Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 16

Projects with a primary purpose of Basic Research - Multisystemic

Project Titles and Keywords

1. **Role of G protein-coupled receptors behaviour and homeostasis**
    - G protein-coupled receptors, behaviour, homeostasis, vasopressin, apelin

2. **Medical countermeasures to dangerous pathogens**
    - Medical countermeasures, infection, protection, defence

3. **Generation and maintenance of transgenic mice**
    - Transgenics, microinjection, mice, genetics

4. **Therapeutic target validation in fibrosis**
    - Fibrosis, signalling pathways, drug targets

5. **Nutrition and lactation in ruminants**
    - Ruminant, Nutrition, Lactation, Metabolism, Rumen function

6. **Breeding and typing of genetically altered Mice**
    - Mouse, DNA binding proteins, signalling pathway, knockout, transgenic

7. **Methods and tools for mouse genome engineering**
    - Mouse Transgenesis Method Development Refinement

8. **Determining pathways that regulate lung inflammation**
    - Lung inflammation, injury, fibrosis

9. **Electrical stimulation of muscle and research in alkaptonuria**
    - Muscle, activity, alkaptonuria

10. **Centrosome biology in mammalian development, aging and stem cell function**
    - Development, stem cells, aging, cell division

11. **The mechanisms underlying mammalian obesity and type 2 diabetes**
    - Insulin resistance, diabetes, obesity, cognitive defects, metabolism

12. **Neurobiology & inflammatory mechanisms**
    - Sensory nerves, cardiovascular, inflammation, neurobiology

13. **Fumarate hydratase in cancer biology and metabolism**
    - Fumarate hydratase, cancer, metabolism, diabetes

14. **In vivo study of sound transmission to the third trimester fetus**
    - Fetus, ear, sound, in-utero, pregnancy

15. **Studies to investigate laminitis predisposition**
    - Horse, laminitis, endothelium, insulin, metabolic

16. **The influence of barometric pressure changes upon membrane physiology and xenobiotic penetration**
    - Air pressure, Penetration of drugs, Skin, Blood flow

17. **Pre-clinical mouse models of human ciliopathies**
    - embryogenesis, development, cilium, ciliopathies

18. **Epigenetic reprogramming in mammalian development**
    - Epigenetics, methylation, germ cells, embryos, diet

19. **Management of genetically altered rodent lines**
    - genetic alteration embryo stem cell

20. **The pathophysiological roles of Podoplanin and its receptor CLEC-2**
    - Podoplanin, CLEC -2, Embryogenesis, Vascular Integrity

PROJECT 1	**Role of G protein-coupled receptors behaviour and homeostasis**	
Key Words (max. 5 words)	G protein-coupled receptors, behaviour, homeostasis, vasopressin, apelin	
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)	Interactions with the external environment can influence our behaviour and often require changes to maintain a relatively constant internal environment in the body, a process called homeostasis. The mechanisms by which we adjust to external threats such as a lack of food or water depend upon both the nervous system (usually for the more rapid responses) and the endocrine (or hormone) systems for more prolonged responses. The cells in the body communicate with one another using diverse array of chemical signals including neurotransmitters, hormones, and environmental cues (e. light and odours). These signals bind to many classes of receptors, the largest of which are called Gi protein-coupled receptors, on the surface of cells in target tissues. Despite a great deal of research over the past decades, the mechanisms by which neural and hormonal factors regulate our behavioural state and control homeostasis are poorly understood. W do not know the full complement of hormones and their	

	receptors that regulate these systems. We do not know the function(s) of many of the G protein-coupled receptors found in the central nervous system and periherai tissues. We do not fully understand how these receptor systems interact. The overall objective of this licence s to gain a greater understanding of the function of G protein-coupled receptors in regulating behaviour and controlling homeostasis. We are particularly focused on the function of G protein-coupled receptors in certain parts of the brain, such as the hippocampus, hypothalamus and the pituitary gland, and in peripheral tissues such as the adrenal gland and fat. We are interested in studying the influence of these receptors in behaviours such as aggression and those induced by stress (e.g., anxiety), and in homeostatic mechanisms such as energy control and the hormonal response to 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)?	G protein-coupled receptors are active in every tissue. They present a wide range of opportunities as therapeutic targets in areas including cancer, cardiac dysfunction, diabetes, obesity, and central nervous system disorders such as depression and behavioural abnormalities. Consequently, G protein-coupled receptors are the core of modem medicine accounting for the majority of best-selling drugs and about 40-50% of all prescription pharmaceuticals on the market. The studies we are undertaking further our understanding of the biology of signalling pathways in cells, which is of considerable academic interest, but, more importantly. may lead to exciting new possibilities for targets for drug therapy. By exploiting genetic and pharmacological approaches we will define the role of particular G protein-coupled receptors in regulating behaviour and controlling homeostasis that may lead to the development of new treatments to correct some conditions such as those involving impaired metabolism and water balance, and those related to chronic stress (e.g. depression).
What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of the 5-year programme, we anticipate that 8,180 mice (the majority of which will be genetically altered) and 1,040 rats will be used in procedures in the project.
In the context of what you propose to do to the animals,	We will experiment on cells in cultures which maybe, derived from animals (which may be genetically altered). However we are limited as to the information

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	that can be obtained from them, and need to study the role of receptors in the complex interaction between the nervous and endocrine systems in living animals. Most of the *in vivo* studies will be performed in mice, although some studies involve rats. Typically we will study the response of animals to mild-moderate stress such as subjecting the animal to a novel environment or requiring it to swim in a tank of warmed water. At the and of the study the animals will be killed and we will measure changes in the level of hormones in the blood, and examine tissues both in the brain and in other parts of the body by means of specialised techniques, which make use of the most recent developments in the field of molecular biology. In some of the studies we may need to inactivate the receptors that control the nervous and/or endocrine responses. This may be achieved for example, by injecting compounds that block the receptors. Surgery is performed under aseptic conditions exercising care and caution throughout the procedures. Animals receive pain killers after any surgery. In other animals we may need to add compounds in order to alter the body's control I systems; such substances may be hormones (for example steroid hormones, related to the type used to reduce inflammation) or substances that act on very specific pathways in the brain. In order to study the effect of genetic influences, we will make use of genetically altered mice; these will be bred under this licence and used for study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The regulation of behaviour and homeostasis by receptors involves complex interactions between many I brain regions and peripheral tissues that cannot be recapitulated in a petri dish. We will carry out extensive basic and mechanistic investigations using *in vitro* and cellular models prior to, and in parallel with, animal experimentation. However, there are no non-sentient systems that model the role of G protein-coupled receptors in the regulation of mammalian behaviour and homeostasis. Animal models provide relevant systems in which pharmacological and genetic manipulations can be performed and receptor expression and function can be measured by using

	anatomical, biochemical and behavioural techniques.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will conduct pilot studies to enable us to accurately determine group size and to reject approaches that are failing to deliver useful information at an early stage. We will also seek to reduce the number of animals studied by careful experimental design, the adoption of sensitive outcome measures with small variation and the study of only the most relevant time points. .
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use mice and rats Much of what we know abofl the expression, regulation and function of receptors in behaviour and homeostasis comes from studies in these species, in particular genetically atered mice. They share many anatomical and physiological features encompassing conserved receptor function with that of humans. and have the lowest neurophysiologica! sensitivity in which the work can be performed. Maximum effort will be focused on ensuring the wellbeing of animals and on optimising environmental conditions so that experiments do not have to be unnecessarily repeated. The rodent stress models that we will use have been employed extensively in many scientific fields for over 40 years, resulting in updated and standardised protocols that are the most refined. We will constantly review the procedures that are used in the programme of work and make any necessary adjustments where possible to refine our procedures and reduce animal numbers.

<table>
<tr><td>PROJECT 2</td><td colspan="2">Medical countermeasures to dangerous pathogens</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Medical countermeasures, infection, protection, defence</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td>X</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td>X</td><td>Forensic enquiries</td></tr>
<tr><td></td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">Infectious diseases caused by dangerous pathogens such as Bacillus anthracis, Yersinia pestis and Ebola virus pose a serious threat to life. Generally, the agents can be spread in food arid water supplies, by biting arthropods and, most significantly in the defence or deliberate release context, by the inhalation route. Currently, there are few licensed vaccines or therapeutics available for these diseases, and many of these are unable to protect against inhalational infection.

Some antibiotic treatments are available for the treatment of some these pathogens but these can only be administered after infection and a positive diagnosis has been made. Many of these pathogens manifest generic symptoms making a diagnosis challenging, particularly in the military theatre or deliberate release environment. This can leave infected individuals vulnerable to severe disease or even death. Furthermore, in many cases the effectiveness of antibiotics to certain pathogens is limited leaving affected individuals unprotected or</td></tr>
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	exposed to long term chronic infection. Therefore, there is a high priority to devise and refine medical countermeasures, which can protect the UK, and it's Armed Forces from infection with such pathogens. If such research is not carried out, no effective countermeasures will be developed to combat a growing threat to the UK population in terms a potential deliberate release, or the UK's security and Armed Forces.
What 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. Evaluation of therapies (or combinations of therapies) that induce higher levels of protection more rapidly, with reduced side effects 2. Improvement of our understanding of host-pathogen interactions 3. Improvement of the delivery of medical countermeasures 4, Development of reagents to enable rapid detection and diagnosis of these pathogens 5. Exploitation of candidate therapies in a public health context
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of animals used will be mice, although hamsters, guinea pigs, rats, ferrets and rabbits may also be used where they represent the most appropriate animal model. We expect to use approximately 6,000 animals per year during this study.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The overall severity of this project is severe. The majority of procedures carried out, for example administration of medical countermeasures or withdrawal of blood samples, will in themselves be of mild severity. However, animals given pathogen are likely to develop disease with associated clinical signs which will be of severe severity and may ultimately lead to death. Animals will not be re-used at the end of a study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are needed to successfully evaluate the efficacy of potential therapies, especially those therapies relying on the involvement of the immune system, because the interactions between the host, the pathogen and the therapy are too complicated to reproduce in the laboratory. However, where

	possible, studies will be conducted in the laboratory using simpler systems prior to use in animals to screen out any obviously deficient therapies (e.g. antibiotics to which the pathogen is resistant).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies will be designed with the advice of a team of statisticians to ensure each study has suitable levels of power without unnecessary use of animals. Pilot studies will be conducted where appropriate to provide information to inform these power calculations. Where possible, studies will be run in parallel to utilise the same control groups. Inbred strains will be used where appropriate to reduce the inter-animal variability and thus reduce group sizes
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 lowest mammalian species that are representative of human infection will be used. In the first instance this will be mice, although hamsters, guinea pigs, rats and ferrets may be used as appropriate. Environment enrichment will be provided appropriate to the species and will include nesting materials, plastic arid cardboard dome houses, chew block and transfer of own scented material following routine cleaning of cages etc. Where appropriate animals will be familiarised to the procedure (e.g. handling, environment) or trained (e.g. placed into exposure tubes for increasingly greater periods of time prior to aerosol challenge) to reduce stress during the actual procedure. For all severe protocols, humane end-points will be used to ensure that the optimal balance is struck between protecting animal welfare and obtaining a full and valid set of data.

<table>
<tr><td>PROJECT 3</td><td>Generation and maintenance of transgenic mice</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Transgenics, microinjection, mice, genetics</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</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>Yes</td><td></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>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 but often lack the skills/expertise to use this technology.

This project has been designed to provide an efficient service for the generation of new genetically altered strains of mice. These new 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 main their proficiency in transgenic techniques 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.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>This project will benefit science through the generation of new genetically altered mouse strains that can be used to better understand how genes control all aspects of mammalian biology. In</td></tr>
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animals could benefit from the project)?	particular, this project will help develop new model 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 no more than 220,000 animals will be used throughout the course of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected 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 for future developments in research, not involving animals, 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 dedicated transgenic service is to establish a pool of skilled people with expertise in core activities so that they remain proficient thus keeping the number of animals to a minimum at all times. New mouse strains generated within this project will be cryopreserved so there is no need to continually breed genetically altered mice if they are not in active research programs whilst preserving these unique strains 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.

<table>
<tr><td>PROJECT 4</td><td>Therapeutic target validation in fibrosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fibrosis, signalling pathways, drug targets</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></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>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Fibrosis of the heart is a major cause of disease such as heart failure and atrial fibrillation. The major aim of this project is to identify new and better targets for treatment and prevention of fibrosis, and understand its mechanisms and consequences.</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>These studies will guide future development of drugs against validated targets, and will help patients suffering from fibrotic disease (e.g. of the heart). They will also help us understand how fibrosis develops in mammalian models. A second benefit is the continued development of improved and refined models of cardiac fibrosis. These refinements will benefit animal welfare.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to</td><td>Mouse, 7000 for breeding, 1200 for fibrosis studies, 5 years</td></tr>
</table>

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?	Adverse effects may result from genetic modification or hormonal induction of fibrosis (e.g. breathlessness or lethargy, high blood pressure, weight loss, muscle weakness, aneurysm), from surgery (pain, wound infection or opening). The majority of mice used for breeding and maintenance will experience harms of mild severity, and for fibrosis studies is moderate. The animals will be killed at the end of the study or if a genetic line is important we will freeze embryos or sperm in case future studies are necessary.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal models are necessary for target validation as cell studies or computer models cannot recapitulate the complexity of fibrosis in an organ, which involves multiple cell types, hormonal and blood pressure and flow changes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The majority of animals are used for breeding, and we will take care to breed the minimum required for our phenotyping experiments. We routinely use statistical power calculations to guide our experimental design so that the minimum number of animals are used. We use cell based and computer studies extensively to predict the best candidates for animal studies, thus maximising the likelihood of success.
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 use mice because this species provides a combination of easy genetic manipulation with cardiovascular system and disease models sufficiently similar to man. The models of fibrosis used are either genetic or hormonal, as these are the major mechanisms known to act in humans, and are less severe than surgical models. We have monitoring regimes developed *with*

(harms) to the animals.	*the advice of the Vet and NACWO (and where necessary the home office inspector) with humane end-points defined to keep within the severity limits.* We keep careful records of expected effects, and this document is made available for consultation by animal care staff. Animals exhibiting any unexpected harmful effects incompatible with a moderate severity are euthanased. All adverse effects are documented and periodically assessed in order to detect sporadic unexpected events. Animals are routinely maintained in a barrier environment and group housed whenever possible. We use minipumps to deliver drugs rather than repeated injections. These are surgically implanted. Infections are minimised by performing recovery surgery under aseptic conditions. Pain and discomfort post surgery is minimised by providing medications, warmth, access to water-softened chow, and fluids (as required).

<table>
<tr><td>PROJECT 5</td><td>Nutrition and lactation in ruminants</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ruminant, Nutrition, Lactation, Metabolism, Rumen function</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>Yes</td><td></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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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim is to provide novel information on the effects of nutrition on physiological and metabolic processes in dairy cows and other ruminants that will increase our understanding of the basic biology and thereby allow development of strategies to improve efficiency, fertility and health of cows, to improve the nutritive value of milk for human consumption, and to reduce the environmental impact of dairy farming.</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>Diet formulation systems currently used for cattle and sheep were developed more than 20 years ago, but modern animals are far more productive than their predecessors. This research will develop more appropriate feeding strategies, leading to healthier, more fertile animals, which are more efficient and have lower methane emissions.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of</td><td>Cattle (up to 1,000 over 5 years)

Sheep (up to 30 over 5 years)</td></tr>
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time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of procedures (e.g. blood sampling, dosing with digesta markers) are mild and cause no more than transient discomfort and no lasting harm. At the end of a set of regulated procedures animals will be inspected by the NVS or nominated veterinary surgeon and either killed humanely by a Schedule 1 method, kept alive at a designated establishment, or discharged from the Act to be killed at a slaughterhouse Up to 16 cattle will be fitted with rumen cannulae to permit sampling of digesta. The operation is classified as moderate and adverse effects are those associated with any gut surgery. Following recovery, maintenance and use of such animals is mild and they can live for many years with no adverse effect. At the end of procedures, animals fitted with rumen cannulae will be killed by a Schedule 1 method. Any animal showing adverse clinical signs will be given appropriate veterinary and husbandry treatment. If it 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	Animals have to be used because of the complexity of digestive, metabolic and synthetic processes in ruminants. In most cases, for example converting feed into milk, there is no substitute for live animals. Some responses to diet, such as changes in feeding behaviour, cannot be predicted or simulated with non-animal alternatives. In vitro systems will be used to supplement or replace some animal studies. For example, we will use mammary cell cultures to study milk synthesis, and fermenters to study activity of

	rumen microbes (although the latter requires animals as rumen fluid donors).
2. Reduction Explain how you will assure the use of minimum numbers of animals	In consultation with our statistician, we will use known variation and predicted responses in power calculations to calculate the minimum level of replication required to provide adequate statistical power for each experiment. When appropriate, we will use covariates and crossover designs to minimise residual variation and reduce the number of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Lactating dairy cows are the target animals for our updated response models, so they are the only animal model appropriate for most of the work. Non-lactating cattle will be used for some studies of rumen function and digestibility because they are easier to maintain. Sheep will be used also, because all feeds in the current national database were evaluated in sheep fed at maintenance. For compatibility, we have to evaluate novel feeds using the same technique. All animals will be maintained to the highest standard of husbandry and care in facilities designed to provide the best possible welfare standards. Procedures will be performed only by suitably competent operatives using appropriate handling facilities to minimise stress on animals. In all cases where there are alternatives, we will utilise the procedure that imposes the least harm to an individual animal.

PROJECT 6	**Breeding and typing of genetically altered mice**	
Key Words (max. 5 words)	Mouse, DNA binding proteins, signalling pathway, knockout, transgenic	
Expected duration of the project (yrs)	6 months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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 objective in this short term licence is breeding and typing of genetically altered mice. 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	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/	

project)?	repair and 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 6 month period the projected use of female and male mice for maintaining the breeding nucleus is 500 for the 14 strains of genetically modified animals we currently use. This is because we maintain the stock lines of genetically altered mice as heterozygotes for all but 3 of our existing mouse lines to minimise adverse effects seen in the homozygous 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?	Most of the procedures we use are mild to moderate. The deletion of most of the genes we work on produce effects that are mild to moderate. Only in two cases of our genetically altered mice do we observe that embryos do not survive beyond 10.5 days. We circumvent this by using refined methods of ablating gene function such that this occurs only in specific tissues or cell types. During embryonic development or adult mice. Any mice exhibiting an adverse phenotype are killed humanely.
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. We also use early zebrafish embryos (stages which are not regulated) for construct evaluation. However, in undertaking studies such as on mammalian development we have had to consider the use of laboratory mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding colony nucleus size is generally kept small with one or two breeding pairs for each strain which is replaced once every 4 months for each strain following a very good colony management strategy.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mouse is the animal of choice since it has many similarities to humans in that there is robust genetic information and the genome is sequenced. Well characterised inbred strains are available. Efficient and reproducible technologies for producing genetically altered mice are also available. Most of our recent genetically altered mice use refined approaches ie conditionally ablating/altering the function of a gene which can be induced in specific to a particular cell type or organ and hence the phenotypes are moderate and minimise welfare costs.

<table>
<tr><td>PROJECT 7</td><td>Methods and tools for mouse genome engineering</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Mouse Transgenesis Method Development Refinement</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>~~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>This project aims to apply and develop new methods for alteration of the mouse genome. It also covers the performance of quality control tasks for current mutant generation processes and products.</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 benefits that are expected from this project include:
- a more efficient use of (fewer) animals for generation of mouse mutants for research,
- the development of methods that will allow making more sophisticated/precise changes in the mouse genes (current methods leave additional alterations in the genome or afford no control over where new genetic material is inserted in the genome),
- introduction of methods for faster and cheaper generation of mouse models (as it will become possible to make the mutation in the mouse strains that are relevant to the research project instead of only the strains that are permissible to current methods)</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>A maximum of 12500 mice over 5 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>Generation of founders:
The generation of Genetically Altered animals involves administration of hormones and implantation of embryos in foster mothers, protocols</td></tr>
</table>

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

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

<table>
<tr><td>PROJECT 8</td><td colspan="3">Determining pathways that regulate lung inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Lung inflammation, injury, fibrosis</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 acute lung inflammation is regulated by the Podoplanin-CLEC-2-Syk pathway and/or whether chronic lung inflammation is regulated by (a) the Podoplanin-CLEC-2-Syk pathways, or (b) vitamin D metabolism.</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">Lung inflammatory diseases such as acute lung injury (ALI) and idiopathic pulmonary fibrosis (IPF) have no current treatment. This project aims to identify key regulatory pathways of lung inflammation to provide novel pathways novel therapeutics may target 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 4,000 animals in total - 1,500 animals for scientific protocols and 2,500 to breed the condition global and cell-specific GA strains required.</td></tr>
<tr><td>In the context of what you</td><td colspan="3">Instillation of substances into the lungs – we expect</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?	adverse effects of a moderate severity such as weight loss and a reduction in respiratory rate. Conditional induction of genetic modulation – we expect adverse effects of mild severity such as minor weight loss. Induction of vitamin D deficiency – we expect adverse effects of mild severity such as minor weight loss When an endpoint is reached, animals will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No *in vitro* techniques are currently available that can fully replicate the complex spatial and temporal interactions within the lung. At present *in vitro* nor *ex vivo* methods exist to model a full immune response. To determine function of a biological pathway in lung during inflammation it is essential to use an *in vivo* assay that reproduces the disease processes as accurately as possible.
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. Small scale pilot studies will be used to determine whether the gene of interest plays a role in lung inflammation/fibrosis prior to a full scale experiment. Statistical analysis from previous studies suggests group sizes of 16 are required to measure significant decline in arterial oxygen saturation at 48hrs (power = 0.9). To maximise the information gained from a single animal we aim to take samples from multiple body sites.
3. Refinement	The mouse has been selected because of established and reliable transgene technology and

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.	extensive literature on lung inflammation models in murine strains with established and reproducible protocols due to the reliable reagents available. We have generated transgenic mice that do not display any adverse effects either before or after candidate gene deletion is induced up to six months of age as compared to their littermate controls. Altered systemic vitamin D levels only demonstrate mild adverse effects. We will only subject animals to these studies when we have sufficient *in vitro* and *in vivo* evidence that the biological pathway is involved in inflammation and/or lung homeostasis. I have significant experience using IT instillations. In addition I have used IT-LPS for the past 3 years thus are very familiar with the monitoring and outcomes of using animals during this protocol. We will use pilot studies containing no more than 20 mice per GA mouse strain to determine optimal dosing of LPS and bleomycin so as to minimise animal suffering. By choosing well established protocols to induce lung inflammation we minimise the unknown effects on the mice and subsequently pain, distress and suffering. Animals that have modified biological pathways that are uncharacterised in terms of their response to lung inflammation will be observed frequently (twice daily) and given glucose-saline when required.

PROJECT 9	**Electrical stimulation of muscle and research in alkaptonuria**
Key Words (max. 5 words)	Muscle, activity, alkaptonuria,
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project observes the changes in muscle tendon and bone in response to increases and decreases in daily muscle activity. Many changes associated with voluntary exercise or electrically activated movement are beneficial, but the best programmes for that exercise are not well understood in term of the cellular responses within the musculoskeletal system. In some cases (such as the rare disease Alkaptonuria which we study in this project), excessive exercise might even accelerate the disease process. We need to understand the signals that cells respond to in terms of the useful responses like increased strength, endurance and ability to reduce the blood glucose level, but also why muscle is lost so quickly when it is not used, in conditions like bed rest during a period of illness, or after a leg fracture.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	If we understand the cellular responses underlying adaptation to exercise we may be able to advise individuals more effectively in terms of the most productive approach to gaining strength or endurance. In situations in which muscle function has been lost, such as laryngeal paralysis, we may be able to advise how best to activate the muscles to restore function. For sufferers of the rare disease Alkaptonuria, we will continue to characterise the disease and to test potential therapies based on replacement of the missing enzyme function or therapies that modify the protein breakdown pathway or avoid the build-up of toxic substances that otherwise cause severe pain and damage to the joints.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice, 200-300 over 5 years Rabbits, 20 over 5 years

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will receive implanted muscle pacemakers to produce programmed activity in one hind limb, designed to interfere as little as possible with normal locomotion and behaviour. Animals will be killed humanely and the muscles taken for analysis after a period of a few weeks of training. Sometimes, the limbs will be scanned by X-ray techniques before the muscles are removed to investigate changes in bone and joint structure. Severity is considered moderate
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no non-animal model of the interaction between muscle, tendon bone and joints. So work that involves the whole musculoskeletal system requires the use of mammalian species which have a very similar arrangement to human.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We make the very best use of every sample, measuring gene expression with sensitive techniques that require very little tissue, and performing microscopic techniques on samples from the same muscles. Because we use implantable stimulators we can exercise one limb so that the other acts as the control (unexercised) limb. We thus do not need an additional group of animals as controls.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The model of muscle activation we use is highly developed making use of miniaturised electronics to produce muscle pacemaker systems that can be programmed by an external controller, like the latest heart pacemakers. That means that the pattern of activity can be adjusted for each individual. We use a standard of technique that mirrors the best veterinary practice. Our model of the rare disease alkaptonuria is much milder than the severe human form, and represents only the early stages of this painful joint disease. Even so, the mechanisms seem to

	be the same so we can still test new therapies because we anticipate that prevention of the early stages will have a significant benefit in later life when the symptoms would otherwise progress to a severe stage.

<table>
<tr><td>PROJECT 10</td><td>Centrosome biology in mammalian development, aging and stem cell function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Development, stem cells, aging, cell division</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</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></td><td>No</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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of our study is to understand why mutations in certain genes cause dwarfism in the human population.</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>Certain mutations in our genes lead to developmental disorders such as dwarfism, small brain or bone and heart problems, but we do not understand why these conditions arise. We propose to use animals to model these disorders in order to improve our understanding of these diseases. Our study has direct relevance not only to the developmental disorders, but also to cancer treatments, since similar mutations are found in many human tumours. We hope that our study will also open up potential strategies for killing cancer cells.</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	Mice (*Mus musculus*) Numbers: 6500 Period : 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Majority of the substances administered are not expected to cause any lasting adverse effects. Of itself, injection of substances will cause no more than transient discomfort and no lasting harm. Cross breeding of some of the animals that carry particular mutations will produce offspring with unknown characteristics, which will be interesting to this project and we will analyse them in greater detail. Tumour burden, if developed, will be limited to the minimum required for a valid scientific outcome. Certain proposed procedures such as abrogation of the animal's own immune system and injection of foreign cells in to such animals might result in moderate severity, but such animals will be kept in a clean and controlled environment and will be closely observed to minimize any suffering. In all cases, the general health and condition of an animal will remain the overriding determinant. Mice will be killed if they show signs of ill health, such as piloerection, hunched posture, inactivity or inappetence, which cannot be alleviated by minor veterinary intervention.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Prior to embarking on animal experiments we will collect as much evidence as possible from cell culture work. There are two reasons why we cannot exclusively use cell culture to obtain results. First and foremost, our study aims to understand mutations that interfere with development, stem cell function and cancer. Since all these are complex physiological processes and due to lack of cell culture systems that mimic this complexity, our work necessitates animal models. Second, for *in vitro* experiments cells must be removed from their natural environment. Interaction with this environment may affect their capacity to divide, differentiate, survive or die, and thus isolated cells may not reflect the process that takes place in

	intact tissues/organs. The transgenic mice selected for this work are valid models for human developmental diseases, as indicated by their shared characteristics.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing the experiments we will perform statistical analyses to ensure that we use the minimum number of mice per group that will be informative, including carrying out pilot experiments. Cryopreservation of embryos from lines that are no longer in active use will decrease the number of animals needed for the experiments. In order to reduce the number of breeding pairs, mice will be bred as homozygous provided they are fertile and have no harmful phenotypes. We will minimise use of animals by teaming up with other research groups interested in surplus tissues from same animals that are not used for this study. To maximise the information from a single animal, we will aim to collect samples from most organs. These samples may be shared with other scientists to minimise the breeding of further animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have already demonstrated that transgenic mice strains can mimic a range of clinical features present in patients with primordial dwarfism. We therefore believe that despite their small size, mice are a good model system to study growth retardation. When similar genes are mutated in lower vertebrates such as zebrafish, the phenotypes are very crude (ie kinky tail) and as such are less informative. We aim to understand a human developmental disease caused by homozygous mutations in a single gene, meaning that all tissues of patients carry only the faulty copy of this gene. To best mimic this scenario, we need to breed the mice as straight knockouts. To avoid unexpected pain and suffering, mice will be bred and analysed as heterozygous animals first. In case of tissue-specific or conditional deletion of genes, we will only use well-established reagents and protocols for their induction.

<table>
<tr><td>PROJECT 11</td><td colspan="3">The mechanisms underlying mammalian obesity and type 2 diabetes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Insulin resistance, diabetes, obesity, cognitive defects, metabolism</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></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>Yes</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">The overall objective of our research is to understand why and how obesity and ageing lead to the development of insulin resistance, type 2 diabetes, cardiovascular disease and neurodegenerative disorders, such as Alzheimer’s disease, and whether we can stop the development of these diseases or reverse it by manipulating the composition of the diet or proteins that are expressed during the development of these.</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">By understanding the molecular mechanisms behind the development of age- and/or obesity-induced insulin resistance we can find novel strategies to combat age- and obesity-induced diseases such as diabetes, cardiovascular disease or Alzheimer’s. Considering that we have an ever increasing ageing population and a rapid rise in</td></tr>
</table>

	obesity levels across the developed world, understanding these is of utmost importance.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse; 7,500, 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The genetic manipulations we use will induce diabetes, Alzheimer's Disease and cardiovascular disease. Some animals will be kept until they are old. Dietary interventions and some genetic crosses we propose in this project, are intended to improve health in mice and also extend healthy lifespan. The level of severity will be moderate, in order to accommodate for the use of some genetically modified mice with diseases. However, we anticipate that our dietary or drug treatments will make them better. Some animals may be exposed to a diet rich in fat, with the major side-effect being that these animals will become obese. Animals for ageing studies will be maintained up until 24 months of age and will be culled if they show any evidence of debilitating disease or pathology. All other procedures are for the purpose of measuring data output (effects on whole body physiology). Surgical procedures to implant minipumps and telemetery devices will be carefully planned and monitored and in order to investigate a specific scientific question (for example if a protein we identified in healthy ageing has the same effect in aged or obese animals). Analgesics will be given. All animals will be killed at the end of each experiment in order to provide biological material for further studies for understanding the mechanisms of ageing and obesity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Obesity and ageing act at the whole-animal level and will depend upon coordinated interactions of multiple organ systems within live animals. Thus these fundamental biological processes cannot be studied realistically in any other manner, for example cell culture or *in silico* modelling. However,

	we will carry out extensive literature searches throughout the time-scale of this project in an effort to continually improve and refine our experimental techniques, in order to avoid unnecessary repetition of experiments, any undue suffering and ultimately to identify appropriate *in vitro* or *in silico* replacements for these animal studies. Wherever possible we will augment our findings in whole animals by using primary cell culture (eg. primary skin fibroblasts, hepatocytes or adipocytes) or commercially available cell lines. Indeed, we have published many biochemical studies using hepatic cell lines for example rather than live mice or primary cells, in order to test out different inhibitors or stimulators of inflammation and stress.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have extensive prior data on the variability in parameters related to obesity and mammalian ageing and will use these data to inform power analyses to establish the minimum numbers of animals required to obtain a reasonable effect size for any particular treatment. We will work with departmental or college statisticians in order that our studies are always undertaken using the minimal number of animals but retaining appropriate statistical rigour throughout. Any pilot studies will be run in such a way that they will be rolled in to the main study wherever possible, so that they are not additional to the numbers ultimately required for the main experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have been shown to be highly effective model organisms to study the basic mechanisms of obesity- and ageing-induced diseases. In addition, the majority of ageing changes seen in mice are also seen in humans. A set of highly refined procedures and extensive training of staff will be used for all studies to ensure consistency in the data output.

<table>
<tr><td>PROJECT 12</td><td colspan="3">Neurobiology & inflammatory mechanisms</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Sensory nerves, cardiovascular, inflammation, neurobiology</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></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>Yes</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">Sensory nerves are the nerves that act to sense and respond to change. However, they work excessively in pain syndromes and insufficiently in cardiovascular disease. At present, we do not know how to regain control in order to protect against excess pain, inflammation and disease progression. The overall objective is to determine how sensory nerves and related mechanisms are regulated and thus increase knowledge. This includes in: i) pain and inflammation associated with joint disease such as arthritis and ii) vascular dysfunction associated with blood pressure control and vascular biology in limbs and skin.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td colspan="3">These studies will enable the knowledge of sensory nerves and their interactions to be increased. We endeavour to pinpoint new therapeutic targets, which will allow new drugs to be developed for</td></tr>
</table>

animals could benefit from the project)?	evaluation in clinical trials for human and veterinary use. Conditions that may benefit are those necessary for healthy ageing such as involving hypertension (leading to cardiovascular disease, the world's biggest killer) arthritis and pain (experienced by up to 50% of the elderly) and skin conditions (such as itching, that distress many).
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate, based experience and experimental design tools, that up to 2,000 mice and depending on experimental need, up to 100 rats will be used each year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There is a need to carry out long-term, in addition to short term, studies; in order to identify disease mechanisms involving sensory nerves. The short term studies (within one day) involve over 60% of the animals and are often carried out under general anaesthesia where the discomfort felt by the animal is minimal. The longer term studies include use of models of inflammatory disease. Inflammation and arthritis will usually be induced by a single injection of a pro-inflammatory substance into the paw or joint. This is carried out under anaesthetic and animals recover without signs of pain. Normally studies of pain threshold involve measurement of the time taken for a rodent to move away from a heat source that is shone onto skin, or a pressure probe. This involves transient discomfort. Some mice undergo a long term model of arthritis (up to several weeks), involving several injections. Whilst this is associated with discomfort, our experience is that mice do not exhibit lack of well being and eat and groom normally. Models involving blood pressure changes can be induced by feeding mice a high fat diet for several weeks, by administering a compound, or by surgery. Blood pressure will be measured either by a non-invasive tail cuff or by surgically-implanted radio-sensors in mild to moderate procedures. In studies that involve surgery, general anaesthesia and pain controlling

	medicines will be used. We examine the effect of drugs that alter the response and use genetically modified mice in order to learn how altered gene products influence disease. These mice do not have any obvious basic health or developmental problems. Rodents under study are closely monitored for signs of discomfort such as excess loss of weight, reduced feeding, grooming and lack of mobility. We provide analgesics and other support and seek veterinary advice as required. Endpoints are defined in each protocol and animals are euthanised if their adverse effects reach these points. Animals undergoing surgical procedures are potentially susceptible to developing infections. To minimise the chances of this occurring, our procedures are conducted using sterile methods, (similar to procedures used when humans are operated on). As a result, adverse effects of infection are minimised. All mice are humanely killed at the end of the experiment and tissues collected for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The factors involved in sensory neuro-vascular biology include the activation of nerves and release of the biologically active substances which act on a range of cells and body systems. It is not possible to simulate these biological systems completely in a test tube. For some parts of our research single cells or tissues are studied in isolation and this is important in order to establish basic mechanisms. The findings need to be evaluated within animal systems as we cannot accurately recreate in the isolated cells in the laboratory the complex structures that work together in the body and in disease conditions such as hypertension or arthritis that include blood vessels, tissues, bones and organs. Therefore, there is an essential need to examine how sensory nerve activation and

	neuropeptide activity influences and interact with whole body systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our breeding programmes are monitored on a regular basis in order to keep numbers to a minimum. We use mathematical analysis to calculate minimal breeding and group sizes. We design experiments based on mathematical calculations to determine the smallest numbers in each group to gain significance, considering the changes expected. i) Using imaging techniques. This allows study of animals by observation and use of fewer animals per group and to obtain better information on disease progression. ii) Implanted sensor probes enable continuous monitoring of the conscious mouse in the home cage. This enables reductions in numbers used, especially with respect to chronic studies, as we can take readings before the induction of the disease model and do a 'before (control) and after (test) evaluation in the same animal. iii) We use a control site within the animal, for multiple site experiments, such as in skin. Additionally, experimental design involving multiple skin sites allows reduction of animal numbers. iv) Cells, fluid and tissues from experiments are used for analysis after the mouse is humanely killed. This enhances the accumulation of knowledge obtained from any one animal undergoing a procedure, thus reducing the number of animals used for any one study. The study is also supported by analysis of human cells where possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general	All our studies will be carried out in rodents, with the majority in mice, where we now have an advanced expertise on murine models of disease and access to genetically modified mice. The animals will be housed in our modern facility with environmental enrichment, designed to allow the animals a wide range of natural behaviours and

measures you will take to minimise welfare costs (harms) to the animals.	some privacy. The husbandry of these species is according to best practice, involving highly trained staff and is under regular review by our institution. Many procedures are carried out in a single day, with anaesthesia used where possible to reduce stress of restraint and injections. This allows us to gain information on mechanisms and modulating systems, with minimal discomfort. Longer term studies involve pre-treatments with procedures such as injections and implantation of sensors, involving anaesthesia followed by recovery. We routinely use medical pain relief at surgery and sterile techniques to reduce the chance of infection. During recovery we monitor animals for adverse effects and treat accordingly. The implanted sensors allow measurements to be carried out on conscious rodents, without them knowing. This both avoids discomfort and stress for the animals and provides very reliable data. We are also actively researching a technique to refine blood pressure measurements and thus reduce welfare implications.

<table>
<tr><td>PROJECT 13</td><td>Fumarate hydratase in cancer biology and metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fumarate hydratase, cancer, metabolism, diabetes</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></tr>
<tr><td>Translational and applied research</td><td>Yes</td></tr>
<tr><td>Regulatory use and routine production</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>No</td></tr>
<tr><td>Preservation of species</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</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>The overall aim of this project licence is to identify and define the metabolic and cancer pathways that support the growth of cells lacking the enzyme fumarate hydratase and allow them/ drive them to cause cancer. Such information can then define routes to therapy for cancer associated with loss of fumarate hydratase, for which there is currently no treatment, and possibly other cancers. Also, information gained about disrupted metabolism associated with diabetes and links to cancer may inform prevention programmes and therapy strategies of patients for both these diseases.</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</td><td>Kidney cancer accounts for around 3% of all cancers. The kidney cancer we are working on is associated with specific defects in the gene that codes for a metabolic enzyme fumarate hydratase (part of the disease hereditary leiomyomatosis and renal cell cancer, HLRCC) and is highly aggressive. No effective therapy exists for this kidney cancer.</td></tr>
</table>

project)?	We hope that by developing and analysing novel mouse models of this cancer and integrating analyses of these with human tumour cells we can provide insights into altered cancer metabolism and a real, innovative route into the design of therapies for various cancers. Also, we hope that these models might be applicable to other metabolic diseases which include diabetes and obesity.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse; approximately 6,000
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will be using mice to model kidney cancer and altered metabolism associated with cancer and diabetes. Mice will develop tumours and/or diabetes. Mice will be culled and tissue harvested for multiple analyses, either at fixed time points, or when the diabetes or tumours have developed and/or there is evidence of weight loss or distress. Some mice will be subjected to blood sampling or glucose tolerance testing. The procedures are deemed to be of mild or moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to study the complexities of either the causes of cancer, or the altered metabolism associated with the loss of the enzyme fumarate hydratase, within the cells of different tissues in anything other than a mammalian system such as the mouse. We have developed cell lines which we use to complement our work; but these cannot address questions about the interactions between cells or the variety of metabolic profiles. Furthermore they cannot model responses of different tissues to nutrients etc in the blood
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use good experimental design and best practice models. We will perform multiple analyses on individual animals to make best use of them and integrate results from different mouse strains in order to make best use of all animals.
3. Refinement	In evolutionary terms the mouse is the lowest vertebrate group for which suitable cancer and diabetic models are available. A large amount is

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.	known about the strengths and limitations of relevant murine models and this, coupled with the relevance to human disease has been considered in our choice of protocols. We will use best practise at all times and continuously re-appraise our approaches in response to the outcomes of our experiments. We have gained considerable experience of these mouse models over a period of many years, and know that the mice need to be monitored carefully for weight loss and distress caused by loss of kidney function and anaemia. We have specific criteria for monitoring this and will cull mice as soon as they become unwell.

<table>
<tr><td>PROJECT 14</td><td>In vivo study of sound transmission to the third trimester fetus</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fetus, ear, sound, in-utero, pregnancy</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>
<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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Preterm born babies are cared for in the Neonatal Intensive Care Unit where they are exposed to high volumes of sound emanating from the equipment such as ventilators, monitoring alarms and pumps that are used to care for them. The noise results in a stressful environment in which these fragile immature babies have to grow. The intense, sustained noises or impulse sounds associated with the use of this equipment are known to have a direct detrimental effect on newborn heart rate and oxygen levels. This may contribute to the general stress of the baby and could therefore delay and/or impair development of the immature baby brain. We aim to develop a physiological sound barrier to be incorporated in incubators used to care for preterm born babies to reduce the risk of immediate and long term side effects associated with the exposure of artificial environment sounds on the immature human brain.</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>This project will improve our knowledge of the acoustic environment of the baby in the womb in-utero during the last third of pregnancy, which is the period when the development of hearing and corresponding auditory cortex takes place in most mammals. This will provide us with the physiological background to better understand how this development is protected in utero from outside sounds. There is mounting evidence that preterm born babies are particularly sensitive to the intense, sustained noises or impulse sounds associated with the used of intensive care equipment</td></tr>
</table>

	and that this non-physiological sound environment may impact their immediate and long-term outcome. The data obtained by the proposed experiments will contribute to the development of neonatal incubators to provide the premature neonate with better protection from artificial sounds and to reduce/prevent some of the long-term side effects associated with prematurity and long-stay in intensive care.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use a maximum of 12 pregnant sheep, 6 at 100-120 days of gestation and 6 near term (140 days). Each experiment will involve 2 animals (experimental and companion). We will allow the experimental animal to recover in the pen from the general anaesthesia and the surgical procedure for 3-4 days to minimize the possible effect of the post-operative stress on both the mother and fetal responses to sound.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We do not anticipate more than a 5% complication rate and we will inform and take advice from The Named Veterinary Surgeon in case of any major adverse events. Minor complications could include a wound infection, haematoma or even a hernia. Before parturition, terminal general anaesthesia will be induced in the ewe using a suitable injectable agent and maintained with inhaled volatile agents. We will perform a laparotomy to identify the position of and remove the monitoring equipment. Ewe and fetus will then be killed by a schedule 1 method while under terminal anaesthesia without recovering consciousness.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to insert hydrophones or any other recording equipment into the amniotic cavity of a third-trimester human pregnancy due to the inevitable associated surgical risk to the mother and likely pre-mature delivery of the fetus.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are currently developing a computer model in collaboration with the department of medical physics & bioengineering at UCL using historical data from the literature and in-vitro laboratory acoustic experiments. If the first series of experiments validates the model we will only need to evaluate the effect of gestational age by comparing data obtained at 100-120 days and 140 days in a series of 6 pregnant sheep.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	The development and anatomy of the hearing system is similar in all mammals including marine mammals. The general anatomy and size of the ewe uterus is similar to the human. The lambs from

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	medium to small breeds are similar in size to human babies, usually between 5 and 12 pounds, with an average of 8 to 10 pounds. The animals will be recovered after the surgical procedure for a few days to ensure that they are healthy before we perform the experiments. We will minimise the animal suffering by using telemetric monitoring without needing to confine the animal's movement.

<table>
<tr><td>PROJECT 15</td><td>Studies to investigate laminitis predisposition</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Horse, laminitis, endothelium, insulin, metabolic</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></td><td>No</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>The objectives of the project are to:

i) Investigate methods to assess the function of the endothelium (blood vessel lining cells) in the whole animal
ii) Study the effects of season, diet and exercise on markers of abnormal endothelial function to determine whether the release of these markers is different between normal and previously laminitic animals.
iii) Study the role of microparticles (microscopic cell particles) in abnormal equine endothelial cell function in the laboratory
iv) Study the effects of short term feeding of various feedstuffs at different times of the year on metabolic responses, fat tissue gene expression and faecal bacteria in normal and previously laminitic ponies to determine whether differences are present which could be used to identify susceptible animals.
v) Undertake a prospective study to identify whether metabolic alterations occur prior to</td></tr>
</table>

	disease onset which could be used to identify susceptible animals.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits likely to derive from this project are an increased our knowledge of the role of abnormal endothelial function, inflammation and insulin in the pathogenesis of laminitis which may allow treatments and prevention strategies to be devised. In addition, by increasing our understanding of why certain individuals are predisposed to laminitis, animals at risk could be identified and preventative management countermeasures instigated in a more effective manner. These benefits they will hopefully lead to a reduction in the frequency of occurrence of this extremely painful equine condition and to improved treatment strategies. which in turn will have a significant impact on equine welfare.
What species and approximate numbers of animals do you expect to use over what period of time?	Adult horses will be used in this project. The majority of studies will use a maximum of 15 previously laminitic and 15 non laminitic animals over 5 years. Up to 400 not previously laminitic animals will be used in a single prospective study conducted over a 4 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 possible adverse affects include anaemia and thrombophlebitis associated with blood withdrawal, collapse following sedation, wound infection in conjunction with fat tissue biopsy, exhaustion associated with over exercising and laminitis following excessive carbohydrate consumption. • The likely/expected level if severity is mild. • At the end of the majority of studies, animals will be inspected by the named veterinary surgeon to determine whether the animals are suitable for continued use in another protocol under this or another project licence, to be kept alive at the designated establishment or to be discharged from the controls of the Act for example to be a companion animal.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to achieve the objectives of this project without using animals as we are studying complex metabolic pathways and physiological responses which vary with daylight length and season that cannot be modelled using isolated tissues, cells or computer simulations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Group sizes of up to 30 animals will be used in the majority of studies. This is in keeping with group sizes that appear in many publications using similar experimental designs. On each occasion, we will use the least number of animals possible to provide reliable and reproducible results based upon the experience of the researchers and evidence from published literature. The proposed number of animals to be used in the prospective study has been discussed with epidemiologists and statistical calculations have been performed to ensure that the minimum number of animals is 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.	Laminitis is a disease which affects horses and ponies and it is therefore most appropriate to undertake these studies in equidae. The unique anatomy of the equine foot and the precarious balance of forces in the blood supply to the foot of this species are central to the pathophysiology of the disease. Thus, our studies to further elucidate the role of insulin resistance, inflammation and abnormal endothelial function in the pathogenesis of the disease require *e*xperiments using whole animals. Whilst we can model some aspects of the disease in the laboratory, ultimately, methods to identify laminitis prone animals have to be tested in experimental horses and ponies (both control and previously laminitic animals) before moving to interventional field studies. The welfare costs to the animals involved in the project will be minimal as all of the proposed studies are mild and the likelihood of adverse effects is very uncommon.

<table>
<tr><td>PROJECT 16</td><td>The influence of barometric pressure changes upon membrane physiology and xenobiotic penetration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Air pressure, Penetration of drugs, Skin, Blood flow</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>
<table>
<tr><td>Basic research</td><td>Yes</td></tr>
<tr><td>Translational and applied research</td><td>Yes
No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes
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
No</td></tr>
<tr><td>Preservation of species</td><td>Yes
No</td></tr>
<tr><td>Higher education or training</td><td>Yes
No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes
No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes
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>The effects of local air pressure changes on penetration of drugs through the skin are presently poorly understood. It is important to understand how drugs work in different air pressures in order to find ways of improving drug absorption. The aim of this project is to try and gain a better understanding how local air pressure changes affects skin function and permeability to drugs. We will measure drug concentrations in skin and plasma to determine how much drug is absorbed. By applying an anti-inflammatory drug to the skin, we will determine whether we can reduced hind paw swelling in rats, through a concomitant alteration of local air pressure and increased drug absorption.</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>This research will contribute to our understanding of how changes in local air pressure influence the skin barrier. This is important because air pressure could alter skin resistance and affect chemical penetration. This project may lead to the development of a novel pharmaceutical dosage form that could be designed to improve delivery of drugs to the skin.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Rat is chosen as the experimental model. Approximately one hundred animals will be used over five years.</td></tr>
</table>

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?	Possible adverse effects include skin irritation after skin administration. To minimise this adverse effect the concentration of the test drug will be kept to a minimum. Paw swelling is likely to occur due to the induction of inflammation. Anaesthesia can lower body temperature. However, body temperature will be monitored and maintained throughout the experimental period. Administration of radioactive compounds to skin may cause a transient increase in skin temperature. In addition, blood sampling may cause temporary discomfort. However, these effects will be minimized since animals will be under general anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To study the effects of air pressure on skin function, the effect on blood flow must be considered and this cannot be done in artificial membranes or isolated skin samples.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before we start any animal experiments we will optimise our experimental approaches by alternative means. Specifically, we will assess the most appropriate range of air pressure to be tested and the drug amounts to be used. A synthetic membrane and porcine skin (obtained from a local abattoir) will be employed before any experiments are carried out in rats. These strategies will enable us to determine the efficacy of our experimental approach prior to the experiments outlined in this licence and will minimise the use of laboratory animals. In addition, rigorous study design with pre-consultation of a statistician will be conducted in order to obtain meaningful results using the minimum number of animals necessary.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Porcine skin is the most suitable animal model in the absence of human tissue to be employed in permeability studies. However, the rat is chosen as the experimental model as it has a close anatomical similarity to human skin, it allows easy access to monitoring the local blood flow and the rat fits the in-house pressure cell that is used to change the pressure applied to the skin.

PROJECT 17	**Pre-clinical mouse models of human ciliopathies**	
Key Words (max. 5 words)	embryogenesis, development, cilium, ciliopathies	
Expected duration of the project (yrs)	five	
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)	Neural tube defects (NTDs) occur in 1 per 1,000 births and cleft lip or cleft palate occurs in 1 per 550 births, and are therefore the most frequent classes of birth defect. The most common genetic causes of NTDs are due to a group of inherited developmental conditions known as “ciliopathies”. Ciliopathies are caused by defects in the structure or function of sensory “antennae” on cells called primary cilia. During normal development, cilia are finger-like projections from cells that detect and respond to chemical or mechanical cues, such as fluid flow, during the formation of the neural tube and other tubular structures. However, defects arise when a key ciliopathy disease gene has errors in its genetic code (known as mutations) that prevent a protein from either being made by the cell or from made properly. This often prevents the protein from being found at the cilia, as would happen during normal development. Ciliopathies share many clinical features such as brain developmental defects (including severe NTDs), defects in eye development,	

	additional numbers of digits and cystic kidney disease. Our proposed work will help to understand the causes of ciliopathies and leads to the use of preclinical animal models in the assessments of possible treatments for these human conditions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	To further understand the molecular roles of key ciliary genes in embryogenesis, we propose to study mice carrying the same defects as those that cause the human conditions. Research work on mouse models of ciliopathies will provide insights into gene function and disease causes of ciliopathies and complex conditions (such as NTDs, cleft lip and palate and eye developmental defects), as well as the functional role of primary cilia in signalling pathways. The understanding of ciliopathies at the molecular level may ultimately result in new therapeutic interventions for both NTDs and cystic kidney disease that may modify disease progression or the long-term outlook of patients with these disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use lines derived from lab strains of *Mus musculus*, the house mouse. Over a period of five years, we anticipate using no more than 1500 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?	Adverse effects will include embryonic and developmental abnormalities, increased pre-or post-natal mortality, reduced growth rate, tumour formation and impaired reproductive performance. These effects are likely to be of mild or moderate severity. If these effects persist or if any substantial sign is evident, animals will be humanely killed by a Schedule 1 method. If developmental defects prevent animals from moving around freely so that they are unable to feed (e.g. retinal degeneration), or if undue suffering is noted, it will be controlled by administration of suitable analgesia, or the animal will be killed by an S1 method. Any animal exhibiting a severe harmful abnormal phenotype will be killed by an S1 method
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As yet, there are no equivalent alternatives to the use of transgenic or mutated mouse models in the analysis of *in vivo* gene function. Our experiments are complemented by cell culture experiments and other *in vitro* biochemical techniques. However, given the complex cell and molecular interactions involved in, for example, the closure of the neural tube (neurulation), the *in vivo* analysis in the context of the whole animal provides the most informative data. Such investigations are impossible in the very limited numbers of human fetuses or patients that are available for scientific research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used in this study is the minimum number required to obtain consistent and verifiable data for each objective, and is based on statistical analyses (power calculations) of our published data. The number of animals used will be minimised by the application of *in vivo* imaging techniques to visualize defects in embryogenesis (namely, cystic changes in the kidneys, brain developmental defects, and additional numbers of digits). This reduces the number of animals required as the same animal can be monitored over time, thus allowing individual animals to be selected at particular time points in embryogenesis and development rather than using a number of animals at multiple time points.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	*In vivo* imaging will also allow the non-invasive antenatal detection of developmental defects and assessment of their severity. This will help us to more accurately determine humane end-points for experiments based on accepted guidelines for animals in embryology experiments and to implement end points.

PROJECT 18	**Epigenetic reprogramming in mammalian development**	
Key Words (max. 5 words)	Epigenetics, methylation, germ cells, embryos, diet	
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)	Epigenetic marks are put upon DNA (the substance of inheritance and cell function) in order to regulate gene activity during development of an organism and in adult tissues, and this is important for normal and healthy functioning of the organism. In germ cells (egg and sperm) and early embryos these epigenetic marks are removed in order to wipe the slate clean, but the removal is not quite complete. External factors such as nutrition can influence epigenetic marking and this can have long lasting consequences for normal development as well as human diseases and ageing, potentially affecting not only this generation but also children and grandchildren.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary benefit will be to publish new information which has wide applicability to other researchers and to pre-clinical (and clinical) scientists. In the longer run, the mechanisms we will discover will allow us to develop diagnostic markers of epigenetic states that are associated with nutrition and disease states such	

	as obesity, diabetes, or heart disease. This could help in the development of treatments (eg for human metabolic disorders). Our work will also contribute to making better stem cells hence helping to develop better and safer strategies for human regenerative medicine.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice and we expect to use about 1550 mice each year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use mice in our studies, and in particular those in which genes have been altered or removed which affect the epigenetic marking process. Removal of essential epigenetic regulators usually leads to early or late embryo lethality, but can have other effects too such as altered metabolism in later life. Dietary manipulations, such as high fat diets, are expected to induce obesity in mice, but the progression of obesity will be carefully monitored and the duration of the diets will be such that harmful complications of obesity are avoided. At the end of the studies, mice will be killed using the most humane methods to collect germ cells, embryos and other tissues in which to investigate effects on epigenetic marks.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals for this study because it is not possible yet to develop mammalian embryos completely in the test tube, nor to reproduce the earliest processes in mammalian embryo development, at which time the epigenetic marks are probably most sensitive to adverse diet and other factors, in purely cell-based systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have been able reduce the numbers of animals needed for these investigations because we have been able to develop highly sensitive methods for profiling the location of epigenetic marks in very small numbers of cells. We are also using cell-based systems where possible; for some stages of early embryo or germ cell development cell-based systems

	are available and we are using these, thus reducing animal numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mice in these studies because in this species we understand the most about where and how epigenetic marks are placed in the DNA, and because we are able to follow the fate of epigenetic mistakes during development in this species in a way that is not possible in other mammals, especially humans. We believe that the processes that put epigenetic marks in place in the mouse are very similar to those in humans, so the mouse is a very informative model. Harm to animals is minimised by using aseptic conditions, anaesthetics, humane methods of killing, and by targeting genetic mutations to the cells of interest to avoid whole-animal suffering. The welfare of each animal is monitored daily by animal care staff, veterinary staff and/or scientists. If, in rare circumstances, an animal has an unexpectedly severe response to a drug or operation, or where an infection develops, treatment is given where possible and, if necessary, the animal is humanely killed.

PROJECT 19	**Management of genetically altered rodent lines**	
Key Words (max. 5 words)	genetic alteration embryo stem cell	
Expected duration of the project (yrs)	5	
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)	This licence is to allow the generation, maintenance and preservation of genetically altered mouse lines for the use of scientific researchers.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Genetically altered mouse lines are widely used in biomedical research. The activity of a particular gene may be disrupted (the so-called “knock-out mouse”) so that its contribution to normal physiology and the development of certain disease states can be studied in detail. Or a very subtle change may be introduced, perhaps to make the gene product more similar to its human counterpart and thus improve the predictive value of the mouse. The potential benefits of ALL such alterations will be described in project licences held by individual researchers. This “service” licence sets out to reduce and refine the use of animals in generating, importing and preserving genetically altered mouse lines at our establishment, by centralising these activities in the hands of skilled and experienced personnel.	

What species and approximate numbers of animals do you expect to use over what period of time?	The mouse is still the most widely used species for genetic alteration. We expect to use about 20,000 mice in standard breeding programmes and a further 3500 in the harvesting of embryos (for genetic alteration or frozen storage). About 3000 animals will have embryos implanted into them, to allow development to normal birth.
In the context of what you propose to 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 shall provide a service to breed and maintain lines of genetically altered mice in which no deviation from normal welfare is expected. We shall harvest embryos or sperm from these animals in order to have a frozen stock (and thereby avoid the need to continue to breed a line that is temporarily not needed). We shall send frozen embryos or sperm to other centres in order to distribute the lines we have and we shall import lines from other laboratories as embryos or sperm too, for "rederivation" into live mice here. Occasionally we may generate entirely new genetically altered mouse lines ourselves, by manipulating embryos or embryonic stem cells before implanting them into adult female mice. Breeding and maintenance are not expected to cause any significant adverse welfare effects. Hormones are used to promote the yield of embryos in some cases, but their administration is also highly unlikely to cause harm. Embryos are usually implanted into pseudo-pregnant females (i.e. mice whose physiological systems have been "fooled" by mating them with vasectomised male animals). This implantation, and the vasectomies, are surgical procedures conducted under general anaesthesia. The procedures are routine, and excellent rates of recovery are expected. All animals undergoing them will receive pain relief after surgery. At the end of a procedures, animals will be killed (some of them for the harvest of embryos or sperm for rederivation or frozen storage) or retained for further breeding under the authority of this licence or other project licences to which the mice might be transferred.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Precise alteration of the activity of a specific gene in mammalian cells still often requires the production of a whole animal carrying the alteration of choice. So, even if most of the scientific effort is devoted to laboratory work on cell cultures, the mouse is still required as a source. Other genes express their activity in a number of tissues and organs and therefore can only be studied properly in the three-dimensional whole organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Centralisation of the activities in one scientific service allows the best use to be made from the fewest number of animals. For example, there only needs to be one stock of vasectomised males (maybe 6-10 at any one time) for the whole of the establishment. All procedures can be monitored for their efficiency and quality and new best practice implemented immediately. Breeding programmes will be under the control of highly skilled animal care staff and will be carefully adjusted to match the service and scientific demands on them.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The major interventional procedure is the surgical re-implantation of embryos into female mice in order to carry them to the normal term and birth. This is a routine procedure and its centralisation in skilled hands ensures that the success rate is very high and that the animals receive the best possible post-surgical care. Non-surgical alternatives have been proposed and we shall continue to evaluate their efficiency. Clearly, if they can be made to work with similar success rates to the surgical procedure, then we will adopt them routinely.

<table>
<tr><td>PROJECT 20</td><td>The pathophysiological roles of Podoplanin and its receptor CLEC-2</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Podoplanin, CLEC -2, Embryogenesis, Vascular Integrity</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>~~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>
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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>To determine the role of the Podoplanin-CLEC-2 signalling pathway during (1) embryonic development of vessels and organs, and (2) maintaining the healthiness of vessels in adults during physiological processes including inflammation and wound healing.</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 potential benefit of this project will be fundamental understanding of the function and regulation of the lymphatic system. By identifying pathways that regulate development of the lymphatics and the way they respond during inflammatory diseases and wound healing 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>Over 5 years, we would expect to use no more than 22,500 mice in total – 7,700 animals for scientific protocols and 14,800 to breed the genetically altered strains required.</td></tr>
<tr><td>In the context of what you propose to 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?</td><td>Breeding of genetically modified animals – we expect adverse effects of mild severity such as minor weight loss. However in some genetically altered animals (e.g. platelet specific CLEC-2 deletion) the resulting lymphatic defects may lead to mild swelling of the paws, which from our previous experience is asymptomatic. If pain is observed, as evidenced by tip-toe walking, animals will be humanely culled however, this is not</td></tr>
</table>

	expected. Induction of conditional genetic modification – 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 upon cessation of tamoxifen treatment with little-to-no lasting effects once genetic deletion has been achieved. Modulation of signalling pathways – we expect adverse effects of mild severity such as weight loss <10% and reduced activity. There will be increased risk of spontaneous bleeding although from our previous experience we do not expect this to happen. Reducing platelet cell numbers – we expect adverse effects of a mild severity including reduction of platelet count by >95%, weight loss <10% and reduced activity. There will be increased risk of spontaneous bleeding although from our previous experience we do not expect this to happen. In addition, this does not lead to hypothermia and has no adverse effects on white cell blood counts Vessel leakage models Intra-tracheal LPS – we expect adverse effects of a moderate severity such as weight loss of up to 20% and reduced activity. Reverse passive Arthus reaction - we expect adverse effects of moderate severity such as increased pain and discomfort which will be treated with pain relief from the beginning of the protocol. Wound repair – we expect adverse effects of a moderate severity such as increased pain, discomfort and loss of weight of up to 20%. These will be treated with pain relief and hydration throughout the protocol. Any animal with infection or weight loss of ≥20% or continued pain will be humanely killed. Chronic inflammatory model TNFΔARE mice – we expect adverse effects of a moderate severity in those mice that carry one mutated TNFα allele, including inflammation manifesting as visible arthritis and mild colitis. We will not be breeding mice with both alleles mutated. We will humanely cull animals that reach 14 weeks

	of age or if they present with joint swelling with absence of grip strength or have weight loss of ≥20% compared to wild type littermate controls. Growth of new lymphatic vessel model – we expect adverse effects of a moderate severity such as increased pain, discomfort and loss of weight of up to 20%. These will be treated with pain relief, hydration and warmth when appropriate throughout the protocol. Any animal with weight loss of ≥20% 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, and platelet function No *in vitro* methods exist to model the complex and full immune response including an intact vascular and lymphatic supply which we believe is regulated by CLEC-2 expressed on platelets. Animal experiments are necessary to ascertain whether the increased expression of Podoplanin observed during inflammation is a mechanistic driver of pathology rather than just a reaction to injury/inflammation. This cannot be achieved using clinical specimens
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical analysis will ensure that we use the minimum number of mice per group that will be informative will be performed. Inducible global knockouts will be used in adult mice 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. We are using a staged approach, involving pilot studies to ensure that an appropriate number of animals are to be used.

	To maximise the information gained from a single animal we aim to take perform multiple *in vitro* analyses on each individual. 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.
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 system that controls blood clotting in 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 on normal and inappropriate blood clotting models, and models of inflammation/vessel leakage in mouse 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. We are using a staged approach, involving pilot studies to ensure that monitoring regimes will be appropriate for each strain used. 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 NVS or NACWO, suffering can be remedied promptly and successfully using no more than minor interventions, such as pain relief and hydration.