drugs that target the receptor proteins described here will be designed. This will help because most drugs in drug discovery fail because either we have not got enough understanding of how the disease tissue works and how best to design drugs against the disease or because the drugs are toxic. In this study we will obtain the information that will allow for the better design of drugs to increase therapeutic efficacy and decrease toxicity • Economically – we work very closely with the pharmaceutical industry and the results of our study will benefit the generation of drugs against key diseases and as such support the activity of major drug companies.
What species and approximate numbers of animals do you expect to use over what period of time?	Time period is five year: Number of mice to be used = 21,000 Number of rats = 2400
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the mice will be used in moderate severity protocols which reflects the fact that we use a prion model of neurodegeneration and that the animals will be subjected to physiological protocols such as fear conditioning that have adverse stimuli. A small proportion of the mice may experience severe symptoms in the course of our experiments. Every effort will be made to ensure the length of time the animals have these symptoms is kept as short as possible. In the end all the animals in this licence will be killed by schedule 1 methods or through carefully prescribed non-schedule 1 procedures. In this licence the animals will be subjected to the following harm: • **Breeding genetically modified animals:** So of the genetic modifications described in this licence might result in a harmful phenotype such as distented bladder, or difficulty in saliva production. This will be mitigated by careful monitoring and making the animals more comfortable (e.g. by

	providing mashed up food to help with eating). • **Blood sampling:** This will be mitigated by good practice from well training of staff • **Anaesthesia** This will be mitigated by good practice from well training of staff • **Administration of drugs and substances:** In this licence we described a number of different routes of administration which include injections and placing very fine tubes into the brain. The route that will be chosen will be well tested in pilot studies to be the one that causes least distress to the animal and the most effective at delivering the drug. If necessary discomfort will be reduced by anaesthesia. Also good laboratory practice conducted by well trained staff will reduce suffering and harm. • **Animal behaviour testing** We describe a number of protocols where animals will be tested for memory loss and anxiety as well as the ability to feel pain. These tests involve the animals being subjected to mild electric shocks and placed in open environments or on run ways. We will reduce the harm to animals by exposing them to as low an adverse stimuli as possible. We also have extremely efficient equipment and very well trained staff that will reduce the harm to the animals. • **In vivo imaging** To monitor brain activity and tumour formation the animals will be placed in imaging equipment. The animals will be anaesthetised during these procedures to reduce harm • **Tumour development** The animals in this study will be induced to have cancer. Careful monitoring of the tumour growth and potential metastasis will reduce the suffering of the animals
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mice provide the best model animals for the study of the receptors as they are readily manipulated genetically. Rats are the best animals to use in some of the metabolic studies described here because they have a metabolism that much more closely resembles that of humans than mice. There are no

	non-animal alternatives as the aim of this study is to take the information that we have gather in our non-animal work and apply it to animals to determine if the principles established in the non-animal models actually are of any significance in animals and potentially humans. This is due to the fact that in whole animals there is a complex interplay between organs and cell types that can not be replicated in non-animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experiments will be very carefully designed so that only the number of animals that result in statistically significant results will be used. We will also be piloting methods to reduce animal numbers by determining if we can monitor the action of our receptors in disease models in a more efficient manner. We will also maintain only those strains that we are experimenting on and keep all others as frozen embryos so that we reduce the number of animals that we breed and maintain.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The main species used will be mice since it is possible to readily manipulate the genome of mice thereby changing the receptor proteins in this species in a way that reveals the function and modes of action of the receptors that relates to the function of these receptors in humans. Other model systems such as yeast, worms and fish do not approximate to that seen in humans in the same way as mouse. We will also use rats but not as many as mice. The rats will be used largely in metabolic studies since they these studies largely do not rely on making genetic changes but rather concentrate on the response of the animals to drugs. As this is the case then whereas mice are closely related to humans with regard to the way the receptors respond, rats are even closer. Hence, where we do not require genetic experiments we will use rats. We will employ disease models, such as prion disease, that are the closest model we have in rodents that mimick human neurodegenerative disease.

Project 20	**Cell signalling in neural and cardiovascular development and regeneration**	
Key Words (max. 5 words)	blood vessel growth, nerve regeneration	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	A healthy nervous system and effective cardiovasculature are essential for the well-being of the adult organism. However, injury and disease often compromises the function of the nervous system and cardiovasculature. The prime goal of this project is to identify molecular and cellular pathways that guide blood vessel or nerve growth in the developing body and to determine how they might be exploited to promote tissue regeneration after injury and disease.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding the molecular and cellular pathways that guide blood vessel or nerve growth in the developing body will help us understand birth defects and also identify targets for novel therapies that promote tissue regeneration after injury and disease.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will breed approximately 40 different mouse strains with genetic modifications to maintain the modifications or to cross-breed them to each other for novel combinations. On average, this breeding programme will generate around 2000 mice per year. Around half of the offspring will be used for experiments. We may also use up to 50 rats per year.	
In the context of what you	We will use rodents in procedures that have been	

propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	mostly classified as not harmful or mild and involve breeding of rodents with genetic modifications or natural mutations to obtain tissues for histological analysis. In some instances, we will have to carry out procedures of moderate severity, which include breeding rodents that have moderately adverse phenotypes due to genetic modifications or natural mutations, inducing injury or disease to examine the molecular and cellular pathways involved, to activate or inhibit novel molecular targets or to administer stem cells or genes for therapy. Importantly, moderate procedures will only be carried out if pilot genetic data or tissue culture experiments indicate a high chance of obtaining essential knowledge to help resolve medical problems. At the end of experiments, animals will be humanely killed in accordance to Home Office guidelines.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Experimental studies utilising bioinformatics and tissue culture have previously identified candidate molecules and cellular pathways that may prevent excessive injury or aid tissue repair and regeneration in vascular or neuronal disease. We will continue to use such methods to identify further candidates, reducing the number of animals required. However, we now need to translate the findings of our in vitro investigations into a physiological and pathological context through developmental biology studies and injury models to determine the precise functions and therapeutic potential of some of these molecules and cells in the intact body, where signalling pathways affect multiple cell types in parallel to orchestrate repair and regeneration. Moreover, in vivo testing of potential new therapies in animal models of retinal disease will be essential before such therapies could be considered for patients. Accordingly, there is currently no experimental approach that can completely replace the use of animals for whole body fundamental and preclinical studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible, we will perform retinal imaging in longitudinal studies of live animals rather than cohort studies to examine how genetic changes or treatments affect eye health, as this will substantially reduce the number of animals required. For experiments requiring the production of tissues, we will harvest multiple organs and share them out amongst team members to advance several research projects simultaneously, rather than allowing each

	team member to produce and use their own animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For this project, it is important to use mammalian models, because its goal is to provide information that can benefit humans. We will use mice and rats as lowest suitable mammalian species, because their cells are structurally similar and employ similar signalling pathways to human cells. Moreover, mouse strains with precisely engineered mutations are available that will allow us to determine the significance of candidate disease molecules in the physiology and pathology of the eye. Natural rat strains with retinal pathologies similar to human retinal diseases are also available and provide suitable models to investigate disease origin and treatment. To minimise the welfare costs to the animals, any potentially painful or distressing procedures will be carried out under general anaesthesia, followed by suitable pain relief and regular health surveillance, as advised by the local Veterinarian and under the care of suitably qualified staff. In circumstances of mild or moderate severity, rodents will be monitored more closely than under regular husbandry practice to ensure that signs of suffering are recognised promptly and treated appropriately under veterinary guidance to prevent suffering, lasting harm or distress.

Project 21	**Study of Infection and Antibiotic Resistance**	
Key Words (max. 5 words)	Antibiotics, bacteria, resistance	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The world is running out of antibiotics. This will lead to the end of modern medicine which is underpinned by the use of antibiotics. For example, without effective antibiotics, organ transplantation, complex surgical operations, cancer treatments may become impossible. The survival of new born babies, those who receive immunosuppressive drugs, intensive care unit patients, patients with pneumonia, deep seated infections and meningitis will decrease. Overall, life expectancy will fall by several decades. The cost to the global economy has been estimated to be $100 trillion, 2% GDP by 2050. The objectives of this project are to investigate new ways of making new antibiotics. In particular, this study proposes to assess the effectiveness of established and new medicines against highly resistant bacteria in animal models of 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 potential benefits of this project are the invention of new ways of making antibiotics. This should lead to new antibiotics entering into development, and eventually these new antibiotics entering the market where they would be used to treat patients who are suffering from highly resistant bacterial infections or prevent them from contracting highly resistant	

	infections.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats will be used. Over 5 years, the proposed work will use no more than 18,500 animals.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	It is proposed to infect animals with bacteria in order to induce an infection. We will monitor progress by examining the animals and by removing blood and other samples to determine the presence of bacteria. Treatment will be given to the animals, in the form of medicines which we think will kill or inhibit the growth of the bacteria. Treatment will be applied topically or by injection, in a similar way that they will be given to patients. The animals will be humanely killed using approved humane methods at the end of the study, the organs of the animals will be removed and will be examined for the presence of bacteria which will determine if the treatment has been effective.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Highly resistant bacteria can be grown in test tubes and antibiotics can be added to these bacteria and survival of the organisms can be determined. But past experience tells us that the environment in a live animal is much more complex than in a test tube. This means that it is necessary to study the bacteria with and without antibiotics in living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Most of the work to identify and characterise new potential antibiotics is performed in test tubes. For example, we test whether or not highly resistant bacteria are sensitive to potential antibiotics. So, only antibiotics which kill highly resistant bacteria in test tubes will be used in the animal models. The test tube experiments will supply key information about which potential antibiotics should be studied in animals and will enable us to significantly reduce the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs harms) to the animals.	Mice and rats are chosen because more past experience exists in other laboratories than other species, upon which we base our proposed animal models. These two species are the most compatible for the evaluation of the efficacy of new antibiotics. It is proposed to utilise optimal experimental techniques with minimal intervention. This will reduce distress in the animals, and will ensure expert preparation of samples for investigation.

Project 22	**Regulation of mammalian Hox genes**	
Key Words (max. 5 words)	Mouse, Hox, gene regulation, enhancer elements	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	One of the central marvels in biology is the means by which an embryo develops from a homogeneous clump of cells to form an elongated, patterned structure with all of its component parts, such as limbs, different types of vertebrae and internal organs forming in their correct places along a head-to-tail axis. We now know that these structural patterns arise first in a pre-pattern of expression of Hox genes, and it is the expressed products of these genes that instruct the embryonic cells on their subsequent routes of development. However, the question still remains as to how the Hox gene expression patterns are themselves first set up. This is the question that I aim to answer in my 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)?	We will learn how Hox genes expression patterns, and thereby body anatomy, along the head-tail axis is regulated. The questions are fundamental, but the findings made may ultimately shed light on the causes and managements of a variety of birth defects in humans.	
What species and approximate numbers of animals do you expect to use over what period of time?	An average of about 54 mice could be used annually in this project which would equate to no more than 270 over 5 years.	
In the context of what you	Mice will be treated in one of three possible ways.	

propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	First, immature females will be injected with hormones to promote egg production. Second, mature, pseudo-pregnant females will be recipients of 2-cell embryos that have been injected with new DNA constructs. Third, males will be sterilized by vasectomy as part of the procedure to provide the pseudo-pregnant female recipients. Mice in the first group will suffer only momentary discomfort. Mice in the second and third groups will experience the moderate discomfort associated with a surgical procedure but this will be minimized post-surgery by analgesics as required, and by use of heated pads to maintain comfortable body temperature. A few genetically altered animals are likely to be produced but these are not expected to develop any harmful deformities that would adversely impact on animal health and welfare. Ultimately, each animal used will be killed in a humane manner.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible we shall utilize cell culture systems in an attempt to minimize animal usage. However, the mechanisms of early development normally occur within small populations of cells, and require a multitude of interactions between molecules and cells that cannot currently be predictably reproduced outside the environment of the embryo. Some results that we obtain in cell culture must therefore be subsequently checked to ensure that they are also valid in embryos.
2. Reduction Explain how you will assure the use of minimum numbers of animals	I shall study those Hox genes for which there is already evidence in published papers for the location of their regulatory regions on chromosomal DNA. This will avoid much of the trial and error approach that was necessary to find these regions in the first place. Most of my work will be done in cultured cells, but once a regulatory region has been characterized in this way I shall need to confirm that this region is similarly required to regulate Hox gene expression along the head-tail axis of transgenic mouse embryos. Transgenic offspring will only be allowed to breed if there is an unexpected requirement - for example, to see how a Hox expression pattern changes in embryos at different developmental times. Transgenic offspring are expected to show no adverse clinical signs, and to behave like normal mice. However, I shall breed the minimum numbers necessary for analysis and maintenance of stock.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are proposed for these studies because we have the appropriate gene sequences. Also, they are small, easy to handle and feed, and have a short generation time with year-round breeding. The genetic modifications that we require in order to analyse developmental mechanisms can readily be introduced into the mouse. Importantly, there are many indications that mouse Hox genes are directly comparable with human with respect to both function and regulation, and our results are therefore likely to be directly applicable to human embryonic development. Hormones or drugs will be given in ways that result in no more than transient discomfort. Any animal that appears to be in ill health, or in the rare event that it fails to recover properly from surgery or fails to deliver its litter on time will be humanely killed without delay. Tissue samples used to test genetic status will be of the minimum size necessary for analysis.

Project 23	**DNA damage response involvement in genome stability and cancer**	
Key Words (max. 5 words)	DNA damage, genome stability, cancer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our recent studies in the nematode worm C. elegans, in mammalian cell culture and mouse models have provided key insights into the roles of the Fanconi Anemia (FA) pathway, and DNA damage response proteins (such as Rtel, ALC1, Rifi) in the maintenance of genome stability. In some cases our findings have suggested potential methods for preventing/treating tumours in patients. This project aims to continue our animal studies to characterize genome instability phenotypes and roles of DNA repair genes in tumorigenesis, neurodegeneration, immune system development and/or infertility.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	These studies will improve our understanding of how DNA Damage response factors contribute to tumorigenesis, immune-deficiency and/or neurodegeneration in humans. Our studies may also lead to new and effective targeted therapies for treating certain human cancers.	
What species and approximate numbers of animals do you expect to use over what period of time?	All of our proposed studies aim to build on extensive groundwork in model organisms, mammalian cell culture and mouse models. Animal experiments remain the only definitive test of whether inactivation/over-expression of these genes is sufficient to promote tumorigenesis, immune-	

	deficiency and/or neurodegeneration. Animal experiments also represent the only feasible method for us to determine if treatment with DNA damage inhibitors or DNA damaging agents are effective in treating tumours. We do not anticipate using more than 5700 mice per year or 28,500 animals 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 level of severity? What will happen to the animals at the end?	We anticipate that the mice used may exhibit increased or decreased spontaneous tumour formation, immune- deficiency and/or neurodegeneration. Animals will be frequently monitored for relevant phenotypes following the NRCI guidelines and those animals developing such phenotypes will be culled for detailed pathological examination to determine the molecular basis of these defects. We are expecting to see not more than moderate signs but as it is not possible to fully predict the nature or severity of any potential defect and for all types of mice, humane end points may be reached on rare occasions.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All our groundwork will be based in mammalian cell culture and C.elegans worm studies. But animal experiments remain the only definitive test of whether inactivation/overexpression of DNA damage response factors is relevant for tumour formation or other disease associated phenotypes. These issues can only be addressed using whole animal models, as modelling tumour development, immune-deficiency and/or neurodegeneration to this level of detail is not possible in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every effort will be made to ensure a minimal and sufficient number of animals is used to provide scientific and statistical relevant results. Whenever it is possible, cell lines will be derived from these animals and transgenic animal lines will be frozen down.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	Although there are species (such as dogs, pigs and non- human primates) that are even more closely related to us than mice, working with these large animals is extremely expensive and is fraught with ethical concerns. With their small size and short generation times, breeding and keeping mice is comparatively simple and inexpensive. In addition, because they have been widely used in research for decades, researchers have built up a detailed

(harms) to the animals.	understanding of mouse biology and genetics and developed Large numbers of tools and techniques to study them. These powerful genetics tools are not yet available for larger mammals. Some of these models can mimic a wide range of human diseases and health problems such as cancer. As noted above, all of our proposed studies aim to build on extensive groundwork in model organisms, notably mammalian cell culture, C.elegans worm studies and during the last 5 years in mouse models. The objectives outlined in this application represent the next and most pertinent step forward in our mouse studies, which is likely to provide novel insights into the impact of these factors on organismal biology and disease. All animals will be monitored closely (following NRCI guidelines) by experienced animal technicians to minimise their suffering during experimental procedures.

Project 24	**Molecular regulation of development**	
Key Words (max. 5 words)	Development, zebrafish, embryo, mechanism	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to increase our understanding of development and diseases at the molecular level. Developmental defects and diseases are remarkably common in both humans and animals and they frequently cause lifelong ill-health and premature death. The vast majority have no known cause, due to the often complex nature of their aetiology and no approved treatments. There is a significant need to the underlying mechanisms of these complex diseases in order to find new avenues of treatment. We model the disease in zebrafish and investigate those animals to find out how molecular pathways are disrupted, how this leads to the disease pathology and identify which aspects of the disease we should be targeting with 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)?	We expect to increase our understanding of several diseases, predominantly neurodevelopmental diseases that affect children and are occurring at an increasingly high rate in the population. This may allow us to propose new treatment strategies for these diseases.	
What species and approximate numbers of animals do you expect to use	We use the zebrafish as our experimental model. The embryos develop externally from the mother and do not become free-feeding and regulated under ASPA until 5 days post-fertilisation. The majority of the	

over what period of time?	diseases we model affect embryos at unregulated stages so most of our studies are not regulated. However, we do need to maintain and breed both normal and existing zebrafish mutant lines that model developmental diseases in order to study their embryos up to 5 days post fertilisation. These lines do come under regulation. At regulated ages, we expect to use 8000 genetically altered zebrafish over a 5 year period, of which all would be classified as non-harmful mutants. Any embryos produced by these zebrafish that are employed in experiments as opposed to breeding will only be used up to 5 days post fertilisation.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The non-harmful zebrafish mutants will be used for the production of embryos between the ages of 4 months-2 years (the period of most effective breeding). There are no harmful effects expected from this breeding strategy. Once the zebrafish have reached the end of their breeding life, they will be humanely euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Vertebrate development requires the co-ordinated growth, differentiation and movement of diverse tissues in time and space. While it is possible to model simple aspects of development in vitro, it is impossible to model the complex events that occur between tissues to form a fully functional embryo. Furthermore, pathogenic processes during disease, and attempts to treat them, involve many cell types and tissues and this cannot be recreated in vitro. It is for these reasons we must undertake experiments upon animals. Our species of choice is the zebrafish which are unregulated up to 5 days post-fertilisation. Most of our experiments are performed on these unregulated animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure minimum numbers are used, we will perform pilot studies and use power calculations to determine the number of animals required and take those numbers into account when deciding on the most appropriate assays to use.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	We use the zebrafish model as a substantive body of work shows that developmental processes are highly conserved between vertebrates and because it is the vertebrate model with the lowest neurophysiological sensitivity. Thus, data derived from our models can

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	be extrapolated to humans and other animals. The protocols to be used are all standard methods in zebrafish research. Due to the wide variety of resources available, we will use existing non-harmful mutants in our research. With the exception of the minor surgery of regenerating fin tissues in order to genotype, all of the treatments are non-surgical. Latest knowledge on analgesia will be applied.

<table>
<tr><td>Project 25</td><td>Investigating the pharmacology of traditional and nanoformulated medicines</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pharmacokinetics, nanoformulation, bioavailability, tissue distribution</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We aim to improve the effectiveness of human medicines by developing better ways of delivering the drugs to the sites in the body where they are needed to work. Drugs investigated will include currently used drugs and also new candidates for drugs. New drug formulations (the way in which drugs are combined with other substances) will be created and, by using animals to measure the concentrations of drug in the blood and tissues following administration of the drug, we will be able to predict which formulations would give us the best results in humans. The data using animals will be used to inform computer models which will inform future human drug trials.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The work undertaken in this project will improve our understanding of what factors in the body can influence how an administered drug works. An exciting area of research in this project will be the investigation of the benefits of using nanotechnology to improve the effectiveness of drug treating human diseases, a field called “nanomedicine”. Nanomedicine is when</td></tr>
</table>

	nanotechnology is used to create extremely small (in the nanometre range) formulations of drug which can improve the ability of the drug to enter the body and to allow for long-acting treatments. This reduces the need for patients to take multiple medications and also can lower the cost of treatment. Although some nanomedicines are already used in patients, some aspects of the technology are still in the early research stage and it is hoped that our research will benefit humans by pushing this research forward to be used in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats, estimated number 1430 over the 5-year project. Mice, estimated number 805 over the 5-year project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Rats and mice will be used. Some will only experience being administered a general anaesthetic, after which the whole experiment will be completed under anaesthetic and the animals will be killed without recovering consciousness. Other animals will receive doses of the potential new medicines being tested after which blood and urine samples will be taken at intervals to assess how the animals re-distribute the medicine within their bodies and how long it takes for it to be eliminated eg in urine. Body tissues will be collected at the end of the experiment after the animals have been humanely killed. The medicines to be given are not expected to cause the animals suffering and the sampling methods do not involve more than transient discomfort. Some animals will be anaesthetised in order to give the medicine by certain routes eg into a vein. At the end of the experiment some animals will be anaesthetised so that a larger volume of blood may be collected and then the animals will be killed without recovering consciousness. No animal is expected to experience more than what is regarded as 'moderate' adverse effects and most will experience only mild and transient discomfort.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project will involve the determination of drug distribution throughout tissues of the body, and the use of living animals is the only suitable approach for this task. Available non-animal models may not include all the cell types or immune molecules that

	may have an important impact on how the medicine is distributed in the body and how the body responds to it.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to assure the use of the minimal number of animals, we will use the following general principles: 1) Wherever possible we will use non-animal laboratory experiments which can give us information on the an investigated medicine 2) Computer programs will be used to simulate the human body, and where possible this system will be used to virtually test the effects and movement of the investigated medicine 3) We will use the correct statistics before any experiment to ensure that only the required number of animals are used 4) As a group we will debate study design and assess each other's experiments to ensure we arrive at the best possible design.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Refinements of our research with respect to animal experimentation include: 1) Tissues from one animal will be used for more than one experiment, maximising the data generated from each experiment while minimising animal usage. 2) Use of mice rather than larger species (rats) where possible.

Project 26	**Utilising MRI to probe physiological state**	
Key Words (max. 5 words)	MRI, contrast agent, diagnosis, disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We seek here to establish that the sensitivity of magnetic resonance imaging (MRI) can be improved so that molecules which feature in cellular processes can be monitored in living systems. We will demonstrate that the improved measurements allow far superior image quality to be obtained whilst facilitating the probing of both health and ultimately the treatment 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)?	By detecting key molecules associated with life we seek to develop methods to monitor a wide range of diseases and thereby improve our ability to both diagnose and treat them.	
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to study mice and rats and over the next 5 years and will use 400 animals.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	We will be injecting agents that have low toxicity and consequently rate the proposed work as being of moderate severity due to the level of harm that is expected to be experienced by the animal over the whole procedure. We expect the vast majority of animals to exhibit no harmful effects. The biggest risk	

end?	to the animal health is that of developing hypothermia when anaesthetised. We will combat this by a rigorous monitoring procedure. We plan both serial and terminal experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use living systems in order to assess the challenges of working with the contrast agents we seek to employ in our magnetic resonance imaging measurements. These studies are essential if the agents are subsequently to be used for the diagnosis of disease in both animals and humans. We cannot mimic the required physiological conditions by in vitro cell culture methods in order to validate these procedures.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our studies will involve the use of several contrast agents. We will start by examining the most promising of these. Experiments will be designed based on both our own findings and published data. We will then develop a standard operating procedure that sets key threshold values to parameters such as dose, signal-to-noise and magnetic state lifetime which reflect key parameters for the future diagnostic use of these methods. These thresholds will then be applied to the new agents in order to minimise the number of steps required to optimise their use. Where possible we will use serial measurements to reduce the number of animals further. Our results will allow us to gain a firm understanding of the number of animals required for procedural development and hence keep this number to a minimum. The methods that we develop will impact widely on the use of animals, and should lead to a reduction in the number necessary for future hypothesis led studies. We also expect our methods to dramatically improve the accuracy of any data that are collected in these studies by increasing the quality of information that can be collected.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	We seek to use mice and rats as part of this work. They will allow us to assess the ability of our new methods to produce chemically resolved images, to define the intrinsic concentrations of agents needed for detection, to optimise the relaxation effects of blood and to establish routes to follow metabolism. Because of the difference in animal size, we expect the rats to produce superior images, however, working with mice will enable use to produce optimise

(harms) to the animals.	protocols that will be needed for future clinical assessments; e.g. the early detection of cancer. We seek to introduce agents that are non-harmful, in conjunction with studies on known drugs. We therefore expect the animals to experience little physiological harm. MRI procedures will be completed with the use of anaesthesia (terminal or recovery) to reduce the harm to individual animals.

<table>
<tr><td>Project 27</td><td colspan="3">Podoplanin-CLEC-2 in Sepsis and haemolytic uremic syndrome</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">CLEC-2, Podoplanin, sepsis, HUS</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>~~No~~</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">To determine whether clot formation and inflammation in sepsis and haemolytic uremic syndrome (a collection of diseases that involve destruction of red blood cells and inappropriate clotting which leads to kidney damage) is regulated by the podoplanin-CLEC-2 pathway</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The potential benefit of this project will be fundamental understanding of sepsis and haemolytic uremic syndrome (a collection of diseases that involve destruction of red blood cells and inappropriate clotting which leads to kidney damage). By identifying pathways that regulate clot formation and inflammation which lead to organ failure and increased death in these diseases, we may reveal potential new targets that may be of therapeutic interest in the future.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Over 5 years, we would expect to use no more than 7,600 mice in total – 5,100 animals for scientific protocols and 2,500 to breed the genetically altered strains required.</td></tr>
<tr><td>In the context of what you</td><td colspan="3">Breeding of genetically modified animals – we</td></tr>
</table>

propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	expect adverse effects of mild-to-moderate severity. The induced lymphatic defects may lead to mild swelling of the paws, which from our previous experience is asymptomatic. However, if pain is observed, as evidenced by tip-toe walking, animals will be humanely culled. Induction of genetic modification within adult mice – we expect adverse effects due to tamoxifen administration of a moderate severity such as weight loss of up to 20% and reduced activity. This is reversed when normal diet is then re-started with little-to-no lasting effects once genetic deletion has been achieved. Sepsis models LPS-induced sepsis We expect adverse effects of a moderate severity such as weight loss up to 20%, reduced activity, fever and piloerection. These mice will be treated with pain relief and hydration when required. Any animal with weight loss of ≥20% and/or continued pain will be humanely killed. CLP-induced sepsis We expect adverse effects of a severe severity such as pain, discomfort, diarrhoea, piloerection, which will lead to huddling and very much reduced activity. These mice will be treated with pain relief and hydration throughout the protocol. The clinical behavioural score will be used to define human endpoints and in particular pilot experiments will be used to determine an appropriate monitoring regime (approx. every 4-6hrs or less if clinical score is high in previous visit). Any animal that is unresponsive to stimulation when handled will be humanely killed. Shiga toxin and LPS-induced Haemolytic Uremic Syndrome We expect adverse effects of a moderate severity such as weight loss up to 20%, reduced activity, reduced kidney function and piloerection. These mice will be treated with pain relief and hydration when required. Any animal with weight loss of ≥20% and/or continued pain will be humanely killed. Animals in all protocols will be humanely killed with or without having blood removed from a major vessel or the heart, which will only be performed

	under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No *in vitro* techniques are currently available that can fully replicate platelets, the major cell type that expresses CLEC-2 Currently no *in vitro* methods exist to model a full thrombo-inflammatory response, including blood vessel damage and clot formation which is associated with organ failure Animal studies are required to determine whether podoplanin-CLEC2 interaction is a mechanistic driver of clot formation and organ failure during clinical disease, which cannot be achieved using clinical samples.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical analysis to ensure that we use the minimum number of mice per group that will be informative will be performed. We are using a staged approach, involving pilot studies to ensure that an appropriate number of animals are to be used. Mice that are genetically altered only after birth will be used prior to any cell-specific genetically altered mice. This will ensure that we firstly identify a gene which shows an effect following our protocols, before generating multiple cell-specific transgenic strains. This will also minimise developmental defects in cell specific knockouts that could compromise the results. To maximise the information gained from a single animal we aim to take samples from the blood under terminal anaesthesia and then from multiple body sites post mortem.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The vascular and immune system of mammals is highly conserved with cell types and mechanisms well-maintained. The mouse has been selected because of established and reliable transgene technology and extensive literature sepsis and haemolytic uremic syndrome (a collection of diseases that involve destruction of red blood cells and inappropriate clotting which leads to kidney damage) models in murine strains with established and reproducible protocols due to the reliable reagents available.

	Inducible transgenic strains will be activated by the most refined interventions possible to minimise stress and pain. In CLP-induced sepsis, we will use a behavioural clinical score indicative of the extent pain and distress that each animal is experiencing to help ensure humane endpoints are maintained. In addition, pain relief and hydration will be maintained throughout the protocol to reduced pain and suffering. In addition, the possibility of using body temperature to predict severity following CLP-induced sepsis will be explore if validation of the equipment is acheived. Multiple blood samples will be taken from the same animal using saphenous vein bleeding to minimise pain. We are using a staged approach, involving pilot studies to ensure that monitoring regimes will be appropriate for each strain used, and that the time point is the minimum required to induce stable clot formation. Mice that undergo procedures and/or mice with uncharacterised genetic mutations will be monitored closely and appropriate action taken if they are deemed to be suffering. Animals will be humanely culled unless, in the opinion of the vet or animal welfare officer, suffering can be remedied promptly and successfully using no more than minor interventions, such as pain relief and hydration. Animals in all protocols will be humanely killed with or without having blood removed from a major vessel or the heart which will only be performed under terminal anaesthesia.

Project 28	**Fungal infection, diagnosis and therapy**	
Key Words (max. 5 words)	Fungus, infection, antifungal, therapy, diagnosis	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives are to investigate how fungi cause infection, including involvement of specific host and fungal factors, and to evaluate new drug treatments for fungal 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)?	Fungal infection affects around one fifth of the world's population at any single moment in time, causing skin, nail, vaginal, oral and bloodstream infections. These latter infections are occur mainly in severely ill patients and are difficult to diagnose, with only limited treatment options available. Understanding how fungi cause disease could identify potential molecules which could be used to design new diagnostic tools, and our role in evaluating new antifungal drugs could accelerate the development of new drugs for clinical use.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use the following species and numbers over the next 5 years: Species: mouse 9700 (includes 3000 GA animals), 100 rats, 80 guinea pigs and 50 rabbits. These are approximate numbers based upon our prior experience, currently funded grants, future grant applications and research plans. The majority of animals used will be mice, but small	

	numbers of rats, guinea pigs and rabbits may be used under specific circumstances, e.g. requirement for repeated blood sampling or a requirement for larger tissue samples for downstream processing.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be used to model fungal infection, with fungi administered via different routes depending upon the infection to be modelled. Some infection models require manipulation of the host, either through maintenance of oestrus (through surgery or oestrogen injection) or through immunosuppression. The host may also undergo blood sampling or imaging. Infected animals will be used to elucidate the role of fungal virulence factors on disease, to evaluate the efficacy of new drugs or new treatment regimens, or to investigate the role of the host immune system or prior infection on susceptibility to fungal disease. Through refinement of our models and monitoring procedures, the vast majority of our animals are classified as mild or moderate, with less than 3% of animals estimated to potentially be classified as severe. All animals will be humanely killed and sampled to provide additional infection-related data at the end of the experiment, enhancing the information gained from each experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Infection is the result of the ability of a pathogen to cause disease and the inability of a host to detect and fight disease due to alterations in the host immune system. Although we can model some steps in infection using laboratory-based model systems, such as fungal interaction with renal epithelial cells, which models the initial steps occurring in the kidney (we cannot yet fully model infection progression nor can we fully evaluate antifungal efficacy in the host without the use of animals. We will continue to monitor the literature for new laboratory models applicable to our area of research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use minimal numbers of animals in our work through careful planning of experiments and using the minimal number of animals in treatments and control groups, but which still allow detection of biologically relevant differences. We routinely use Power analyses to determine the minimum number of

	animals required for any experiment. We will continue to develop and evaluate new techniques to allow us to follow infection in individual animals, rather than sampling groups of animals at each of the time points of interest.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The majority of our work uses well-characterised fungal infection models, which we have been working with for over 10 years. The majority of these models are based in mice, with other species used only when larger biological samples are required as part of the study. We have already refined our models by significantly reducing the length of infection (3 days compared to 28 days). New models will be developed through pilot studies using small numbers of animals. We will minimise harm to animals by putting in place clear and careful monitoring systems. Anaesthesia and pain relief will also be administered where appropriate and procedures will only be carried out by highly trained and competent personal licence holders (trained postdoctoral scientists and PhD students from my research group and the technicians based in the animal facility).

Project 29	**Nuclear reprogramming by Xenopus eggs and oocytes**	
Key Words (max. 5 words)	nucleus, reprogramming, Xenopus	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective is to understand the molecular mechanisms of nuclear reprogramming by Xenopus eggs and oocytes. It is unknown what molecules in an egg are needed for nuclear reprogramming.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Improved possibilities for cell replacement in humans for diseased or aged tissues. If we could identify normal components of eggs that have a reprogramming capacity, these molecules might be obtained from natural sources and might be used to help reprogram cell nuclei without the need for nuclear transfer.	
What species and approximate numbers of animals do you expect to use over what period of time?	Xenopus laevis, 1900 over 5 years	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No adverse effects expected from any of the procedures that will be undertaken or from any of the genetic alterations that may occur. At the end of procedures animals are either killed or will be returned to the normal stock tanks and re-used.	

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No alternative non-animal based models currently exist that can be used for this study. Reprogramming is not successful for the eggs and oocytes of any animals other than those of Xenopus laevis, when the aim is to analyse molecular mechanisms. Eventually we might be able to use purified chemicals from natural sources and so reduce the number of oocyte experiments needed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We could reduce the need to use animals if in vitro extracts of eggs and oocytes could be made effective. There is no prospect of this at present. The use of animals will be the minimum sufficient to obtain scientifically valid data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Only the oocytes of Kenopus have a sufficiently large germinal vesicle for these experiments. Fertilized Xenopus eggs provide a more efficient test of our procedures than can any other animal. Note that the material available from one egg of Xenopus is equivalent to 4,000 eggs of a mouse or other mammal, All the experimental procedures will be of minimal invasiveness. Progress towards refinement would include the use of purified molecules from natural sources to replace the need to inject oocytes.

Project 30	**Complex mammalian responses to alkylating agents**	
Key Words (max. 5 words)	DNA damage, DNA repair, metabolism, alkylation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Exposure to environmental chemicals that can damage our DNA, the long polymer that makes up our genes, pose a significant threat to human health. If not repaired appropriately, DNA damage reduces cell survival. DNA repair mechanisms protect against DNA damage and are important in preventing disease, especially diseases associated with ageing, like cancer and neuronal degeneration. However, chemicals that damage our DNA are also used in the clinic, for the treatment of cancer. Therefore, DNA damage is important for the causation of cancer but also for the treatment of cancer. The objectives of this project are to understand how DNA damage triggers cell death, the role of cellular metabolism in survival to DNA damage and how cellular division or proliferation influences cellular responses to DNA damage.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary potential benefit relates to the generation of novel knowledge about the mechanisms used by mammalian organisms to respond to physiologically relevant DNA damage with emphasis on understanding DNA damage-induced cell death and the role that DNA repair plays in this process. The public will benefit from the outcomes of our research firstly by the advancement of scientific knowledge	

	(dissemination of results during the lifetime of the project), and longer term because of the potential to improve health and well-being with concrete implications for neurodegenerative diseases and cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	The species used is the mouse, *Mus musculus*. I expect to use a maximum of 2000 animals over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We propose to treat animals with compounds that modulate DNA damage levels using non-toxic doses. The treatment will be done by injections into the abdominal cavity and not expected to cause any harm or lasting pain. We will also modulate the amount of light to which the animals are exposed. We do not expect harm or adverse effects and the likely/expected level of severity will be mild. Genetically modified animals generated (protocol 1) will be used either for experimentation (protocols 2 and 3) or killed by humane methods at the end of their breeding cycle. Animals used in regulated procedures (protocols 2 and 3) will be killed by humane methods at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The study of the effect of a particular protein in a mammalian organism is best achieved through studies using genetically modified animals, where this protein has been removed. A protein is a substance produced by a gene that is involved in creating the traits of the body, such as hair color, or is involved in controlling the basic functions of the body, such as DNA repair. A gene is the biologic unit of heredity and made of DNA. Studying the mechanisms regulating mammalian DNA repair, as well as the effects of DNA damage induction requires *in vivo* work for recording whole-animal behaviour and assessing the tissue-specific differences in the response to DNA damage.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Periodic review of our breeding scheme as well as careful statistical analysis to determine optimal treatment group sizes. Power calculations are also used for sample size estimation.
3. Refinement	The mouse is the species of choice because it allows for genetic manipulation of its genome. Mice also

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	represent a mammalian system that is well characterized and available in defined genetic backgrounds, greatly reducing the number of variables that could influence experimental outcomes. We improve animal's welfare by enriching their environment by providing shredding, chewing and tunneling materials. We minimise welfare costs by using a refined treatment which we optimized in a previous programme of work and which was shown to lead to no measurable harm to the animals.

Project 31	**Polyclonal antibody development**	
Key Words (max. 5 words)	Polyclonal, Antibody, Immunisation, Rabbit, Sheep	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Production of critical binding reagents for use across the spectrum of scientific applications from fundamental research in human and animal healthcare, through to applications in diagnostics and therapeutics.	
What 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 academic applications, the binding reagents will assist in the fundamental understanding of complex biological events, including post-translation modifications. For applied research and product development, binding molecules will underpin advances in early disease detection and modification, and substantive advances in molecular therapeutics and imaging.	
What species and approximate numbers of animals do you expect to use over what period of time?	The target species are sheep, rabbits and chickens, on the basis of their ability to create antibodies of exquisite affinity and specificity towards small molecules, defined epitopes of larger molecules and post translational modifications. 1500 sheep, 1400 rabbits, and 200 chickens 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	The level of severity is Mild for all species. Adverse reactions are unlikely, but may be expected to be localised to the site of injection, manifesting as	

level of severity? What will happen to the animals at the end?	slight swelling and inflammation. Animals at the end of a procedure, if deemed fit and healthy may be entered into the breeding group, or non-immunisation blood donation group for serum protein collection. Alternatively, rabbits would be euthanised using a schedule one method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Despite the emergence of non-antibody binding molecules that are created through molecular biology techniques, applications and adoption of the molecules is insufficient to replace the need for antibodies. Further, with the dynamic shift in fundamental biologic research towards post-translational modifications, there is a growing demand for highly specific antibody reagents to underpin discovery and product development.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A major drive towards exploiting the tremendous capacity of the immune system will aim to validate the utility of multiple antigen immunisation. If successful, this practice would markedly reduce the numbers of animals used in producing high quality antibody reagents. Continued refinement of protocol will increase the rate of success with challenging antigenic sequences reducing the frequency of repetition and elongation of protocols.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Cross-bred animals retain a healthy vibrant immune system that produces antibodies of exquisite performance. Keeping these animals in open floor pen holding and in stimulating environments as close to natural as is possible helps maintains health and diversity. Stress free, comfortable animals produce the best and strongest immune responses. Close working and handling of animals in open social groups will enable prompt detection of a reduction to health. Highly qualified technicians and veterinary support will ensure any deviation from health will be detected immediately.

Project 32	**Mechanism underlying diet induced metabolic disorders**	
Key Words (max. 5 words)	High Fructose, high fat, sucrose, ketohexokinase, NAFLD	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There are more than 3000 rare inherited human disorders with the genes still being unidentified in most cases. Despite their rarity, these conditions often offer unique insights into gene function and the cause, development and effects of disease. When combined with the ability to generate and study a mouse mutant of the corresponding gene, very rapid increases in understanding are possible. Even after the gene is identified, the mechanism by which mutations lead to the pathology in each disease is often not apparent. To remedy this and develop rational therapeutic approaches, two downstream routes of investigation are particularly valuable. One is genetic, the subject of this project licence, the second is structural analysis of the gene product, with a view to drug design. These approaches are complementary and the *in vivo* disease models, created by gene targeting in mice, are essential for testing the conclusions of *in vitro* structural studies. The combined approach offers the best chance of leading to new therapies and so to an improvement in human health. Fructose is a major dietary sugar. Various studies over the years have shown both that small amounts of dietary	

	fructose can have positive effects in healthy and diabetic subjects but chronic high-fructose diets (as common in the West) increase the likelihood of hyperglycaemia, hyperlipidaemia, obesity, non-alcoholic fatty liver disease (NAFLD) and diabetic complications. Our primary interest is to understand the role of specific genes in these disorders. Metabolic experiments will be carried out on genetically modified mice to study the role of fructose and nature of fructose metabolism in metabolic disorders alongside study of the function of certain genes in rapid clearance of dietary fructose from the circulation.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This study may provide insights into the development of fatty liver and associated metabolic disorders; and also identify potential biomarkers or targeted therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice; maximum of 5000 (maintenance of colonies and experiments) over 5 years of time
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Transgenic mice will be maintained to provide the animals required for the studies. Mice (males and females separately) will be randomised into different groups and fed on a range of normal to high-fat and high fat high sucrose diets. Mice receiving low calorie diets may have restricted growth, these animals will be monitored and weighed and will be killed if found to be suffering unduly. Adverse effects due to diabetes or related diseases will be monitored using a diabetes score card system which will be modified, by experience, to identify at an early stage and therefore minimise any animal suffering. Administration of glucose may cause transient discomfort during the injection but has no other adverse effects. Administration of an accepted dose of insulin has no adverse effects other than transient mild discomfort during the injection. Hypoglycaemia, if proven by blood testing, will be treated by administration of glucose and withdrawal or reduction in dosage of the drug. Animals exhibiting any

	abnormal /harmful effects will be killed humanely. At the end of the experiment, animals will be killed humanely and the tissues and blood will be used for biochemical studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In this project we are investigating disease and its developmental mechanism which involves complex interactions of different genes in different cell types in the whole body. We have considered in vitro systems like cell cultures and micro-organisms like yeast and bacteria. Such systems cannot replicate the complexity involved in metabolic and physiological disorders. Physiological effects in species other than mammals may not be relevant to human metabolic disorders such as diabetes, fatty liver and cardiac diseases. Therefore, a mammalian model is required for understanding the metabolic relevance of the system under study.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Analytical procedures will be optimised, using available frozen mouse tissues, and cell lines, before use on new transgenic mouse tissues. This study is designed based on the previous experience. Wherever suitable, cell lines will be derived from transgenic animals for further detailed investigation. The experience of well-trained staff will ensure that the lowest number of animals possible will be used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse has been proposed in this study because of its (1) close genetic and physiological similarities to humans (2) natural development of diseases that affect the systems, including liver, heart and brain. (3) lowest degree of neurophysiological sensitivity and ability to multiply faster, reproducing as often as every 63 days. A score card system specific to the project, has been developed to assist in identifying any adverse effects at an early stage.

	For DNA genotyping, the mildest appropriate method of sampling will be performed under general or local anesthesia. Animals showing adverse effects will be killed humanely.

Project 33	**Pathophysiology of the unfolded protein response**	
Key Words (max. 5 words)	Protein misfolding, Endoplasmic reticulum, Unfolded protein response Protein Chaperones, Diseases of aging	
Expected duration of the project (yrs)	Five years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Proteins are linear polymers of amino acids and are essential components of all living things. To function properly proteins need to be maintained in their correct structure, which, in the case of a linear polymer that folds onto itself to create a defined three-dimensional object, means that protein folding must be maintained. Some of the most prevalent diseases affecting the world's aging population are associated with protein unfolding and misfolding. But to date the insights into mechanisms of diseased progression have not found their way into therapeutics for neurodegenerative, metabolic and inflammatory conditions associated with protein misfolding. Mounting evidence suggests that one way to attack these common conditions is to tweak the normal defence mechanism cells deploy to cope with proteins that misbehave. Experiments conducted in cultured cells and in simple organisms have suggested ways this might be achieved, but to progress further with such promising leads we need evidence that these manipulations of the cell's defences are relevant to wellbeing of complex	

	animals. This unmet need can only be addressed by performing regulated experiments in organisms that resemble us humans in the way they cope with the protein folding problem.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Demonstrating the utility or futility of a given manipulation of the cells' defence against protein misfolding diseases in a relevant animal model is crucial to pointing the way to develop fruitful therapeutics and away from fruitless ones. And are the cornerstone to develop drugs in the future
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats are close enough to us to be physiologically relevant, obviating the need to experiment on even more complex and sentient organisms. We anticipate the need to use up to 5940 rodents (5715 mice and 225 rats) spread over five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most experiments proposed will lead to no discomfort beyond that experienced by any rodent bred in captivity and residing a modern animal facility. The most demanding experiments involving surgery, genetic alteration, compound administration and food deprivation will lead to no more than MODERATE transient discomfort to the animals and these will affect less than ¼ of the total animals we expect to use. The animals will be humanely killed at the end of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst experiments with simple organisms (flies, worms) and cultured cells can (and will) suggest ways in which tweaking the normal defence mechanism cells deploy to cope with proteins that misbehave *might* be beneficial as treatment of human disease, it is impossible to justify the considerable resources needed to develop these ideas into drugs, without validation experiments in a relevant organism. Rodents share with us many of the same adaptations to rogue proteins and thus represent the simplest, practical, meaningful system to gage the physiological implications of any therapeutic approach suggested by non-regulated research.
2. Reduction Explain how you will assure the use of minimum numbers	Power calculations will be made to ensure that what is deemed a-priori to be a significant effect can be detected with the number of animals assigned to that

of animals	experiment. This quantitative assessment ensures that the minimal numbers of animals are used and that animals are not squandered on underpowered experiments. The most important aspect of reduction is a strategic design feature: We will only progress to test in rodents ideas that are soundly supported by cell culture experiments or experiments with simpler organisms. The experiments in rodents too will be structured as funnel with shorter and simpler experiments at its top; testing (and rejecting) ideas/hypothesis with very few ideas/hypothesis percolating to the bottom, where longer and more complex experiments will test only those hypothesis that have passed the earlier stringent rejection criteria. Thus the burden to experimental animals is matched to the potential benefit to humans and animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats are close enough to us to be physiologically relevant, obviating the need to experiment on more complex and sentient beings. The use of mice is predicated on the existence of well-developed techniques to manipulate them genetically, which is necessary to critically test hypothesis and on the existence of well validated mouse models of human diseases associated with misfolded proteins. To minimise animals suffering we will exploit, when needed, the capacity to limit the effect of a genetic manipulation to certain cells and tissues in mice (so called conditional genetic manipulation). This may limit the harmful effects of a mutation, whilst enabling the study to go forward. Rats will be used only in those experiments where large amount of tissue are needed for biochemical analysis.

Project 34	**The immunobiology of mycobacterial infection**	
Key Words (max. 5 words)	Tuberculosis, Mycobacterium, vaccines, chemotherapy	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Tuberculosis is a major threat to global health and claims about 1.5 million lives per year. Current drugs are effective in treating infections, but there are an increasing number drug resistant infections, and the treatment takes six-months and has unpleasant side effects, making the development of new drugs a priority. The current BCG vaccine works in children but is not effective against adult tuberculosis in those parts of the world with the highest incidence of disease. In addition the WHO estimate that 2 billion people carry latent *M. tuberculosis*; a reservoir of infection that will be with us for many years to come.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Given the major threat of TB to global health, there is an urgent need for new vaccines to prevent active disease, as well as to prevent reactivation of latent infection. New drugs to treat latent infection and/or shorten treatment are also a priority. The aim of our research is to understand the biology of tuberculosis in terms of the interaction between host and pathogen with a view to improving control of Tuberculosis, and to test new vaccines and drugs using our bioluminescent system where possible.	

What species and approximate numbers of animals do you expect to use over what period of time?	We use the mouse as a model of Tuberculosis, and will use a maximum of 2,500 mice over the 5 year period. Up to 1,000 are used for pathogenesis research, 1,000 for therapeutic intervention research and the other 500 for breeding purposes.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will infect animals with the bacterium that causes Tuberculosis in humans, *Mycobacterium tuberculosis,* and this is classified as moderate severity. In practice we monitor the animals for evidence of symptoms e.g. ruffled fur, abnormal respiration, reduced mobility and changes in body weight, and stop the experiment before they reach this advanced stage of disease. Animals are humanely culled at the end and we use the tissues and organs to measure what has happened during the infection and/or treatment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The interactions between bacteria and the infected human are very complex and at the moment we need to use animals to study the progress of an infection and how best to either prevent it with a vaccine or treat it with a drug. Current non-animal alternatives cannot reproduce all of the complexity in this system. Our collaborators have made some progress in the development of *in vitro* granulomas to monitor the early stages of infection and the factors involved; where possible will use this model as a replacement. The inbred mouse model used here is well characterised and clinical signs of infection are readily recognisable; it also causes the least overall harm compared to other animal models of tuberculosis whilst still achieving the objectives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our bioluminescence imaging tools allow us to detect bacteria within intact living animals in real-time. This results in a substantial reduction in both the numbers of animals used during experiments and the level of suffering.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	The mouse model causes the least overall harm compared to guinea pigs, rabbits and non-human primates, which experience more severe disease. Inbred mice are widely accepted as models for human infectious diseases; their homogeneous genetic background reduces the number of animals required, compared with outbred animals. The microorganisms used will include laboratory and clinical isolates as well as attenuated bacterial strains

(harms) to the animals.	such as BCG, to ensure the best picture is obtained of bacterial virulence and the progress of infection. All animals will be housed in groups with appropriate environmental enrichment and fed according to current 'best practice' at Imperial College Central Biological Services. Monitoring animal weight and observing behavioural changes during the course of infection allow us to put in place humane endpoints to minimise animal suffering. Bioluminescence imaging helps in this, since we can monitor the progress of infection and/or treatment and terminate experiments as early as possible thus minimizing harm.

Project 35	**Understanding how parasitic worms (roundworms) have evolved and developing new drugs to treat diseases caused by them**	
Key Words (max. 5 words)	Parasite, Trichinella, novel drugs, anthelmintics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Roundworm parasites are some of the most successful parasites and have a huge impact on human and on animal health. The World Health Organisation estimates that more than 2 billion people carry infections with these parasites, with the majority of these corresponding to soil-transmitted gastrointestinal infections. Parasite infections cause increases in morbidity and mortality; the majority of debilitating worm infections occur in the tropics, where they exacerbate the effects of poverty and malnutrition. Worldwide most domestic animals, especially livestock, carry worm infections and this has an enormous impact on the animals themselves but also on the economic sustainability of rearing livestock. The drugs that are used to treat roundworm infections have been available for decades but the use of the same, limited set of drugs for many years has led to the development of resistance and there is now an urgent need to develop new drugs. The aim of this project is to understand how these parasites have evolved, why they are so successful and how we can treat or prevent diseases. We are doing this by identifying genes that are specific to roundworms and studying how these genes regulate the growth, development and reproduction of the worm. Using	

	this information we have developed a method for identifying potential new drugs that would kill or debilitate the worms without affecting the human or animal host. The knowledge we will gain from working with this worm will be applicable to other roundworm species and, in some instances to flatworm parasites as well.
What 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 two benefits arising from this work. The first is that we will have a better understanding of how these parasites have evolved and how their development is controlled. The second is that the method for screening that we have developed will identify candidate drugs or molecules that can be developed into effective treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	We infect predominantly mice (a maximum of 200 over a 5-year period) and occasionally rats (maximum of 60 over a 5-year period).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals are orally infected with the worm, which is a parasite of the intestine in the early stages and of the muscle in the later stages. When in the intestine it causes symptoms similar to mild gastroenteritis that can last for a few days; while in the muscle it may cause some mild muscle stiffness.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This is a parasite and requires an animal host as it cannot be grown outside of an animal. Animals are used to produce the parasites on which we carry out our investigations. Therefore, the large majority of our work is carried out in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use the minimum number of animals to maintain the infection and provide sufficient parasites to study the effects of drug exposure and to be able to study the parasite genome and gene expression. Having worked with this animal parasite model for many years we determined the maximum infective dose required to establish good infections and the minimum numbers of animals required to ensure recovery of sufficient parasites to carry out the in vitro studies.
3. Refinement Explain the choice of species and why the animal model(s)	We use an infective dose of parasites that is just sufficient to establish an infection and minimise adverse effects. We use older and larger animals,

you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	who can tolerate the parasite better and this also reduces the likelihood of adverse effects. The work involving animals is carried out by very experienced technicians and the animals are closely monitored to ensure the severity of the procedure does not exceed a mild severity A Named Veterinary Surgeon is on site for the vast majority of the time to provide advice should this be required.

Project 36	**Immune regulation of tissue repair in ageing**	
Key Words (max. 5 words)	Immunity, senescence, ageing	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The Aim of this project is to understand how the immune system regulates tissue repair during health and how it becomes dysfunctional with ageing, with a view to developing new ways to promote tissue repair and function in the elderly.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	**1: Identify which** immune cells are dysfunctional with ageing and impacting on tissue repair. This is worthwhile, as it would directly identify which cells we can target therapeutically to promote tissue repair and ameliorate age-related degeneration and disease. **2**: **Determine** the relative contribution of cell autonomous and non-cell autonomous factors contributing to this dysfunction. This is worthwhile, since it would further clarify if the cells identified in 1) are affected due to intrinsic defects or due to altered micro-environmental factors, which we need to understand in order to devise a therapeutic strategy. **3**: Test if promoting immune-system function can rescue tissue repair**,** by **3.1)** genetically manipulation and **3.2)** testing selected therapeutic drugs that may be used for clinical translation in humans. Genetic manipulation of a particular mechanism is	

	necessary to provide proof-of-principle evidence that such mechanism is a worthwhile target for promoting tissue repair, and warrant further drug testing and/or new drug development for therapeutic strategies. A pilot screen with candidate-driven drugs that are most of them used in the clinic is worthwhile, as it would find a new use for an already approved drug, which would speed up translation into the clinical setting.
What species and approximate numbers of animals do you expect to use over what period of time?	I will use zebrafish as an ageing model because I have shown that, similarly to humans and in contrast to inbred lab mice strains, removing telomerase from zebrafish leads to premature ageing in the first generation. The total number expected to be used are 16000 fish over the course of 5 years, but most will be used for breeding many of which will be used in experiments before the fish reach an age when they come under the Act. Of these 16,000 fish only 4,500 will be used in more invasive procedures in fish of an age where they are protected under the Act.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most animals will be used for breeding and generating transgenic and/or mutant lines. To understand how tissue repair is impaired with ageing and the interplay with the immune system requires animals to age, whether naturally, or in the presence of mutations that mimic premature ageing syndromes in humans. During the course of their life-span premature aging will result in weight loss, this work will identify any correlation between this weight loss and inflammation and muscle wasting. This, however, will only happen to maximum 50% of the animals used in this project. Animals will be closely monitored, particularly after the age of 9 months when weight loss is accelerated. Fish will be grown to selected time-points, where they may: - Be culled for post-mortem analysis of fixed tissues or isolated cells from selected tissues. - Undergo specific tissue regeneration challenges and culled at selected time points post-procedure for post-mortem analysis of fixed tissues or isolated cells from selected tissues. - Undergo selected drug treatments aimed at improving tissue regeneration and culled at selected time points post-procedure for post-mortem analysis

	of fixed tissues or isolated cells from selected tissues.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Understanding tissue repair requires *in vivo* studies using an animal model, since these are processes that depend on the interaction between different types of cells in a tissue and organismal context, meaning it cannot be modelled in any meaningful sense *in vitro*. We will, however use Integrated *in vitro (for testing defects in single cells)* and *in silico* work alongside *in vivo.* Furthermore, simpler questions will be, whenever possible, addressed in larvae forms. Where studies have been done in other systems, and the data available, these studies will not be repeated in this model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have sufficient pilot data to perform *a priori* power calculations to estimate group sizes-generally maximum of 6 per group. When this is not the case, pilot experiments with few animals will be performed first. We will obtain data for multiple tissues of the same individual at the same time and, when possible, over-time. Experimental versus control groups will be siblings whenever possible to reduce variability, thereby reducing the number of animals required for achieving statistical significance.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	This is a model I pioneered in characterising, not only in a premature ageing mutant line but also in naturally aged wild type zebrafish. This means I have sufficient data and assay expertise to minimize potentially harmful side effects caused by technical inexperience and to estimate the number of animals required for the different assays, thereby minimizing surplus for most of the proposed procedures. All procedures described have been extensively used and/or described by me or by collaborators, which again minimizes potentially harmful side effects caused by technical inexperience and minimizes the number of animals required for the different assays. There are no severe procedures predicted in this research programme. Procedures will be tested first in few animals in pilot

	experiment (up to 3 of each experimental and control group).

Project 37	**Control of Central Nervous System Autoimmunity**	
Key Words (max. 5 words)	Multiple sclerosis; neuroprotection; repair, autoimmunity; spasticity	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Multiple sclerosis (MS) is disease of the brain and spinal cord affecting over 2.5million people worldwide and costing the UK economy over £1 billion pounds per year. Over 6,000 people in the UK are diagnosed annually and most will be unemployed, divorced and severely disabled within 25 year of diagnosis. This is one of the few diseases that Society considers to be eventually worse than death. We believe that effective treatments will delay the development of disability. Whilst (1) relapsing neurological attacks in MS have some treatments, which have significant side effects, there are no treatments to slow (2) progressive nerve loss or promote (3) repair agents. Furthermore (4) symptom control agents have side effects that limit their use. We are part of a team aiming to develop treatments in each of these four areas for MS, and use of animal models of MS is one part of this objective. We aim to examine a target pathway for each of these research areas such that we can support development of these treatments to become real drugs, whilst uncovering the biology of MS. Using these approaches we have generated ideas about treatment, which were moved from animal studies to human benefit. We aim to do this again	

	under this licence and use the animal studies to find new treatments and support the development of drugs that we already think will be of benefit 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)?	We aim to identify pathways to treatments that slow or stop autoimmunity, in a way that is relevant to many conditions diseases. We can identify pathways to treatments to control loss of nerves, which are relevant to many human neurodegenerative conditions and we can identify pathways to repair damage from MS and other diseases and we can identify pathways to control neurological symptoms. We will support development of treatments hopefully leading to human benefit.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and expect to use about 13,500 mice (most used in breeding experiments to maintain the mouse colonies) over 5 years. These will be used for the development of treatments to control neurological disease using models of ascending severity in a 3Rs relevant structure. Only approximately 500-750 mice a year will be used in experiments relating to severe neurological disease.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We aim to induce and treat autoimmune disease of the nervous system in mice. We can do this in two ways. We will inject nerve or eye proteins under the skin in such a way that it stimulates the animal's immune system to attack its own nerves, or in some limited cases the retina. Alternatively we will use an animal whose immune cells are genetically-programmed to attack the nerves and stimulate them to migrate into tissues to the body. In both cases when the white blood cells get into the nervous system they trigger a disease cascade causing altered/blocked nerve signalling. This can cause a variety of neurological signs. In some cases this may mean transient loss of vision, which does not really affect the mice, as vision is not a major sense in mice as they live in the dark. Alternatively this can lead to transient tail and hindlimb weakness and sometimes limb paralysis and loss of feeling, due to the nerves not transmitting impulses or damage to the nerve cells. The animals can develop increasingly severe neurological! movement disability that accumulates with time as the number of attacks increase, which can occur every 3-5 weeks, until slow continual nerve loss occurs and relapsing attacks stop, just as happens in MS. Some of these signs of disease, such as bladder incontinence and muscle spasms only

	become manifest after the occurrence of significant disability in both animals and humans. We use a number of less-severe models, which show no neurological problems, to address issues such that use of animals with severe problems are avoided. At all stages of the disease we have defined endpoints that are specified in the licence to ensure that animals are killed before their suffering may exceed what has been defined as appropriate for the aims of the study. We work closely with people affected by disease and are open about our animal studies and openly discuss their value and their limitations, which we seek to minimize. The animals will be culled as required by Law at the end of the experiments and tissues used to compare the effects seen in humans.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives do not offer the complexity of studying and modifying an disease caused by the immune system that causes nerve damage to the nervous system, which can be used to identify and develop treatments of multiple sclerosis. We are developing “replacement” alternatives for some aspects of the project and use animals, when alternatives do not exist to address the issues
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use animals to test hypothesis and our experiments contain sufficient animals to determine whether treatments work or do not work. The numbers used are based on statistical advice and supported by considerable experience that we have using the models. We have developed systems that are reproducible and can translate animals work into human benefit. This reproducibility allows us to reduce the number of animals used experiments. In addition to 3Rs benefits it also makes no economic sense to use animals when they are not needed
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We are working with some of the few animal models in the World that shows the full complexity of the human condition. We identify the problems in human biology that we need to address; we generate the experimental tool such as a cell culture or animal model to investigate the issue. We have then been able to move a/the solution back to create human benefit. Some of the models have been refined such that we can obtain information without the severity of many multiple sclerosis models used by others. We have refined our studies to minimise animal and human costs to produce data that is relevant to the

	development of pharmaceutical drugs and created studies that address unmet clinical needs.

Project 38	**Studying macrophage plasticity in inflammatory disease**	
Key Words (max. 5 words)	Macrophages, inflammatory disease, genomics/genetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Inflammatory conditions comprise a broad range of diseases where current therapies lack specificity. For instance, when the filtering unit of the kidney (i.e. the glomerulus) becomes chronically inflamed and there is a risk of kidney failure, current therapies are mainly based on corlicosteroids, which are non-specific anti-inflammatory agents that affect many tissues within the body causing adverse effects such as weight gain, bone thinning, diabetes and high blood pressure. There is a scientific need to understand how the inflammation is caused (what are the different steps, mediators and their relative importance) in organs/tissues such as the kidney, lung, skin and the joints. Understanding inflammation is the first step in designing new therapies that would target the inflammation alone, rather than having off- target side-effects. This project's objectives are to understand how a specific cell type called the macrophage drives inflammatory responses causing damage to tissues. This cell is present in almost all inflammatory reactions and its activity can be modulated from pro-	

	inflammatory to anti- inflammatory. This suggests the macrophage is a promising target for development of new, more effective and safer therapies for inflammatory 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 potential benefits likely to derive from this project are as follows: 1. Understanding how the macrophage behaves in diverse inflammatory conditions will be crucial in identifying and targeting molecules that are specific to this cell type. 2. There is an increasing research focusing on the macrophage world-wide. This cell fascinates scientists because it can have a broad activity range referred to as plasticity. Depending on the tissue environment, this cell may exert either toxic or anti-inflammatory effects. Understanding the function of the macrophage using animal models of human disease will be important in determining its complex role in inflammatory disorders in humans. 3. The results obtained from this project will help the scientific community in general as part of the collaborative effort focusing on understanding the macrophage role in disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice — 4700 /5 years Rats — 7300 /5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Our protocols have been designed to reach a moderate level of severity at the maximum. Every animal undergoing recovery surgery will receive adequate and timely painkillers to reduce pain or discomfort after the procedure. Signs for infections will be dealt by applying the appropriate treatments and immune-compromised animals will be held in designated facilities. Animals will be humanly killed using approved methods at the end of the protocol. Any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health deteriorates significantly will be killed by a Schedule I method. All the procedures will be carried out while working closely with experienced animal care staff.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of animals is essential for our work, since it is only by study of the intact animal that we will be able to work out how inflammatory disease is caused and what therapeutic approaches might be of benefit. We also use cell culture experiments, where possible, to address specific questions about cellular mechanisms of disease, but this cannot reproduce the complexity of the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The models of inflammatory disease which we have developed are generally extremely reliable and reproducible, so we can obtain scientifically significant results from small groups of animals. As stated above, numbers are also reduced by using in vitro methods where applicable.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	This application includes both rat and mouse models of inflammatory diseases, since each species has advantages for certain studies. One advantage of mouse models has been the availability of a wide range of genetically modified mice which can be used to determine the effect of specific genes on the disease model being studied. However, there have been major advances in rat genetics over the last few years, and several genes involved in macrophagedependent inflammatory disease in the rat have been shown to be relevant in the related human disease. Also, recent advances in techniques mean that specific genes can be deleted in rats in order to study their relevance in particular disease models. We therefore wish to generate and breed genetically modified rats in order to investigate the more accurate models of human disease that can be produced in the rat as compared with the mouse. Because of the application of 3 Rs in all of our studies involving inflammatory disease in experimental animals, we assess the outcome of the experiments by using histology, at a relatively early stage of disease such that no animals suffer from the clinical effects of prolonged inflammation (i.e. kidney or lung failure. Any animal which becomes unwell during the course of the experiments, for any reason, will be humanely killed. Another aspect of refinement is the usage of recently available data-rich techniques such as Next generation sequencing methods. In recent years, we have collected and analysed these kinds of data from

	multiple tissues from single animals in various models of inflammatory disease. We have established a data analysis pipeline which allows identification of molecular targets in silico using computational methods. This minimises experimental approaches by offering a hypothesis-driven focused strategy in a specific model involvinq the macrophaqe.

Project 39	**Regulation of cell polarity in vertebrate development**	
Key Words (max. 5 words)	Embryo, development, cells, regenerative medicine	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Repair or replacement of diseased or damaged tissues and organs is an achievable but currently distant objective for most tissues. One of the major challenges is to reconstruct the normal architecture of tissues. The basic biology of how normal tissue architecture is generated by cells during embryonic development is the major area of investigation of this project. In particular, how does tissue achieve organised shapes by directional growth? How do the cellular components we know of that control cell polarity organise polarised cells in intact tissues? How do these relate to the action of chemical signals that influence embryonic development in general?	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding how tissues get their shape (morphogenesis) will be the basis for fine-tuning reconstructive, reparative and regenerative therapies of the many organs and tissues of the body where structure matters.	
What species and approximate numbers of animals do you expect to use over what period of time?	We use Xenopus frogs. Typically, about 100 females will undergo procedures each year, but provided they remain vigorous and healthy (which they usually do) they thrive and can be re-used over many years. About 100 males are sacrificed as this is needed to	

	be able to do in vitro fertilisation.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There are no adverse effects of the ovulation procedure as such, although there is always the risk of the occasional (although rare) infection or physical injury in the course of handling individuals in a population. Usually, this can be treated through standard veterinary care, but animals showing any signs of untreatable stress will be sacrificed by an approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Development of tissues is a complex process and cannot be reliably mimicked by cells grown in a dish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce the numbers of animals needed for the research by choosing Xenopus frogs, which lay hundreds of eggs at a time. We mimic natural mating stimuli and frequency to maximise the yield without stressing them. Re-using the animals after ample rest periods means that they do not have to be killed as a result of the procedure. Animals have been know to live and thrive under these conditions for many years.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Frogs are tetrapod vertebrates, like us, and they have been used for the investigation of embryonic development for over one hundred years. In this time it has been shown that the basic mechanisms we are interested in are shared between frogs and humans, making this a great model system. Not only the fecundity but also the large size of the embryonic cells, the ease of doing embryonic surgery, the ability to track cells and inject them with gene expression-altering reagents and the large knowledge-base surrounding this species make this the most refined system for these investigations.

Project 40	**Treatment of abnormal retinal development**
Key Words (max. 5 words)	Mice, Albinism, L-DOPA, Treatment, Vision
Expected duration of the project (yrs)	5 year(s) 0 month(s)

Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions 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)	Albinism is a condition characterised by pigment deficiency and abnormal retinal development, resulting in visual impairment. Unfortunately, there are no treatments available for infants and young children affected by albinism. Normal retinal development is known to continue at least until 5 years of age and there is evidence to suggest that retinal development in children with albinism is also ongoing, although occurring more slowly and in an abnormal pattern in comparison to normal controls. L-DOPA, a signalling molecule which is essential for normal retinal development is deficient in albinism. Exactly how L-DOPA deficiency results in abnormal retinal development is unclear. It is also unclear if L-

	DOPA supplementation in early infancy/childhood can help to correct abnormal retinal development in albinism and optimise visual function.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We aim to: (a) establish proof of concept for the earlier treatment of albinism with L-DOPA; (b) define the therapeutic time window within which L-DOPA treatment will be effective at rescuing abnormal retinal development in albinism and (c) explore the biochemical mechanisms underlying abnormal retinal development in albinism and potentially identify novel therapeutic targets.
What types and approximate numbers of animals do you expect to use and over what period of time?	We would estimate a total of approximately 300 animals for the project. Exact numbers will depend on the results of breeding and the experimental data. Mice have been chosen as the knockout mouse is the only animal model of albinism in which preliminary data exists for the use of oral L-DOPA (including drug dosages and formulation) in rescuing retinal function. The minimum number of animals will be calculated for each part of the project based on power calculations and statistical estimates.
In the context of what you propose to do to 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 administration of L-DOPA to mice may result in side effects similar to those experienced by humans including nausea, loss of appetite, movement disorders, sleep disturbances and agitation. Close attention will be made to the health and behaviour of the animals throughout all experiments and when at rest. Appropriate action will be taken where an animal is thought to be distressed at any time. Eye-drops will be used in very similar techniques to those employed in humans with the same use of local anaesthetic drops and general anaesthetic where appropriate. Some examination techniques may include restraint for short periods and pre-injection of visualising substances such as fluorescein as is used in humans. Minimal discomfort is expected from these procedures which will be limited in number and frequency. In order to record eye movements in some mice it will be necessary to attach a small fixing plate to the head by an adhesive using a surgical technique. This will be done under general anaesthetic with appropriate subsequent pain-killers and a long recovery period. Subsequent eye movement tests will be done by minimal animal contact and are limited in

	time and frequency. Some animals experience discomfort but in most cases only in the period immediately after surgery. Additional pain-killers will be used as necessary.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	In order to establish L-DOPA as a treatment in infants and young children with albinism, it is necessary to define exactly when treatment will be effective. In humans, albinism is a very large and variable group of conditions, making it extremely difficult to diagnose the specific subtype at an early age. This limits the precision with which the potential therapeutic window can be determined, necessitating the use of a genetically well-characterised animal model of albinism, where it is possible to test the efficacy of L-DOPA treatment at specific and earlier ages. In addition, the safety of L-DOPA treatment has not been established in infants under 3 years of age and it would be unethical to commence L-DOPA treatment trials in human infants and young children prior to demonstration of proof of concept and safety in animals first.
2. Reduction Explain how you will ensure the use of minimum numbers of animals	The numbers of animals needed will be determined by accurate statistical calculations, allowing us to use the minimum number of animals possible per protocol and by adopting a longitudinal study design. In order to minimise the numbers of mice needed & any possible distress, the functional & anatomical effects of administering L-DOPA to mice at different ages on retinal development will be determined by performing longitudinal, non-invasive eye examinations.
3. Refinement Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For this study, I have chosen the only genetically well characterised animal model of albinism in which preliminary data exists for the use of oral L-DOPA (including drug dosages and formulation) in rescuing retinal function. In addition, electroretinography (ERG) have been established as a measure of visual function in both control and albino mice. Optical coherence tomography (OCT) retinal imaging in mice has also been shown to be reliable. This makes them an ideal model for longitudinal monitoring of retinal development response to L-DOPA treatment. We will reduce suffering by minimising the number

	and frequency of procedures, minimising the stress and suffering of mice during procedures and reducing the number of animals used. For example, for eye examination, animals will need to be restrained. We have established protocols for causing minimal stress and minimising the time of restraint. Strict rules will be applied to the number of procedures or examinations per week and per animal and for the duration of the technique. All procedures will be completed with close attention to animal stress signs and general anaesthetics, local anaesthetics and systemic pain killers will be used as they are when these procedures are carried out in human children.

Project 39	**Blocking senescence in ageing**	
Key Words (max. 5 words)	Ageing	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Testing potential anti-ageing and anti-cancer drugs in mammalian models. Using genetically altered (prematurely aging) and wild type mice, we will test a potential anti-ageing drug to assess whether it reduces clinical signs normally associated with age, and reduces the incidence of spontaneous tumour formation (Identity of the drugs cannot be publicly disclosed at the moment due to pending IP applications).	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	These experiments could define novel treatments to ameliorate the symptoms of ageing.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. 500 per 60 months.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will	Some of the mice will be used to maintain breeding colonies. If the drugs tested work as expected, mice should experience better ageing and a prolongation of their lifespan. Mice will be culled to study their tissues at	

happen to the animals at the end?	different ages. Blood will be drawn at different times. Animals will be injected or dosed via gavage to study the effect of drugs. The drugs that are going to be used have no recorded side-effects in mice. Injecting contrast agents and anaesthetics may be needed for imaging purposes to assess tumours appearance. Also, animals will be kept alive for longer than usual for lab mice due to the nature of the experiments, with may inflict loss of muscle tone, locomotion, weight and changes on appearance The level of severity for this animals will be moderate. All animals at the end of the experiments will be culled under Schedule 1.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Preliminary experiments have already been performed in human cells in culture and flies. Experiments in a mammal model are necessary in order to bring these drugs to potential clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The design of the experiments on mice will include randomization, blinding, and factorial design. Advices will be obtained Bioscientific Events Ltd and from biostatisticians at the University of Leicester.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the organism of choice because they are well studied, their immune functions are similar to humans, and many reagents exist permitting detailed experimental analyses which are recognized internationally. Non-mammal models of ageing have limited use in advanced studies due to the difference in the molecular pathways involved in the process. Mice are genetically well studied and are the simplest mammalian model to study ageing We will run pilot studies with a small number of animals to make sure that the methods used and drugs don't affect they welfare above the limits stated on our licence. And if the pilots are successful we will expand the study to bigger group tests, so we can have significantly difference in biostatical results, proving that the drugs we use have an impact on improving animal welfare and lifespan. The drugs used are known to have minimal side effects and we expect them to actually improve the fitness of the animals, not harm them.