Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 19

Projects with a primary purpose of: Basic Research into the Cardiovascular, Blood and Lymphatic System

PROJECT TITLES AND KEYWORDS

1. **Neural regulation of cardiovascular function**
    - Blood pressure, sympathetic, hypertension, homeostasis, autonomic nervous system.

2. **Neural control of myocardial excitability in cardiovascular disease**
    - Heart, brain, autonomic nervous system, free radical, cyclic nucleotides

3. **Characterizing how lipids regulate cardiovascular disease and bleeding**
    - Atherosclerosis, lipids, bleeding

4. **Therapeutic neovascularisation for ischemic disease**
    - Neovascularisation, Ischemia, Cardiovascular disease

5. **Molecular Regulation of Heart Development**
    - mouse, embryo, development, heart, vasculature

6. **Prevention of Organ Injury Following Cardiac Surgery**
    - Cardiac Surgery, Organ Failure, Transfusion

7. **Interplay between oxygen and iron in hypoxia-induced pathology**
    - Iron, hypoxia, heart, muscle, placenta

8. **Inflammation and Atherosclerosis**
    - Inflammation, Atherosclerosis. Collar, Disease, Therapy.

9. **Provision of biological materials**
    - Blood, Macaque, *in-vitro*

10. **Thromboinflammation in thrombosis in veins**
    - Thrombosis, inflammation, mast cells, T-cells, platelets

11. **Genetics of Cardiovascular Development and Function**
    - Heart development, heart disease, genetics

12. **Studies on the Mechanisms of Atherosclerosis**
    - Accelerated atherosclerosis, restenosis, hypercholesterolaemia

13. **Regulation of Haematopoietic Development**
    - Haematopoiesis, Angiogenesis, Embryogenesis, Leukaemia

14. **Production of antibodies for research purposes**

- Production of antibodies for research

15. Regulation of Heart Development in Vertebrates

- Heart, Cardiogenesis, Morphology, Non-compaction

16. Endothelial dysfunction in pulmonary hypertension

- Vessel wall thickening, lung, hypoxia, inflammation

17. De novo generation of haematopoietic stem cells

- Blood, stem cells, genetic program, haematopoiesis, transplantation

18. Antibody production

- Antibodies, immunisation

19. Regulation of blood vessel maintenance and repair

- Blood vessels, heart attack, aneurysm, cardiovascular disease

20. Adaptive mechanisms of the ischaemic myocardium

- Myocardial infarction, gene-modified animals, redox, cardiac remodelling, heart failure

21. Characterising & inhibiting vascular disturbances

- Microcirculation, stem cells, inflammation

22. Regenerative capacity of skeletal and cardiac muscle

- Cardiac stem cells; skeletal muscle stem cells; repair, regeneration; myocardium

23. Regulation and mechanism of platelet activation by GPVI and CLEC-2

- Platelets, signal-transduction, haemostasis, vascular-integrity

24. Cardiovascular effects of uraemia

- Kidney disease, heart failure, uraemia, diabetes

25. Systems Biology of Cardiac Biophysics

- Heart Rhythm, Infarction, Ablation, Fibrosis

26. The regulation and role of protein kinases in the heart

- Heart failure, the failing heart, heart disease, protein kinases, phosphorylation

27. Mouse models of cardiovascular development and disease

- heart, cardiogenesis, CHD, mouse models

28. Control of early heart development in vertebrates

- Heart, embryo, frog

29. Molecular mechanisms of development

- Angiogenesis, inflammation, drug screening, molecular targets

<table>
<tr><td>PROJECT 1</td><td colspan="3">Neural regulation of cardiovascular function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Blood pressure, sympathetic, hypertension, homeostasis, autonomic nervous system.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>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">The aim of this project is to understand the brain’s involvement in generating high blood pressure and to gain mechanistic insight as to how and why this occurs so that new therapeutic strategies can be harnessed. This is particularly important since ~14% of hypertensive patients are resistant to contemporary drugs or cannot tolerate medications.

The causes for high blood pressure, which affects 1 in 3 of us, are unknown in 95% of patients. Given the poorly tolerated side effects of blood pressure medications and the numbers of patients that become resistant, new therapeutic strategies are urgently required. High blood pressure causes stroke, heart failure/attacks and kidney damage costing significant reductions in life quality, suffering and medical expense to the state. It appears that in most patients with high blood pressure there is a central nervous system dysfunction. Whilst we understand what has changed, we do not know why</td></tr>
</table>

	this has occurred. Our primary aim is to understand the neural regulation of the circulation and breathing (as this influences blood pressure) and to determine the changes that occur during the development and maintenance of high blood pressure in animal models of this human syndrome. We hypothesise that there are genomic changes within areas of the brain controlling blood pressure that reduce or restrict blood flow into these areas of the brain triggering high blood pressure. It is this that we wish to study.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The results from these studies will provide us with fundamental new information relating to how the body receives, handles and then acts upon changes in blood pressure. It will reveal the way in which the body normally responds to these stimuli and reveal how this is altered in conditions of high blood pressure. We will obtain a better understanding of the genes in the brain that are responsible for high blood pressure and how external factors interact with them. As such, it will help in providing much needed information that will assist in the design of new medicines and/or therapies to treat people and animals who suffer from high blood pressure and related diseases. As such it is envisaged that this work will be of significant benefit to the large number of patients who are stricken with hypertension and other related diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	It is expected that no more than 7,000 rats and 1,700 mice will be used during the course of this project. Rats are the most established animal species for understanding the central nervous control of the circulation and respiration and there are established models of hypertension in rats that share commonalities with human hypertension. We have much experience (20 years) with these animal models. Mice are required because there are some excellent models of human diseases (e.g. Rett syndrome) and allows us to use transgenic animals. For all experiments we use power calculations to ensure that the minimal number of animals are

	used to achieve biological significance. In all experiments, the design is carefully planned to ensure maximal data output from each animal.
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 proposal will use a number of animal models of high blood pressure and heart failure. The reason for using more than one model is that high blood pressure is most likely due to a number of factors such as stress, high salt intake, chronic pain and excessive autonomic nervous activity. Equally, it may be due to increased susceptibility due to the genetic background of an individual. Hence, we have chosen models that reflect these distinct facets to get a full understanding of human cardiovascular disease. From previous research we know which regions of the autonomic nervous system control the heart and blood vessels. We also know from studies in man, and from animal models of hypertension, that there are changes in the activity of nerves that control the cardiovascular system in the high blood pressure condition. Interestingly, these changes precede the onset of high blood pressure suggesting a possible causative link. Changes in autonomic control of the cardiovascular system are now used clinically as prognostic indicators of cardiovascular disease. In addition, most medicines that are routinely prescribed to patients to lower blood pressure also affect autonomic nervous activity. Hence we will: 1). assess how genes in the brain affect the development and maintenance of high blood pressure 2). demonstrate how genes affect the susceptibility for development of high blood pressure triggered by external factors such as a high salt diet, stress and/or altered levels of circulating hormones; 3). determine whether mechanisms controlling

	blood flow into areas of the central nervous system controlling blood pressure are dysfunctional in conditions of high blood pressure. 4. examine the role of homeostatic reflexes in control of blood pressure in disease states and understand whether manipulation of these systems could provide therapeutic benefit in animals with high blood pressure. ***Procedures:*** We will use animals with hypertension that are genetically pre-disposed and will induce hypertension by pre-treating dams and then study their offspring. Other methods include infusion of hormones, high salt diet, applying stress, occlusion of major blood vessels, including those to the kidney or brain, or removal of a kidney. Experiments will be carried out in rats and mice under terminal anaesthesia (i.e. the animals will not wake up from the anaesthesia). In these studies, a number of measurements can be made that are not possible in conscious animals such as activity from nerve trunks or recording from single brain cells or imaging of processes operating within the brain. However, in order to make definitive measures of blood pressure it will be necessary, in some cases, to make measurements in conscious animals. In these cases, we will use the most modern methods that allow remote recording of blood pressure (via radio waves) such that the animal is undisturbed, unrestrained and behaving naturally in its home cage. These experiments will permit us to look at the long term effects of genetic manipulation using viral vectors, drugs and experimental perturbations on blood pressure control. This is crucial as high blood pressure is a chronic disease which develops over time. Our experiments aim to reproduce this. Heart failure will also be studied by permanent occlusion of a coronary artery.

	Expected adverse events: Our animal models of hypertension are of severe severity. In any model of hypertension, stroke may occur. Should an animal exhibit signs of stroke or weakness they will be immediately killed and the NVS/NACWO consulted. Where there is a surgery with recovery wound breakdown may occur and will be minimised by good surgical technique and appropriate asepsis and if it does occur it will be repaired under general anaesthesia. Post-operative pain will be controlled by use of analgesics . Infection around wounds may occur and in consultation the NVS and/or NACWO consulted antibiotics administered. After cannulation, cannulae may become dislodged. This is likely to prove fatal. An animal suffering as a result of haemorrhage will be immediately killed. After CNS surgery intracranial bleeding may occur and the animal will be killed immediately. Following cannulation of the aorta for radio-telemetry ischaemia in the hind body may occur. To avoid this, appropriate training will be given to minimise the occurrence. Any animal showing signs of more than minor distress will be killed. Hypothermia may occur as a result of general anaesthesia, this will be minimised by provision of supplementary heat both during anaesthesia and during recovery. Studies under anaesthesia will include continuous physiological monitoring which will provide an online assessment of the level of anaesthesia, which can also be assessed using noxious pinching of a paw or the tail. The level of anaesthesia will be adjusted accordingly. In the event of blood loss (hypovolaemia), animals may receive saline solution by infusion. To study heart failure this will be induced by coronary artery ligation; this may kill some animals due to fatal arrhythmias, which makes this a severe procedure.
Application of the 3Rs	
1. Replacement State why you need to use	Since the nature of the work is to look at the regulation of physiological systems, in vivo studies on conscious and anaesthetised animals

animals and why you cannot use non-animal alternatives	will be essential. The proposed studies can only be carried out in living animals since the aim is to study integrative physiological and pathophysiological processes that are controlled by the central nervous system and which cannot be replicated in computer-based model systems or in isolated tissues. Thus, to fulfil the objectives the cardiovascular system must be maintained functionally intact so that the functional consequences of experimental perturbations can be determined at the systems level.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Over the term of the last licence efforts have been made to reduce the total number of animals used: 1). We have adopted modern methods of measuring blood pressure using radio-transmitters. This is viable for 3 months unlike conventional catheters which block. This increased longevity has reduced animal numbers and avoids the need for chronic indwelling arterial catheters, which can be life threatening if they are pulled out by the animals, and cause infection which affects data interpretation. 2). Our *in situ* intra-arterially perfused decerebrate preparations, which are insentient, are typically used in preference of anaesthetised in vivo animals as they avoid the depressant action of anaesthesia and give data that are more analogous to that obtained from the conscious animal. Moreover, multiple reflexes can be stimulated countless times allowing us to perform many experiments in the same preparation rather than separate in vivo animals. 3). We will use acute brainstem slices in vitro that more reliably give long-term intracellular data than in vivo (and in situ). Numerous slices can be made from a single rat so it is likely that many more recordings can be made from a single rat brain when sliced compared to an in vivo situation. Neurones can be functionally identified by retrograde transport of a fluorescent dye. Thus, statistical power will be obtained from fewer animals. However, data interpretation of these experiments is open to question since the type of neurone cannot be tested in a physiological environment where all connections are intact. Thus, our approaches of in situ and in vivo animals will be needed where these connections are maintained. 4). We have invested a lot of effort into culturing brainstem slices, which remain viable for weeks. This together with cell lines has reduced considerably the need for many of the in vivo

	experiments since many chronic tests (i.e. virally mediated gene expression) can be performed on this type of preparation. However, in terms of assessing physiological function, in situ and in vivo approaches are 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.	By far the bulk of our studies will involve anaesthetised animals, insentient animals (decerebrated under deep anaesthesia) or radio-telemetry. Whilst the latter does involve a surgery for implantation of the transmitter for recording blood pressure, this then allows the animal to live unrestrained, unstressed, unhandled in its home cage oblivious to the fact that we are recording its blood pressure. Experimental time is kept to a minimum. Animals are checked at least once daily seven days a week. Any signs of lethargy or ill health are dealt with immediately. We have an excellent animal facility and staff including a NVS and NACWO who are always at hand to advise us on best practice. In animals that will be anaesthetised we will check for the depth of anaesthesia regularly by noxious pinching and from the stability of their blood pressure, heart rate, respiration, end tidal CO2, body temperature and blood gases. The animals are therefore kept in excellent physiological condition.

<table>
<tr><td>PROJECT 2</td><td>Neural control of myocardial excitability in cardiovascular disease.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart, brain, autonomic nervous system, free radical, cyclic nucleotides</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five 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>Many cardiovascular diseases (eg heart failure, hypertension, post myocardial infarction) are also diseases of the autonomic nervous system. The autonomic nerves increase and decrease the hearts excitability during times of stress and rest, however, their impairment can trigger cardiac arrhythmia. In particular, sympathetic activation, high levels of circulating adrenaline, and impaired cardiac vagal activity are all negative prognostic indicators for sudden cardiac death and strong independent predictors of mortality. Yet the communication between the neuron and target heart cells is still poorly understood and remains a major therapeutic target.

Our research aims to understand how the chemicals released from these nerves control the rhythm of the</td></tr>
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	heart during normal and abnormal activation. Specifically, we are interested in the intracellular messenger systems in the neurons that control the release of neurotransmitter. In particular we want to establish whether a fast acting chemical messenger released as a gas, nitric oxide, or peptides released by the heart itself, are important in initiating a cascade of chemical events that regulate the activity of ion channels on cell membranes that affect the excitability of the heart. Importantly, we also want to establish where in the cardiac-neural axis these changes occur, since both central (brain) and peripheral neural sites (close to the heart itself) have been implicated in cardiovascular disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	If we can identify the key target proteins in the nerves from diseased animals then we can target the genes that encode these proteins with a gene transfer strategy. Current therapy on the heart cells themselves is still sub-optimal. By controlling the release of chemical transmitters from the autonomic nerves we may be able to get better regulation of the chemical transmitters which can trigger arrhythmia in diseased states.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the period of five years we expect to use 3000 mice, 2000 rats and 750 guinea-pigs.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We are propose to characterise new genetically modified mouse models as well as generating mouse models of human disease, notably mild cardiac hypertrophy which we could also test new therapeutic strategies. Rats that show spontaneous hypertension will also be studied. Characterisation would typically include non-invasive (*e.g.*, echocardiography/MRI) and/or invasive (*e.g.* LV haemodynamics as terminal procedures) evaluation of cardiac mass and function under anaesthesia, and *ex-vivo* investigation using myocardial tissue and cells. Mice and rats might undergo voluntary exercise (by using a wheel available in their cage) or be administered agents that are used or considered

	for use in human disease and have their blood pressure and heart rate monitored by implantation of radio-telemeters. Cardiac hypertrophy will be induced by administration of isoproterenol via an osmotic mini-pump (essentially a small capsule) implanted subcutaneously, a treatment that induces an increase in heart size and remodelling. The progression of the disease will be closely monitored by non-invasive techniques and invasive cardiac investigations as terminal procedures. Expected side effects of the procedures may cause respiratory problems and thoracic pain of moderate severity. Some animals will receive gene therapy to correct defective protein signalling in an attempt to rescue their diseased state. At the end of the procedure the 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	We require studying animal models of heart disease because exclusively *in vitro* studies would not recapitulate the complexity of the pathophysiological situation found *in vivo*. The use of animals is therefore essential for assessment of the components of cyclic nucleotide signalling in the complexity of the mature intracellular environment and within the whole organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals required has been carefully determined based on previous experiments, and is the minimum required to achieve statistically significant results in our investigations. Everything has been done to ensure that the numbers of animals used are as small as possible. Through years of basic research experience, the applicant and his collaborators are familiar with the use of adequate statistical tests to ensure that a viable minimum is used. Power calculations are routinely performed by the applicant when experiments are designed. The minimum numbers of animals required have been carefully reviewed by the funding agencies and have been based on power calculations.

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*** Experiments will be performed on mice for a number of justified reasons. First, the mouse has a predisposition for voluntary wheel running and will self-limit its performance when tired. For this reason it behaves like a human. Secondly, the mouse will also be used to study various mutations of NOS and reactive oxygen species (ROS) signalling pathways since these mutations are not available in other species. Moreover, the transgenic approach allows specific mutations in ROS pathways to be selectively studied to test their role in the neural control of cardiac function. Thirdly, we have created a cardiac neuronal NOS overexpression model, which will increase specificity of targeting to study normal and diseased phenotypes chronically. These site-specific vectors minimise off target effects to the animal and are seen as a major refinement and advance from the previous PPL. Finally, gene transfer strategies have already been developed for the mouse. Gene transfer will be used to establish proof of principle of signalling system with genes targeted via adenovirus, which gives short acting expression (peak expression 5 days). Gene transfer will be targeted into the mouse, the spontaneously hypertensive rat (an established animal model of hypertension, with which we have experience of working) and the guinea pig. ***RAT*** The best model for hypertension is the spontaneously hypertensive rat which we have a lot of experience with regarding gene transfer and autonomic phenotyping *in-vitro* and *in-vivo.* Like the mouse, volitionally wheel running will occur so animals will self-regulate their activity. ***GUINEA-PIG*** we have a great deal of experience with *in vitro* experiments performed using guinea pig tissue to dissect out the role of the signalling pathways. Again, the increased size of the tissue provides advantages over the mouse, and dissection of guinea pig tissue for *in vitro* preparations with intact sympathetic and/or parasympathetic innervation gives a higher success rate than is seen with the rat. Moreover, the electrophysiological profile of the guinea-pig is similar to the human compared the rat or mouse.

	As well as refining our viral vectors to be site-specific to minimise promiscuous gene transfer *in vivo*, we have also developed a technique to transfect cells *ex vivo* at reduced cost to the animal. Additionally, we are building cardiac-neural co-culture systems to study arrhythmia in a dish, but tissue must first be removed from the animal regarding unregulated procedures. Finally, data will be used to validate our recent computer models of cell-cell communication which will also add to *reduction* and *replacement* over time. All invasive procedures will be performed under anaesthesia and the animals will be continuously monitored post-surgery and will always receive analgesia. Any animal showing signs of uncontrolled pain will be humanely killed.

<table>
<tr><td>PROJECT 3</td><td colspan="3">Characterizing how lipids regulate cardiovascular disease and bleeding.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Atherosclerosis, lipids, bleeding.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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>
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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 colspan="3">This project aims to understand the role that particular lipids (fats) play in causing cardiovascular disease and in regulating blood clotting. Work from our group has discovered several new lipids that are made by human circulating blood cells. Our preliminary studies indicate that these can regulate immune responses in vitro of relevance to cardiovascular disease and we now wish to determine their in vivo actions.</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">Lipids play a major role in the pathogenesis of cardiovascular disease, but the mechanisms of this are still not fully understood. The potential benefits to of this work could include new treatments to prevent cardiovascular disease in susceptible individuals. We may also uncover new agents that could be developed for treatment of either bleeding or pro-coagulant disorders</td></tr>
<tr><td>What species and approximate numbers of</td><td colspan="3">Up to 12,500 mice over 5 year period</td></tr>
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animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will be bred together to generate new genetically modified strains. This might result in unexpected illness that will be closely monitored for. Based on published literature this is unlikely to be greater than mild/moderate severity. Appropriate control measures are in place to ensure that all animals will be monitored closely and appropriate action taken. All animals will be killed at the end, either by terminal anesthesia or an approved human method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are required for this work because we cannot test the effects of lipids in cardiovascular disease using cell models. Up to now, all of our work on these lipids has been on cells and to further our understanding of their roles in disease we now need to conduct in vivo experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Based on previous work using these atherosclerosis prone mice (carried out in the lab or our collaborators in Oxford), appropriate group sizes that give a defined statistical power will be used. Results will be monitored as they are undertaken to determine whether subsequent experiments could use less if 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 measures you will take to minimise welfare costs (harms) to the animals.	Mice do not normally get cardiovascular disease on high fat diets. However, mice with a mutation in the ApoE gene (a protein that is important in handling plasma lipids) are susceptible to (i) elevated plasma lipids and (ii) atherosclerosis. These mice are a robust murine model of cardiovascular disease that have been extensively characterised over the last 15-20 years. This makes them an appropriate model to study the pathophysiology of this disease. To minimise harm to the animals, they will be monitored daily (more often when undergoing procedures) and where there is any concern, advice will be sought from the Named Veterinary

	Surgeon and Named Animal Care and Welfare Officer, before appropriate action taken.

<table>
<tr><td>PROJECT 4</td><td>Therapeutic neovascularisation for ischemic disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neovascularisation, Ischemia, Cardiovascular disease</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>
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<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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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>Ischemic disease is the most common cause of death and morbidity in the UK. The goal of the project is to identify and develop new strategies to increase the growth of new blood vessels for treatment of ischemic diseases such as peripheral arterial disease. Peripheral arterial disease results in obstructions of arteries, limiting the blood supply to organs. The most common site of peripheral arterial disease is the lower extremity. Of note, no medical therapies are effective in improving perfusion to the lower extremity in patients with peripheral arterial disease. Surgical and catheter-based “revascularisation” approaches can improve considerably the preservation of life and limb. However, many patients with generalized occlusive disease have no option for revascularisation. Moreover, re-stenosis complicates up to 25% of revascularisation procedures. Clearly, a need exists to develop new treatment strategies for patients</td></tr>
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	who are not suitable for the current procedures of revascularisation. "Therapeutic neovascularisation" is a promising strategy to relieve the ischemia effects. Although a series of clinical trials in therapeutic neovascularisation have been completed, the degree of success in these studies has been limited. This has spurred interest in identifying novel therapeutic targets and strategies that enhance blood vessel growth and improve the outcome of ischemia.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	• The project will allow us to have better understanding of the factors controlling the growth of new blood vessels after ischemia, and to shed light on novel targets that can be exploited to treat ischemia (limb ischemia, in particular) • The knowledge gained from the project will lead to development of new medicines and approaches in the future, which can improve the prognosis and symptoms of patients suffering from ischemia.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use **mouse** model in our project for several reasons: - Mouse genes can be readily modified to alter expression of key molecules allowing us to study the gene function in ischemic disease context. - Mouse hindlimb ischemia model mimics many aspects of human peripheral arterial disease so it is a useful model to understand the molecular basis of the growth of new vessels in the context of ischemia. - It has proven previously to be an instrumental tool for discovering targets for therapeutic drugs in cardiovascular diseases. Estimated numbers of animals to use is **5400** mice over 5 years
In the context of what you propose to do to the animals, what are the expected adverse	• In order to study drug candidates on the process of blood growth, we test it first in model of neovascularisation. The drug is mixed with a gel that is injected directly or encapsulated in a small

effects and the likely/expected level of severity? What will happen to the animals at the end?	cylinder under the skin of the animal. Several weeks later, blood vessels will invade the gel. The animals will be terminated humanly to measure the effect of drug on the blood vessel growth. This protocol is moderate severity. No major adverse effects are expected. • The femoral artery supplying the hindlimb muscles will be permanently blocked in one side, to cause ischemia. Following the surgery, the effect of the therapeutic intervention on the blood flow in muscles and limb function will be determined using non-invasive techniques such as laser Doppler imager and voluntary running wheel over an extended period (up to 28 days). This protocol is a moderate severity. Surgical discomfort and the pain that could arise from ischemia will be alleviated by appropriate painkillers. Rarely (<5%), some animals will develop necrosis of the whole foot or severe immobility. We therefore monitor animals regularly and euthanase by human method at an early stage. • All surgical procedures are done under general anaesthesia, so the animal does not suffer during the procedure. • The numbers of animals will be minimised by careful experimental design and appropriate statistical analysis. • All the animals will be humanly killed at the end of experimental period and tissues will be collected for further biochemical and histological analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Some experiments will be performed in cell to understand the molecular basis of blood vessel formation and to help to choose the best therapeutic approaches to take forward. However, the use of live animals is unavoidable since cell models lack the blood flow and interaction between blood vessels and immune system that are typical of living tissues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	• Generally, the primary cell screens for new therapeutic targets will limit the numbers of animals required for the following evaluation phase in animals. In particular, we use well-established cell models that reflect many aspects of blood vessel growth.

	• The use of non-invasive Laser Doppler Imager for measuring the limb blood flow allows us to study the same mouse at different time-points. • We use standard statistical methods to determine the minimum number of animals necessary to obtain meaningful results.
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.	• Animals are housed in social groups and are provided with environmental enrichment. To minimise adverse effects after surgery they are routinely provided with painkillers, softened food, extra heating, and fluids. • As in humans, some animals (<5%) will show necrosis of the whole foot or severe immobility that is inevitable when limb ischemia occurs, and we therefore monitor animals regularly and euthanase by human method at an early stage.

<table>
<tr><td>PROJECT 5</td><td>Molecular Regulation of Heart Development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>mouse, embryo, development, heart, vasculature</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>(Yes)</td><td>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>Purpose of the project.

This project uses genetically altered mouse lines, to investigate processes in the embryo and foetus that are involved in assembly of the heart and which, if impaired, might predispose to, or cause, heart defects. A significant by-product of these studies is to uncover molecular pathways and cell biology in the developing heart which might be extended to the diseased adult heart to repair any injury. The work plan consists of: (i) identifying genes important in the shaping the developing heart and those specifically involved in the formation of the cardiac muscle, the outer layer of the heart called the epicardium and the coronary blood and lymphatic vessels; (ii) investigating the embryonic and foetal processes that lead from gene function to heart assembly and, moreover, from gene defect to heart defect; (iii) identifying new methods or pathways for</td></tr>
</table>

	preventing heart defects by 'correcting' heart development in the embryo or foetus and iv) ultimately uncovering embryonic cell potential which might be reactivated in dormant adult heart cells to instrument repair following a heart attack. **Clinical unknown.** Heart defects that manifest at birth affect 1% of all pregnancies world- wide and, adult cardiovascular disease is the biggest world-wide killer, therefore, in combination this represents an enormous burden on society. Many children with heart defects require corrective surgery, transplantation and extended (even life-long) medical care. For example, children with hole in the heart or defective blood vessels require surgery, often repeated as the child grows older. Moreover, our studies in the embryonic heart may help identify how to stimulate gene pathways and cells in the adult heart to initiate repair of damaged muscle and blood vessels following a heart attack.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	**Expected benefits.** These include: (i) increased understanding of embryonic heart development, both normal and abnormal leading to heart defects manifesting as congenital heart disease; (ii) improved methods of genetic diagnosis and genetic counselling, which should follow from discovery of genes that cause heart defects in mice, provided the findings are confirmed in human studies; (iii) identification of new pathways that might be amenable to drug treatment (iv) identification of pathways of development that might be recapitulated in the scenario of adult heart “repair”.
What species and approximate numbers of animals do you expect to use over what period of time?	**Numbers of animals to be used.** In this project, the majority of mice will be used for purposes of breeding to maintain colonies of genetically altered strains. It is extremely difficult to estimate the numbers required as this depends on strength of effect. We estimate that up to 2500 mice per year will be involved in the breeding

	programme, of which 1000 are used solely for breeding and genetic typing using DNA obtained from an ear punch. 50 female mice will receive hormone injections to produce large numbers of fertilised eggs (e.g. for embryo freezing to preserve valuable strains) and a further 50 females will serve as uterine foster mothers for implanted embryos (e.g. when re-deriving a strain for importation or health purposes). The remaining 1200 mice will be mated to produce pregnancies containing embryos and foetuses for the study. Mated mice will be killed to remove embryos and foetuses for the studies, or recycled into the breeding colony.
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 are associated with making new genetically modified mouse lines, via generation of vasectomised males, administration of drugs to females to induce super-ovulation and embryo transfer. These procedures are anticipated to be mild in severity, but adverse events associated with pain sensation in each case will be countered by administration of analgesics. Breeding and maintenance of genetically modified lines in the vast majority of cases (87%, ie. 10,000 of the total 11,500 animals within the project) will have no outward phenotype and lie within a mild severity category. In approximately 1-2 lines (total of 1500 mice) there may be increased severity of adverse effects as associated with cardiac insufficiency (including increased heart rate, breathlessness, inactivity and failure to feed). These animals will be monitored and where distress exceeds a moderate severity limit they will be humanely culled (by a schedule 1 method). In the experimental protocols, the harvest of blood from rats, will incurr transient stress associated with the induction of terminal anaesthesia. In the studies involving administration of substances to look at effects on embryonic growth and effect on heart development during pregnancy, treated adult female mice may experience adverse effects related to the substance including shallow, rapid breathing, increased heart rate, uncoordinated or slowed movement or failure to thrive. These mice will be monitored and where

	distress exceeds a moderate severity limit they will be humanely culled (by a schedule 1 method). Embryos will normally be studied at an early stage of development, before pain or other sensations have been acquired. These embryos are killed almost the moment they are taken, so there is minimal potential for suffering in any case. Wherever possible, experiments will be done using embryos cultured in a test tube. This minimises the number of pregnant mice that need to be used, since embryos from a single female can act as both 'experimental' and 'control' treatments. Moreover, use of culture studies minimises the number of procedures that need to be carried out on pregnant females.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research into heart development concerns the processes by which the embryo and foetus develop the specific shape and function of the heart. Mice have a four chambered heart which in terms of the way it develops and the genes/proteins and cells involved in its formation are very similar to humans. Embryonic heart development is a four-dimensional process (i.e. varying in space and time), and, therefore, requires the analysis of either whole developing embryos and/or isolated embryonic hearts. Direct genetic studies of foetal (embryonic) humans are difficult practically, and only descriptive analysis is possible, with experiments ruled out on ethical grounds. Tissue culture systems, although they can provide useful information on certain molecular or cellular phenomena, cannot mimic the complexity of a functioning organ such as the heart, let alone the developing embryo. Computer simulations can be valuable in extending theoretical approaches to embryonic (heart) development, but cannot tell us about real biological situations, such as those occurring in the embryo.
2. Reduction Explain how you will assure	We have a high level of expertise in our lab and, therefore, can ensure that our experiments are correctly designed and conducted and as much

the use of minimum numbers of animals	data collected as possible at any one time. It is true that the scientific strength of any conclusion drawn is largely dependent upon the strength of the effect seen, however, we continuously analyse the data generated from these studies and as such can very clearly identify early in the study how many samples will be required to produce a strong (statistically significant) scientific conclusion. Should these numbers exceed our expectations we will be able to promptly make adjustments to ensure the minimal number of animals are 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.	We have chosen to study a mammalian species, the mouse, so that the principles emerging from our research have the greatest chance of applying to the human situation. Mice have also been used throughout scientific history and are still in use today as models of development. Due to this the mouse model is the most refined choice for our studies, utilisation of other models would either require the use of more animals so that the appropriate genetic tools can be generated or would not accurately reflect human heart development and hence, would reduce the clinical application of the data generated from this study

<table>
<tr><td>PROJECT 6</td><td>Prevention of Organ Injury Following Cardiac Surgery</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cardiac Surgery, Organ Failure, Transfusion</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></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>1. To refine our existing model of organ injury to increase its homology to adult cardiac surgery. As a next step we propose to incorporate median sternotomy into the model to reflect surgical trauma; a key contributor to postoperative inflammation.
2. To refine our existing model to more accurately reflect patients with comorbidities such as diabetes.
3. To continue our evaluation of interventions that may attenuate organ injury</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>1. Acute lung and acute kidney injury are common life threatening diseases with no effective treatment. Making our model more representative of clinical cardiac surgery will increase the likelihood that new treatments we develop in the model will be effective in clinical trials
2. Our existing model was also performed in otherwise young healthy pigs. Cardiac surgery patients commonly have comorbid conditions however that increase the risk of organ failure. Principal among these is</td></tr>
</table>

	diabetes. New treatments must be effective in those patients at greatest risk. We will therefore develop a model of diabetes and evaluate the interaction between this condition and CPB in the genesis of AKI. 3. We propose to evaluate two reno-protective interventions; Aqix and Aprotinin, two interventions that have immediate clinical relevance in cardiac surgery. These results will influence the design of future clinical trials. 4. We propose to develop new and safer transfusion strategies; specifically to determine how the additive solution Rejuvesol reduces port transfusion inflammation, and whether this inflammation is offset by improved oxygen delivery in the setting of severe anaemia.
What species and approximate numbers of animals do you expect to use over what period of time?	232 swine up to 1 year old, weighing 30-100kg, 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?	All of the procedures described in the current licence are defined as moderate, and all animals are killed at the end of the experiments. Animals will be exposed to risk, commensurate with the complex surgical procedures that are to be undertaken. Up to 5% of animals may fail to complete all stages of the procedures described. The most common complications being acute heart or lung failure. These have in the past been attributed to acute transfusion reaction (approximately 4% of all transfusions), or as a result of hypotension during surgery. The investigational team is highly experienced however having undertaken over 150 of these procedures in the last 5 years.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	1. Post-surgery inflammatory organ injury is a consequence of both systemic and local organ specific processes cannot be modelled well in vitro.

use non-animal alternatives	2. Interventions have both systemic and local effects, and evaluation of these, as well as systemic and local toxicity is important considerations in translation. 3. We have developed in in-vitro model of inflammation however this must be seen as complementary, rather than an alternative to the use of a pre-clinical swine model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be reduced by: 1. Study designs powered to detect differences in clinically relevant endpoints, as determined by our previous experience with these models. 2. Factorial study designs that increase study power whilst utilising smaller numbers of animals. 3. Common control groups for different projects with standardisation of protocols and endpoints across studies. 4. Parallel development of an in vitro perfusion system that facilitates mechanistic analyses, preserving large animals for the study of clinical risk factor interactions and pre-clinical evaluation of novel interventions.
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.	1. Rodent models, the mainstay of research into inflammatory organ injury, have not yielded important clinical benefits despite decades of research. 2. Swine are the standard for the pre-clinical evaluation of novel interventions in cardiovascular disease because of their homology to human physiology and pathological responses. 3. Reproducible, reliable and relevant data requires close attention to minimisation of pain and suffering, the maintenance of optimal haemodynamics, temperature and oxygenation and standardisation of the animals' experience. To achieve this we have developed a large multidisciplinary team that includes surgeons, anaesthetists, clinical perfusionists and technical staff. This team has a strong track record in delivering reproducible results and high quality translational research with this model.

<table>
<tr><td>PROJECT 7</td><td>Interplay between oxygen and iron in hypoxia-induced pathology</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Iron, hypoxia, heart, muscle, placenta</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></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 the studies is to understand the effects of changes in iron and oxygen levels on the function of the lung, cardiovascular system, muscle and placenta. Indeed, these tissues are known to be sensitive to changes in oxygen levels (hypoxia) and there is some evidence that this sensitivity (hypoxia maladaptation) may be prevented or ameliorated by changing the iron status through supplementation. The project formally examines the effect of iron modification on the pathophysiological hypoxic response in these tissues.</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 understanding gained from our studies will help design human studies to assess the benefits of iron supplementation in conditions such as pulmonary arterial hypertension, chronic obstructive pulmonary disease, pre-eclampsia and reduced exercise capacity. There is already evidence that iron supplementation can prevent pulmonary</td></tr>
</table>

	hypertension associated with altitude hypoxia. By exploring this same paradigm is other biological tissues, it will be possible to help design iron-based therapies for hypoxic maladaptation in those tissues.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 9000 Rats 3000 Over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most animals will undergo an intervention to alter their nutritional status (mainly iron), followed by application of physiological stress in the form of hypoxia. Alteration of nutritional status may be associated with mild to moderate weight loss and, if carried out in younger animals, may slow growth. Prolonged hypoxia exposure is designed to induce the pathological maladaptation that mimics human disease, and as such this intervention will cause a mild to moderate disease phenotype, which is dependent on the genetic strain used. After dietary modification and hypoxia exposure, animals will undergo a battery of minimally invasive tests to measure cardiovascular function (MRI/MRS imaging under anaesthesia), respiratory function (plethysmography), and skeletal muscle function (treadmill exercise). These tests per se are not associated with any major adverse effects. However, anaesthesia and implantation of a telemetry device may be associated with risk of mild to moderate weight loss and infection respectively. All animals will be killed at the end of the protocol, either by schedule 1 methods or under general anaesthesia for removal of organs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The questions cannot be addressed in cellular systems or in non-mammals because they require intact physiological effects and depend on the complex pathways that exist in tissues and that

	depend on many cell types.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers to be used are based on statistical power calculations. Most of our readouts use non-invasive techniques will be used to enable multiple measurements and re-use of the animal in longitudinal studies, which will reduced the number of animals required to achieve statistical significance.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats were selected because their physiological responses to iron and hypoxia mimic closely those see in humans. These models are the most suitable for addressing the key questions set out in our objectives (i.e dissecting the role of specific pathways at the molecular level), because these models are amenable to genetic manipulation which allows mechanistic studies by using knockout models. To minimise welfare costs to the animals, we have refine our protocols as follows: Treadmill exercise protocol: a nitrile glove and a dark background are used to encourage the rodents to run, instead of previously used electric shock. Also, in the hypoxia exposure step; most experiments will include an adaptation period where O2 levels are dropped gradually to allow the animal to adapt to the lower O2 levels. Surgery: General and local anaesthesia, accompanied by appropriate analgesics will be used to minimise pain and suffering. Where new lines of genetically altered animals are used, their phenotypes will be observed closely, and any unexpected harmful effects detected quickly so that the animal is culled humanely. These animals will first be bred under a “moderate” protocol, so that they are observed more closely, and only transferred to a mild breeding protocol if their phenotype is deemed mild.

<table>
<tr><td>PROJECT 8</td><td colspan="3">Inflammation and Atherosclerosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Inflammation, Atherosclerosis. Collar, Disease, Therapy.</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">Cardiovascular disease is the biggest killer Worldwide. Despite new therapies being developed, 70% of events such as heart attacks and strokes cannot be prevented. Inflammation is a key component of the development of cardiovascular disease (atherosclerosis). In addition, having another inflammatory disease such as arthritis can make the heart disease worse. The pathways linking inflammation and cardiovascular disease development are not fully understood.

Our objective is to investigate the link between inflammation and atherosclerosis by determining the molecules and pathways involved. This will enable us to identify possible points of intervention against which new therapeutics can be developed and may lead to disease prevention.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td colspan="3">Cardiovascular disease is a big social and economic burden on society. If we can increase our understanding of the relationship between</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	inflammation and cardiovascular disease we will enable the development of novel therapeutic strategies or agents. Furthermore, if we can understand why patients with inflammatory diseases such as arthritis get more cardiovascular disease compared to the wider population, we may be able to prevent this phenomenon occurring. It is likely that the most effective therapies will need to be targeted to a specific stage or time-point in disease development for the most successful outcome and thus understanding the role of inflammatory molecules and pathways at all stages of disease is key. Increased scientific knowledge will lead to the development of new therapies and thus lead to reduced suffering, reduced mortality, increased lifespan and reduced burden on health systems.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice approx. 9-10,000 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the animals in the mild breeding protocol will experience no adverse effects and will help us to obtain the genetic makeup of animals we need for the other protocols. The moderate breeding protocol covers situations such as crosses where the phenotype is unknown. The main protocol involves placing a small encircling plastic “collar’ on the carotid artery under full anaesthesia. It usually produces no adverse effects and will generally only amount to ‘mild’ severity. Pain relief will be administered before surgery. The inflammations caused by the induction of arthritis are designed to be background conditions for the development of the lesions within the ‘collar’ device. The manifestations should not lead to a situation requiring killing because severity limits have been reached. Monitoring and defined ‘end points’ will allow us to avoid exceeding our severity

	limits. The scanning procedures are performed under general anaesthesia mainly for restraint and immobility. The procedure is not pain-inducing and the main concern is keeping the animal warm and hydrated. The irradiation protocol is a way of changing the makeup of the bone marrow without a prolonged breeding program to produce genetic variants. If cellular replacement is successful there should be minimal adverse effects. Once the purpose of the procedure has been attained, all animals will be painlessly killed.
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 because no 'test tube' (*in vitro*) techniques emulate the flow characteristics of blood vessels susceptible to atherosclerosis. The 'whole body' inflammatory responses that may influence atherosclerosis are not confined to a single tissue. Rather, the inflammatory processes that occur in arthritis (for example) are dynamic and highly complex, involving movement of cells or agents from distant sites via the circulation. Hence, modelling the effects of treatment on atherosclerosis must at some stage involve whole animals, rather than isolated tissue extracts. A large proportion of our work is *in vitro*. We have exceptional access to clinical material that provides the opportunity to study atherosclerotic cells and tissues from human sources. This is a huge head start in identifying the key pathways and mediators and immediately reduces the need for preliminary screening in animal models. It cannot replace however, the unique whole body scenario
2. Reduction Explain how you will assure the use of minimum numbers of animals	The same techniques used *in vitro* can be used to study the tissues retrieved from animal models. In order to maximise the information extracted from each experiment and to avoid unnecessary repeats, we will measure a variety of parameters: 1) the size of the vascular lesions and the type of cells

	contained in them will be evaluated by histology or similar techniques; 2) the number and type of cells recruited to draining lymph nodes and spleen will be analysed by a cell analysis method called flow cytometry 3) cytokines and other mediators will be quantified at lesion sites, lymph nodes and spleens via histology or genetic analysis; 4) cytokines and other systemic inflammatory markers and lipid levels will be assayed. With the knowledge gained in the laboratory, more targeted studies in animals will reduce the numbers required. The ability to use genetically altered animals to verify the specific objectives will increase our certainty. For experiments deemed necessary, the minimum number of animals required has been determined using power calculations to allow generation of meaningful data within the constraints of experimental variability. Statistical power analysis allows us to predict the numbers of animals that will be needed to detect significant differences. Same sex animals will be used generally throughout the study to reduce experimental variability and enhance reproducibility. The experiments will be performed in a logical sequence and the preliminary experiments will be used to confirm whether reliable and significantly significant data has been recovered using the smallest number of animals possible. Because of our unique position in relation to access to clinical material, and experts in inflammation and atherosclerosis, we feel we can make best use of the facility to study the described models in mice. Our group contains scientists uniquely experienced in this area of *in vivo* and *in vitro* work and we expect our methods to continue to develop and refine.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Mice and rats are the lowest vertebrate groups on which well-established models of atherosclerosis have been developed. Mice are preferable to rats because of the greater availability of genetically modified animals and reagents specific for this

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	species. Post surgical pain relief will be provided in all cases unless doing so might significantly affect the experimental outcome. In this situation, we believe opiate type analgesia to be optimum to avoid conflict with the aim of the procedure i.e. anti inflammatory agents in a model of inflammation. Our protocols are now refined to produce the conditions we require and negligible adverse effects on the mice. Good husbandry, compliance with dosing guidelines, monitoring and defined ‘end points’, all help to avoid or minimise suffering. Dosing volumes and routes are all specified. Substances administered to animals by injection or orally will be made in the smallest volume commensurate with the aims of the procedure. Alternative methods of administration will always be considered.

PROJECT 9	**Provision of biological materials**	
Key Words (max. 5 words)	Blood, Macaque, *in-vitro*	
Expected duration of the project (yrs)	Five years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
	X	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)	To supply blood and tissue from rhesus macaques to scientists for use in *ex-vivo* studies	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Scientists require primate blood and tissues for a variety of purposes; these include making comparisons across species and for use in immunological studies, which are more effective when using a species more closely related to humans. Non-human primates could also benefit from this project. The welfare of primates used in research is important and scientists are continually looking for better measures of primate welfare. Some of these can be found by analysing blood and tissue. Macaques in the breeding colony can be used as a control for macaques undergoing procedures with a higher level severity at other institutions.	
What species and	We expect to use up to 20 rhesus macaques for	

approximate numbers of animals do you expect to use over what period of time?	blood and tissue collection under non-recovery anaesthesia over the five year period. Approximately 30 male rhesus macaques will be used for collection of small volumes of blood under sedation or short-term anaesthesia.
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 risk of adverse effects to the animals used in this study is very low. The centre has collected blood from recovery animals over a number of years and has had few adverse effects The volume of blood collected is kept low to minimise the risk of anaemia and animals are checked over by a veterinary surgeon following the blood collection. The expected severity level is mild for recovery animals. At the end of the procedure the non-recovery animals will be humanely killed and the recovery animals will be returned to the breeding stock. Some of the recovery animals will be reused on the same protocol but no more than four times per year with a minimum of one month between procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal tissue and in this case primate tissue is required for a wide range of experiments that cannot be replaced with non-animal alternatives. These include toxicology and immunological studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As a breeding colony we have animals that come to the end of their natural breeding life and need to be humanely killed for health, welfare or colony management reasons. By taking blood and tissue under non-recovery anaesthesia we minimise the need for production of additional animals to supply these products and we can also provide tissues from a single animal to multiple users.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	The unit's expertise is with rhesus macaques. The products provided by this licence are intended to support and assist the development of methods and experiments that require primate tissue. The animals remain in their breeding groups and are

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	kept in large enclosures with high levels of enrichment. This gives them the opportunity to perform a wide range of natural behaviours including mating and foraging for food, They are cared for by highly experienced animal technicians. As the blood and tissues can be collected at the unit there is no need to transport the animals removing possibility of stress due to transportation.

<table>
<tr><td>PROJECT</td><td colspan="3">Thromboinflammation in thrombosis in veins</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Thrombosis, inflammation, mast cells, T-cells, platelets</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></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">In this study, we plan to focus on mechanisms of how thrombi are developing in veins. We wish to uncover how immune system, that normally protects us from infection, participates in thrombosis. At present, it is unknown how different components of immune system, such as mast cells (allergy regulators), lymphocytes (microbe killers) and platelets (contributing in bleeding arrest), are involved in development of blood clots in veins.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">This work is expected to:
1) advance our fundamental scientific knowledge of how anti-bleeding and anti-microbe systems work together in our organism to regulate thrombosis;
2) provide new information about how different cells and molecules interact in this process;
3) based on the obtained knowledge, this study will help to develop new drugs that would efficiently prevent and/or treat thrombosis in veins (for example, in patients after major surgery, in individuals with paralysed legs or arms or even in long-haul flights) without inducing preventing blood ability to clot normally.</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">2000 mice</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td colspan="3">After surgical procedure that we plan to use (laparotomy followed by ligation of a large blood vessel Inferior Vena Cava) to mimic vein</td></tr>
</table>

effects and the likely/expected level of severity? What will happen to the animals at the end?	thrombosis mice usually completely recover within an hour. After this time, mice behave normally and access food and water after surgery, have no bleeding or any problem with incision site in all days after surgery. Mice shall not demonstrate any major discomfort after the operation. To make sure that mice wake up and recover normally they will be observed for days after procedure. During the course of experiments, we will inject different substances; such injections do not result in any detectable adverse effects in mice. Drawing blood from superficial vessels or tail bleeding assay might induce a mild discomfort very shortly after the procedure and no further suffering is observed. We expect the level of severity to be up to moderate at maximum. At the end of each experiment, mice will be humanely killed by methods approved by the Law.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are several reasons why the proposed research can not be accomplished without using animals: 1. At present, there is no non-animal approach or method that would allow to recapitulate in a test-tube the entire complexity of factors regulating thrombosis in a whole organism; 2. Mice are currently a “gold standard” for pre-clinical studies of inflammation and thrombosis because basic mechanisms regulating these processes are similar in mice and humans; 3. Genetically modified mice allow for targeted and precise testing of the role of certain genes, receptors or cell types in the disease; In all experiments, in which convincing results can be obtained without involving animals, an alternative "test-tube" approach will be employed. Prior to starting animal experimentation, we will undertake a comprehensive literature search and scientific discussion to ensure that the factor (e.g., cell or molecule) that we plan to study is indeed very likely to be involved and also to exclude the possibility that a similar study has already been published.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The following measures will be undertaken to minimize the number of experimental animals: 1. E*xperience of scientists in different countries and literature data*. During last years, researches have accumulated a substantial experience with the

	proposed animal model. Based on his experience and results published by multiple groups that have used this model, we will anticipate minimal expected number of animals per experimental group needed to obtain a statistically relevant result. For all procedures, we will constantly look in the scientific literature for methods to decrease the number of animals and consider whether the use of animals is necessary to address the experimental question under investigation; 2. *Using power calculations*. In order to predict minimally possible number of mice, we will perform a special statistical evaluation called power calculation for each research procedure. This will allow us to limit the amount of animals to the absolute minimum necessary to receive convincing and unambiguous information
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.	*Choice of specie*. Mouse is a “gold-standard” model for preclinical modelling of thrombosis. This is because of the large number of available genetically-modified mutants, basic similarity between mechanisms of blood clotting, thrombus formation, and vascular wall diseases in mice and humans. *Choice of models and methods*. Breeding and maintenance of genetically-modified mice are performed in a special unit that has the very highest standards of care and animal husbandry. *Minimization of animal suffering*. All mice will be given a special medication that removes pain prior to surgery and after surgery, as required. Mice will be monitored after surgery and animals demonstrating signs of discomfort will be immediately withdrawn from experiment and humanely killed. Experiments will be performed only by well-trained staff to minimize duration of the procedure and any technical mistakes that could lead to additional suffering. Normally, the proposed procedure leads to a minimal (actually, close to zero) mortality and is well tolerated by mice, which already as soon as 60 min after surgery start to actively move in the cage and demonstrate normal behaviour. Mice normally access food and water and do not lose weight.

<table>
<tr><td>PROJECT 11</td><td colspan="3">Genetics of Cardiovascular Development and Function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Heart development, heart disease, genetics</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">Congenital heart defects are one of the most common birth defects, affecting 1 in 145 new born babies and heart disease is the main cause of death in the UK, accounting for approximately one third of all deaths. Many of the causes of congenital heart defects still remain undiscovered and the link with the development of heart disease in the adult has not been extensively researched. Using genetically modified mouse models, the objectives of the project are to study and understand the genes which are important for the development of the heart and to investigate what happens to the heart, when they are mutated/altered or their level of expression is less than normal. Furthermore, this will be extended to explore the role of these genes in the adult heart and investigate the link between their role in development and if this is abnormally reactivated in a diseased heart. The cellular and molecular mechanisms that control cardiovascular development and function will be investigated.</td></tr>
</table>

	Therefore, this research aims to identify and understand genes and their signalling pathways which are important for cardiac development, but are also involved in the progression from a healthy normal heart to a diseased heart in the adult.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Abnormalities in heart development and function seen in congenital heart disease and adult heart disease are a huge healthcare burden, at both extremes of life, in the developed world. Therefore, by extending our understanding of the genetic pathways that control heart development and function it may be possible to devise screening protocols for unborn babies and adults to identify any potential risks.
What species and approximate numbers of animals do you expect to use over what period of time?	The mouse will be used as our model as their heart is very similar to humans. These are genetically modified mice which allow us to target genes of interest in particular cells of the heart. This project will require complex breeding between different mouse strains and it is estimated that no more than 16,325 adult mice will be used throughout the 5 years of the project licence. The majority of these mice are used solely for breeding or to produce offspring for experiments. This includes 4,100 adult mice which will be used to study gene function in adult heart disease. In the majority of cases the mice will be investigated as foetuses or neonates and we will not exceed 9,600 and 1,200, respectively, as detailed in the project licence. As approximately 8 embryos are present within each litter, this allows us to minimise the numbers of adults used to generate offspring for analysis. In all cases, power calculations will be carried out to ensure that enough animals are used to gather scientifically valid data, but that excessive numbers are not used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will	Most of the mice used in this project will need to be identified by examining the mouse's DNA. This will be extracted from an ear clip from each mouse. This procedure is quick and should only cause mild, short lived pain. The majority of the research will

happen to the animals at the end?	involve the use of embryos or foetuses for the analysis of the heart. This will involve mating genetically modified mice and then killing the pregnant females to collect the unborn embryos/foetuses. In some cases the pregnant female will be administered substances by injection or oral routes, but we do not expect this to have any adverse effects, but they will be closely monitored. A number of mutant mice will be allowed to live into adulthood. The workload of the heart will be increased by exercising the mice and by pregnancy, and their heart function assessed and will be compared to wild type mice under the same conditions. These mice will be closely monitored but the level of severity of any abnormality in the heart will be low. After examination and collection of data, these mice will be humanely killed and their hearts removed for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For our research we are dependent on examining the development of a mammalian heart in an attempt to understand how congenital heart defects and adult heart disease develop in humans. The mouse is an ideal model as it has a similar heart and blood vessels to humans and it can be used to investigate the role of different genes in the different cell populations which form the heart. There are no suitable cell culture systems that could be used as an alternative as it is important the heart remains intact to identify any abnormalities.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of litters collected for analysis of the embryonic hearts will be closely monitored and each embryo will be used for a number of experiments, maximising the information which is obtained, therefore minimising the number of pregnant females used. All of the experiments will be carefully planned to use the minimal number of mice.
3. Refinement	There are a number of genetically modified mice already used extensively in research which allow

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 different cell populations which are required for the development of the heart, to be targeted. These can be used to investigate the role of specific genes during heart development and in adult mice, making the mouse the perfect model. The pregnant female mice will killed humanely and any mice that have been injected, or subjected to stress on their hearts will be closely monitored.

PROJECT 12	**Studies on the Mechanisms of Atherosclerosis**	
Key Words (max. 5 words)	Accelerated atherosclerosis, restenosis, hypercholesterolaemia	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Atherosclerosis underlies coronary heart disease (CHD), the major cause of mortality and morbidity. The mechanisms causing atherosclerosis include cell migration and proliferation, and these are promoted by cytokines, and growth factors. Several factors are known to enhance atherogenesis: mechanical injury (e.g. following balloon-angioplasty, equivalent to angioplasty used to treat CHD), immune stimulation, and enhanced free radical production. These factors also enhance heat shock protein (Hsp) expression. We therefore aim to investigate the role of Hsps in accelerated atherosclerosis. Growth factors and cytokines (including erythropoietin) have an important role. Our previous work has indicated epidermal growth factor receptor (EGFR) may be involved in early atherogenesis. Effects of some dietary factors (e.g. trace elements and antioxidants) are unknown and will be studied. Hsps, erythropoietin, specific peptides of Hsp and erythropoietin, inhibitors of	

	EGFR and EGF, including specific neutralising antibodies, will also be investigated. We aim to investigate the factors contributing to coronary heart disease (CHD) using established mouse, rat and rabbit models. Arterial injury will be combined with high cholesterol diets, and specific antagonists to growth factors, or cytokines given locally or systemically.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our project will lead to a better understanding of the role of heat shock proteins, specified cytokines (including erythropoietin) and growth factors in atherogenesis, and potentially how they may be inhibited. This may lead to the identification of new therapeutic targets.
What species and approximate numbers of animals do you expect to use over what period of time?	Our experiments will use rabbits, rats and mice. The hypercholesterolaemic rabbits and genetic murine models (apoE and LDLRj are well-established models of atherosclerosis. We plan to use approximately 390 rabbits; 110 rats; 230 mice over *5* years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Changes in eating habit. Animals will be monitored for weight change. If >15% of starting body weight returned to normal chow diet, or withdrawn from study. Monitored for efficacy of anaesthesia during surgery, and for pain post operatively. Post operative pain relief analgesia with opioids and non-steroidal anti-inflammatory agents. Post-operative infections to be treated expediently with appropriate antibiotic. Post-operative bleeding will be monitored, and corrected as required. All animals will be killed by appropriate methods at the end of the experimental protocol.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of animals in studies of atherogenesis is unavoidable. Currently there are no representative models of this complex disease. However, we will use parallel cell culture studies; and tissues will be used for ex-vivo studies of endothelial function and lesion characterisation.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental design and methods previously discussed with a statistician. There will be a careful use of control groups, and group sizes will be minimized, whilst maintaining sufficient power, by using appropriate pilot studies in order to determine the correct experimental conditions. The experimental design will typically consist of parallel groups of animals, randomised to active intervention, sham, or placebo control. Several active intervention groups will be used where possible to limit the requirement for control animals. A pilot study will generally be run prior to the full scale study, to optimise dosing and undertake pharmacokinetic studies if necessary. In vivo experiments will be complemented by ex vivo and in vitro experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the only available species where LDL', ApoE' knock out (ko) animals are commercially available. The models of dietary atherosclerosis are the least severe approach (mild) for native atherosclerosis. The rat carotid is a well-established model of restenosis (moderate severity). The combination of hypercholesterolaemia and arterial injury will use the ko mouse or cholesterol-fed rabbit. Elements of continued-use represent an integral part of the project necessary for exploring the mechanisms involved in the accelerated atherosclerosis (moderate severity). Appropriate environmental enrichment will be provided (periods in floor pens, or with nesting material) to encourage desirable natural behaviours and improve welfare. The frequency and volume of blood sampling will be limited and will be undertaken by experienced staff. Animals will be given a tranquilliser prior to bleeding when necessary. For animals undergoing a surgical procedure, they will be tranquilised prior to using a general anaesthetic. They will be carefully shaved and skin prepared. The level of anaesthesia will be regularly monitored during the procedure (corneal and pain response). Post-operatively each animal will receive prophylactic pain relief, and will be monitored

	until fully recovered. They will be monitored daily by experienced staff for signs of discomfort (wound licking) and complications (wound dehiscence, discharge).

PROJECT 13	**Regulation of Haematopoietic Development**	
Key Words (max. 5 words)	Haematopoiesis, Angiogenesis, Embryogenesis, Leukaemia	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	formation of the blood system during embryonic development and more specifically how this population of unique blood cells is generated during that time. In this project, we will define the role of novel regulators of embryonic and adult blood formation. We will investigate the development of leukaemias upon alteration in the expression of these novel regulators. Finally, we will investigate the similarities in the formation of blood Throughout adult life, blood, cells are continuously produced by a population of unique blood cells that is generated during embryonic life. To date little is known about the molecular mechanisms that control the vessels in embryonic development and in cancer development.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work is interesting in its own right, and it should help efforts to understand haematopoietic development and hoW changes in this process might result in leukaemias. More than half of young patients and about 90% of older patients still die from leukaemias. Failure of initial treatment and relapse after remission are the main obstacles t.o cure. Better understanding of the disease is needed to design novel treatment.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used in this project. We anticipate using around 9500 animals during the 5-year period of this project licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The level of severity will be moderate for all experiments. Adverse effects might encompass mild discomfort and stress from initial treatments such as injection. The study of leukaemia might cause dverse effects such as anaemia or bone marrow failure. All animals in these experiments will be closely monitored for sign of ill health. At the end of all experiments, animals will be humanely killed by an approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This programme of research is based on extensive experimentation using in vitro culture systems. A large amount of pilot experiments and optimization are performed using in vitro assays. As a result, this minimizes considerably the number of experiments to be performed in animal, Unfortunately, to date no in vitro assays can be used to unequivocally test the potential of haematopoietic stem cells that are characterised by long-term engraftment and multilineage potential. Furthermore, the homeostasis of the haematopoietic system can only be assessed in vivo and finally the micro-environment specific to the various anatomical regions where haematopoiesis emerges during embryonic development cannot be reproduced in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	On regular basis, we will seek advice from our in-house statistician and particularly before starting specific experimental approaches. This will allow predicting the treatment regimens and size of the cohorts needed to answer the questions we are investigating. Furthermore, we will mostly perform established experimental procedures that are described in the literature. The response of wild-type and eventually transgenic animals, can be anticipated from the published work. Pilot experiments with small numbers of mice will be initially performed to establish the strength of the phenotype. The number of animals required for these

	experiments will be a function of the number of genes studied and the strength of the phenotype. The use of in house expertise in procedures and animal welfare, the maintenance of animal health and welfare by IVC caging, the use of aseptic techniques and the use of animals of defined status does limit the numbers of animals required. The use of new technologies such as imaging of live animals under anaesthesia also contributes to reducing the number of animal used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have been chosen for the study because they represent the least sentient species from which meaningful experimental data can be generated, while exhibiting considerable genetic and biological similarities to human with regard to their haematopoietic system. They are the best characterized species to •test the function of haematopoietic stem cells and experimental protocols are particularly well established for this animal model. Other less sentient non-mammalian species, such as Danio rerlo or Xenopus, lack a haematopoietic system that is comparable in complexity and anatomy to that of Homo sapiens. Furthermore, there is considerable experience in the wider scientific community regarding the use of mice as a valuable model system for haematopoiesis and haematological malignancies and many reagents exist for the phenotypic characterisation of mouse cells. By contrast, there is no published experience to date on the xenotransplantation of haematopoietic cells into fish or amphibians. The techniques used in this project will involve minimal distress to the animals, while ensuring scientific quality. Close monitoring of the health of experimental animals and use of analgesia will ensure that suffering due to the experimental protocol is minimized. We will also ensure that protocols are continuously reviewed, revised and refined to ensure that the research methods used are the most appropriate and the number of animals used minimized.

<table>
<tr><td>PROJECT 14</td><td>Production of antibodies for research purposes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Production of antibodies for research</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To produce polyclonal and monoclonal antibodies specific for a range of novel biological molecules</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>A key feature of antibodies is their exquisitely specific interaction with the antigen which they recognize. The specificity and avidity of this interaction permits antibodies to be used as tools to give insights into many different biological processes. These processes include infections by viruses and bacteria where the study and application of antibodies will ultimately lead to improved measures for the diagnosis, prevention or treatment of infections. Important human and/or animal viruses being studied at the establishment include influenza viruses, human immunodeficiency viruses, respiratory syncytial virus, adenoviruses and rotaviruses. Additionally, antibodies are invaluable tools for the study of fundamental biological processes in plants, bacteria and</td></tr>
</table>

	animals.
What species and approximate numbers of animals do you expect to use over what period of time?	50 rats and 250 mice over the course of 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Likely levels of severity are mild with few adverse effects expected. <u>Injection with antigen and adjuvent</u> According to the suppliers, and our own experience, adverse reactions due to adjacent are rarely seen with non-ulcerative adjutants. Should they occur they are in the form of a slight subcutaneous lump at the site of injection which resolves without intervention. <u>Blood sampling</u> While mice and rats rarely show any adverse effects from having been bled from a superficial vein potential adverse consequences are pain, infection, thrombosis, phlebitis and hypovolemia. The likelihood of these effects are <2%. Animals will be inspected immediately following bleeding and checked the following day. All animals will be killed 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	While it is possible to generate antigen-specific antibodies *in vitro* the application of these techniques remains limited and most require the use of animal- or human-derived material . The only fully *in vitro* approaches require microbial systems to generate antibody fragments with varying ranges of avidity for the target antigen. These molecules are used for specific purposes but do not present the full range of activities and applications seen with conventional antibodies raised *in vivo*. In particular, antibody fragments and antibody-like molecules generated *in vitro* are not readily useable in detecting and quantifying the target antigen using current standard protocols.

	Antibodies will only be produced *in vivo* under this license if no *in vitro* alternative is available or if the *in vitro* approaches are unable to produce a suitable alternative with the required properties for the research. Following immunization for the production of polyclonal antibodies the antibodies are obtained from the serum of the immunized animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used to raise antibodies will be kept to an absolute minimum for each immunogen.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats will used as these have been shown to be capable of producing good antibody responses and will provide sufficient sera for all practical purposes. It is necessary to use mice for the production of monoclonal antibodies as mouse B cells are required from the production of hybridoma cell lines using standard techniques.

<table>
<tr><td>PROJECT 15</td><td colspan="3">Regulation of Heart Development in Vertebrates</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Heart, Cardiogenesis, Morphology, Non-compaction</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>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>Yes</td><td></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">Congenital heart disease (CHD) is the commonest birth defect and its global medical burden exceeds that of many other complex diseases such as diabetes, hypertensive heart disease, asthma, or rheumatoid arthritis. Research to uncover the genetic basis of CHD is essential to facilitate improvements in genetic screening and counselling. Chromosomal causes and heritable syndromes account for approximately one fifth of the disease, but clinical genetic studies have only identified the genes responsible in a few cases, where mutations in single genes account for the malformation. The bulk of inherited CHD is believed to result from multiple genetic causes in which the impact of any one gene mutation is affected by individual genetic variation, other gene mutations and crucially, a range of environmental factors.

A fundamental prerequisite for understanding CHD is identification of the genes and gene regulatory</td></tr>
</table>

	interactions that regulate normal heart development. This encompasses not only the genes necessary for appropriate differentiation of the various cardiac tissues, but also the genetic factors that control normal morphogenesis of the heart and its constituent parts. Research over the last decade has identified a number of these genes and from their study it is clear that rather than being a linear, causal chain of events, heart development (like other aspects of embryo development) is more accurately viewed as the result of a complex web of genetic interactions, which is itself modulated by environmental influences. Mapping this genetic network and understanding how it is regulated both spatially and temporally is the focus of this research programme. Our studies focus on the role of one key gene regulator that lies at the heart of the cardiac genetic programme, the homeobox protein, Nkx2-5, mutations of which are a known cause of CHD and which can result in a wide range of abnormalities. To begin understand the diverse effects of such mutations, our goal is to identify the genes this protein regulates during normal heart formation and the manner in which it does so. We will achieve this through a combination of computer bioinformatic methods, in vitro studies and, where necessary, by manipulating activity of the gene in transgenic animals. Formation of the heart is a complex process in which tissue differentiation is intimately linked to formation of an entire organ. We currently know little about how the genetic programme of heart development regulates such complex changes in cardiac tissue organisation underlying heart formation, although precision in the three dimensional changes in composition and shape are critical for proper heart function. Our research programme incorporates novel, high resolution imaging methods to map the changing patterns of gene expression and cell lineage as the heart develops, in order to gain insights into the manner

	in which regulators such as Nkx2-5 impact on heart morphogenesis. One of the dramatic changes in 3D structure that occurs during heart development in the foetal period is the transformation of the muscular wall of the heart ventricles from a spongy network into a compact, thick wall. Failure or misregulation of this process is a proposed cause of non-compaction cardiomyopathies. We are investigating this possibility, using a transgenic mouse model which mimics a human mutation in the muscle protein ZASP which has been identified in studies of familiar left ventricular non-compaction.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By identifying the network of gene activities necessary for normal heart development, this work should lead to a better understanding of the origins of many congenital heart disorders. This will allow genetic screening procedures for such conditions to be established and could suggest novel routes for therapeutic treatment. For heart muscle cells in particular, identification of genes capable of initiating and controlling muscle differentiation has great therapeutic potential since it may suggest a way to promote regeneration of healthy muscle in patients suffering from inherited disorders or heart disease. Identification of regulatory genes may provide a route to stimulate replacement of diseased heart tissue or scar tissue with healthy cardiac muscle. Furthermore, by advancing our understanding of the genetic controls regulating heart development in the embryo, it will become possible to identify environmental, nutritional or behavioural factors that influence the genetic network and suggest ways in which the incidence of congenital heart abnormalities can be reduced. Studies of a mouse model for non-compaction cardiomyopathy could shed important new light on the origins of this disease and provide insight into the normal developmental controls in the embryo heart that are disrupted in this condition. This could provide a way to develop pre-symptomatic

	diagnostic markers for the disease suitable for translation into clinical practice. Integrating the study of cardiac gene regulation with the analysis of 3D heart morphology and an assessment of cardiac function, we will gain a better understanding of the links between gene activity, formation of the heart organ and cardiac function. This will not only be important for our understanding of congenital heart disease, but is also likely to shed light on maladaptive molecular changes that are common endpoints of diverse cardiac disease.
What species and approximate numbers of animals do you expect to use over what period of time?	3000 Mouse (Mus muscularis) 5 years 5000 Frog (Xenopus laevis, Xenopus tropicalis) 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 bulk of our work will utilise existing transgenic mouse and frog lines in which the genetic alterations are known to have little or no adverse effect on the animal. Most studies will utilise embryos from these lines, obtained at different stages of gestation. Both adults and embryos will be sacrificed using approved methods before their tissue is used for experimentation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studies of heart morphology and the manner in which genetic changes are linked to changes in heart organ morphology can only be performed using animal models since no cell line can reveal the interrelation of accurate heart 3D structure with cardiac function. Furthermore, few stable cardiac cell lines are available for studying aspects of cardiac function and none of these accurately reflects either the gene expression or functional activity of the entire organ (which is composed of several different cell types).
2. Reduction Explain how you will assure	Much of our analysis of gene regulatory interactions will be based on in vitro studies of protein:DNA interactions and bioinformatics analysis of our data

the use of minimum numbers of animals	and the considerable databases that are publicly available to the research community. Where possible, any experiment involving the use of animals will only be undertaken after power analysis, using the appropriate minimum numbers to obtain statistically significant results. Creation of reference 2D and 3D image datasets for heart development at each embryo stage will provide a resource that will obviate the need for a considerable portion of the histological analysis that is currently undertaken in biomedical research, including our own. For this reason, our data will be made freely available to the research community.
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.	Many aspects of heart development have been highly conserved through evolution, despite fundamental differences in heart structure (such as the number of heart chambers). As a result, we are able to use embryos from lower vertebrate animals (frogs) for a significant portion of this work. Such studies will help direct the research using the mouse, which provides a much closer model of human heart development.

<table>
<tr><td>PROJECT 16</td><td colspan="3">Endothelial dysfunction in pulmonary hypertension</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Vessel wall thickening, lung, hypoxia, inflammation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)

Pulmonary hypertension (PH) is a term that describes raised blood pressure pressure in the lung. Blood vessels in the lung become narrow and this restricts the amount of oxygen that can be delivered to the body. Current therapies delay progression of the disease but do not reverse the condition of provide a cure.

Our group works on the role of cells that constitute the inner lining of the blood vessels in the lung and play a key role in disease. These cells, called the endothelial cells, decide how much oxygenated blood gets through the lung. Our aim is to establish causes why the endothelial cells do not function properly in pulmonary hypertension and establish optimal treatment to prevent the disease.</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></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"><u>General study objective:</u> to develop treatments that improve function of cells covering the inner surface of blood vessels in the lung (endothelial cells) and prevent development of pulmonary hypertension.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td colspan="3">There is a major need for treatments that improve the prognosis of pulmonary arterial hypertension. The annual mortality with current treatments is</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	around 10%. An effective inhibitor is needed to restore normal function to the cells covering the inner side of blood vessels in the lung so that more oxygenated blood can flow through the lung.
What species and approximate numbers of animals do you expect to use over what period of time?	5 years, maximum 200 rats/year, 200 mice/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?	The severity of procedures used in this project is mild-moderate. All animals will be killed Schedule 1 or under terminal anaesthetic at the end of experiment. Any animals that are not fully recovering from the treatment during the experiment, and show signs of pain or severe distress will be killed by Schedule 1.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro assays with the use of human or animal cells cannot adequately model the complete array of processes involved in pulmonary vascular remodelling. Lung tissues from PH patients are not available. Therefore, further in-vivo work is required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of in-vitro methods, human studies or ex vivo Schedule 1 organ culture (see above) limits the numbers of animals required for the in-vivo investigation stage. We intend to use rats and mice as these animals provide well established, internationally recognised models of pulmonary hypertension. The size of cohorts required for the study is based on published data from our laboratory and other national and international centres. Writing a well defined study protocol for every experiment will allow to reduce the number of 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 intend to use rats and mice as these animals provide well established, internationally recognised models of pulmonary hypertension through chronic exposure to hypoxia (severity: moderate) and monocrotaline injection (severity: moderate). Chronic hypoxia represents the least severe form of pulmonary hypertension but does not demonstrate marked vascular remodelling and so is limited as a means of investigating vascular cell turnover and survival. Monocrotaline is given as a single injection but causes progressive pulmonary vascular endothelial damage , leading to pulmonary hypertension. This appears 6-8 days and pulmonary pressure rises progressively to a new level at 24-38 days. Animal tolerate this with little evidence of illness for the first 3-4 weeks but then develop progressive dyspnoea, and weight loss and die at around 42-48 days. Animals will be assessed twice daily for signs of ill health and killed humanely when they show signs indicative of poor prognosis to avoid unnecessary suffering. All the therapies currently approved for treatment of pulmonary arterial hypertension underwent development in these experimental models prior to clinical studies. The project will examine the efficacy of treatments in the least severe form of pulmonary hypertension and only take those that show promise into experiments with more severe forms of the condition.

PROJECT 17	**De novo generation of haematopoietic stem cells**	
Key Words (max. 5 words)	Blood, stem cells, genetic program, haematopoiesis, transplantation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Clinical bone marrow cell transplantations into leukemic or blood-defective patients have demonstrated the medical value of hem atopoietic stem cell (HSC) regenerative therapy. However, the lack of a sufficient number of donor (or patient-specific) HSCs remains the overriding constraint to treatment. This is because very few people donate these rare bone marrow or umbilical cord blood HSC for clinical use. Also, even if more donors could be found, t is important that the donor HSCs are matched to the patient, to avoid the patient's body from rejecting the transplanted donor cells. It is unknown how the body makes HSCs. We do know from the work in my laboratory, that they are made only during a brief period of time and are made from the cells that line the blood vessels at early stages embryonic life. If we can determine how these valuable stem cells are made, we should be able to engineer HSCs outside the body. The objectives of this study are to identify the genes that play a role in HSC generation and use these genes to make HSCs from other cells in the adult body (for example, the cells that line the blood vessels could be an abundant	

	source) and very importantly, to make HSCs that are matched to patient. To do this we will use mouse models in which the HSCs are marked by molecules that can be detected under fluorescent light. We will also use mice that have defective HSCs to help us identify and study the genes that are important in the production of HSCs in the mouse embryo. The fluorescent and defective HSCs will examined under the microscope to observe their movements in the embryo. They will also be sorted from the rest of the cells of the embryo and transplanted into adult mice, to test whether the HSCs can produce the entire blood system. In some cases, we will transplant these cells into neonatal and immunodeficient mice to determine the potency of the HSCs. We will look for what genes are turned on in the HSCs in attempts to identify new HSC genes. Once we have identified the key genes for HSC generation in the mouse embryo, we will introduce these genes into mouse and human blood vessel cells so as to induce them to become HSCs. If we are successful, we will optimize this method in order to produce large number of patient-specific HSCs.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Each year around 1,800 people in the UK will need a hematopoietic stem cell transplant. Most of these patients will not be transplanted since there are not enough donors of HSCs and these donated HSCs are not matched to the patient. The potential benefits likely to derive from this project are in the ability to produce patient-specific HSCs. This should allow for clinical transplantation of all patients needing a replacement for a defective blood system. Since the cells have come from the patient, they are exactly matched and should not be rejected.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse models of that allow the study of HSC generation. Mice are easy to breed, have a blood system and blood stem cells very similar to those in humans. Mice can be translated with blood stem cells in the way that human patients transplanted in the clinic. In addition, mouse strains exist in which they are defective in blood stem cell production, allowing us to determine what genes are important in blood stem cells. Approximately 24,500 mice will be used over a period of 5 years. To generate new genetically modified mice we will need 2000 animals. The breeding and maintenance of these mice for our studies requires 18,000 animals

	and 4,500 animals will be needed as recipients for in vivo transplantation experiments to test for blood stem cell function.
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 proposed study involving mice is expected to have only mild to moderate affects. These are related to blood stem cell deficiencies and are found only in early stage mouse embryos. The proposed blood stem cell transplantations in mice are similar to those observed in human medicine. Genetically modified animals will be generated and used for breeding and maintenance of the mouse lines (mild effects). These animals will be used to generate the embryos and cells we need for transplantation experiments. The females will be sacrificed under Schedule I method, to obtain embryos. The mouse colony will be maintained to a minimum number and aged adults will be sacrificed by Schedule 1 method. Mice showing adverse effects caused by irradiation and hematopoietic reconstitution (e.g. lethargy, weight loss) will be provided with supportive treatment and monitored for changes in health status. If monitoring indicates deteriorating health-, animals will be monitored more frequently, provided supportive treatment and/or culled and examined for blood failure. Most recipient mice used in transplantation experiments will be sacrificed under Schedule I method at the end of each experiment. Some mice will undergo blood modulatory treatment and then be sacrificed for blood tissue analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our mouse embryo studies are the pivotal to understanding how blood stem cells are generated. To date, no research laboratory has been able to generate blood stem cells from in vitro cultured embryonic stem (ES) cells or any other cell type. Blood stem cells do not survive in culture — they either change their haracteristics and are no longer stem cells or they die. Hence, we can only study blood stem cells within the context of the whole animal. Only by testing putative stem cells by transplantation into mouse recipients are we able to determine whether a stem cell has been generated. We will replace the mouse model when we are finally

	able to generate transplantable blood stem cells from ES cell cultures or from the gene transfer studies as proposed in our research project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are carefully planned and controlled so that only the minimum numbers of genetically manipulated mice are maintained in the colony. Research staff meet routinely to discuss colony size for each mouse line. When necessary, genotype analysis is by quick PCR method to identify genetically modified mice, so as to keep the number of mice in our colony to a minimum. Since, we need mostly females for mouse embryo production, the number of males for matings are kept to a minimum. To ensure high breeding efficiencies, females are screened for oestrus cycle before being mated with a male (rate of successful plugging is about 60%). Mouse lines not in current use are frozen as sperm. For transplantation experiments, we will use statistical methods to allow the maximum acquisition of data, with minimum mouse 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.	Mice are used in these experiments because they are the best and most accessible model for study of blood stem cells. Following WWII, studies on the effects of irradiation have shown that blood stem cells are depleted and with bone marrow transplantation, stem cells can be replaced to rescue the entire blood system. Various strains of inbred mice, the ability to make mutants, the availability of reagents to sort mouse stem cells and the short 2 year life span of mice, all contribute to my choice of this animal model for our experiments. Furthermore, the blood system of mice is very closely similar to that of the human blood system and supports lmost direct translation to human clinical study. To minimize welfare costs to the animals, research staff are fully trained for the procedures they are responsible for in their specific experiments and will follow recommendations from the animal facility staff for further refinement of techniques. More specifically, for transplantation experiments, the irradiation procedure to ablate endogenous HSCs uses a split dosing of irradiation (4 hour interval) so as to minimize stress. Other ablative technologies to be used include myelo/immunoablative reagents that are used clinically in human bone marrow transplantation therapies. These adult treated mice and neonatal

	mice (myelo/immunodeficient) also serve as recipients to measure less potent blood stem cells. Mice are given specific bedding materials during breeding and after irradiation and transplantation, and other environmental enrichments (chew blocks, tunnels) for comfort and warmth. Antibiotics are provided in the drinking water for animals undergoing immunocompromise to prevent any risk of infection.

<table>
<tr><td>PROJECT 18</td><td>Antibody production</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Antibodies, immunisation</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></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></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>Provide a service for raising polyclonal and monoclonal antibodies in llamas, rabbits, mice, chicken, sheep, guinea pigs, goats and rats.</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>Production of entirely new antibodies to scientific targets of interest that can be used as a) tools to advance basic science, b) development of clinical diagnostic tools and c) contribute towards new drug discovery and development.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Llama 50
Rabbit 250
Sheep 250
Mouse 1000
Guinea pig 125
Rat 375</td></tr>
</table>

	Goat 250 Chicken 125
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be injected with an immunogen via a peripheral site with optional booster injections and test bleeds to determine if antibodies have been produced before antibodies are harvested using a method appropriate to the species used (e.g. Schedule 1 termination, exsanguination under terminal anaesthesia, final blood sampling from a peripheral vein). Granulomas and sterile skin abscesses may occur but the protocol is designed to minimise this possibility (<1%). There is very small chance (<0.5%) that the immunogen may cause an adverse systemic effect. Overall, antibody production is of mild severity. Immediately at the end of antibody production, animals will be humanely killed by a Schedule 1 method with the exception of llamas, goats and sheep that may be re-used or re-homed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	An intact immune system only available in a live, host animal is required to generate highly specific antibodies, thereby necessitating the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Typically only 2-3 animals are required to successfully produce specific antibodies. Thus only 2-3 animals will be used in each attempt to create a specific antibody before additional animals are used if the initial attempt is unsuccessful. In the case of llama, goat and sheep use, the long lifespan coupled with the mild nature of antibody production supports re-use without compromising welfare which will reduce overall number of animals used.
3. Refinement Explain the choice of species and why the animal model(s)	In all cases, the lowest order species possible will be used. The minimum number of animals required to ensure

you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	successful production of an appropriate amount of antibody will be used. Suffering will be minimised by sequential injection of immunogen in successive animals in order to reduce the harm caused if any systemic adverse effect is caused. Careful consideration will be given to injection volumes used, needle gauges used and blood volumes drawn Strict criteria determining suitability for re-use of llamas, goats and sheep will be adhered to. Clearly defined endpoints for all procedures prevent the possibility of undue suffering and minimises any necessary discomfort experienced by the animals. Once a previously unavailable antibody has been produced, the use of in vitro or synthetic techniques to produce further antibodies will be undertaken where scientifically possible.

PROJECT 19	**Regulation of blood vessel maintenance and repair**	
Key Words (max. 5 words)	Blood vessels, heart attack, aneurysm, cardiovascular disease	
Expected duration of the project (yrs)	Five	
Purpose of the project as in ASPA section 5C(3)	No	Basic research
	Yes	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	No	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Defects in the structure and function and blood vessels cause a range of diseases including heart attacks, strokes and cancer. We are investigating certain proteins which regulate blood vessel structure and function in order to determine how they contribute to disease and decide whether they can be used to keep blood vessels healthy and prevent the development of disease.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding how the proteins which regulate blood vessel structure and function are produced and regulated will provide a valuable insight in to how diseases develop. We hope that the information derived from this project will identify new targets which could be developed to create new ways to prevent and treat these diseases in future.	
What species and approximate numbers of animals do you expect to use over what period of	We plan to study 2000 mice over the next five years.	

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?	In many cases, we will be able to examine blood and tissue samples collected after humane killing of mice. In other cases, we can study mice using scans similar to those in humans (for example ultra- sound or magnetic resonance scans (MRI)) whilst they are under an anaesthetic. In some animals, mice will undergo surgery to create diseases similar to those in humans (for example heart attacks, aneurysms and blood vessel damage). Mice will be under anaesthesia for surgery and will receive painkillers as they recover. Most animals are then humanely killed within 2-6 weeks to study the effects of disease. However, as in humans, a minority of mice might die suddenly.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The diseases we are studying are complex. Whilst we can conduct much of our work in cells in the laboratory, we cannot fully recreate all the processes which occur in the body to cause diseases like heart attacks or aneurysms. In order to identify new ways of preventing and treating disease in humans, it is necessary to carry out some aspects of our research in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are able to minimise the number of animals used by careful design of our studies, combining scans, blood tests and detailed examination of tissues collected after the animals are humanely killed.
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 chosen the mouse as it is relatively straightforward to alter the genes of mice in order to study the effects of specific proteins in normal function and disease. The mouse is one of the lowest order mammals in which is it appropriate to study human disease.

<table>
<tr><td>PROJECT 20</td><td colspan="3">Adaptive mechanisms of the ischaemic myocardium</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Myocardial infarction, gene-modified animals, redox, cardiac remodelling, heart failure</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>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 colspan="3">This project aims to investigate the changes that occur within the cardiovascular system after a heart attack and during the subsequent development and progression of heart failure. It aims to identify new therapeutic targets and treatments for this condition.</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">Heart failure is a common condition that affects up to 2% of adults. There are over 100,000 heart attacks every year in the UK and this is the commonest reason for the development of heart failure. The morbidity and mortality of heart failure is unacceptably high despite the use of multiple treatments. Newer and better treatments are therefore required. A better knowledge of the mechanisms that underlie the development and progression of chronic heart failure after heart attack is essential to develop new therapies.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of</td><td colspan="3">A total of up to 5,000 mice or rats over the lifetime of the project.</td></tr>
</table>

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?	A proportion of animals may die at the time of initial heart attack or during subsequent development of heart failure. Some animals may gradually develop signs of heart failure such as rapid breathing, loss of weight, or reduction in activity. Animals will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because heart failure is a complex condition involving the interactions of several body systems, there is no feasible alternative to the use of animal models. It cannot be reproduced in cell systems nor is it amenable to computational modelling due to the numerous uncertainties and unknowns regarding mechanisms.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will follow principles of good experimental design to ensure clear answers to questions being addressed while using the minimum number of animals. For many studies, we will use non-invasive techniques that allow serial assessment of cardiac function, allowing reduction in numbers. This is especially valuable when assessing the impact of medicines aimed at preventing or slowing disease progression. Wherever possible, detailed studies of cellular mechanisms will be conducted in cultured cells in the lab.
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 study will be performed using mice and rats because all relevant methods and techniques are successfully established in these species, and because of the availability of genetic alterations in these species. These help to study specific biochemical pathways with a view to understanding disease progression and interfering with it to provide new treatments. Animals will be closely and regularly monitored during the study. Any clinical problems will be dealt with in consultation with the veterinary surgeon. If necessary, in cases of severe distress, animals will be humanely killed.

<table>
<tr><td>PROJECT 21</td><td colspan="3">Characterising & inhibiting vascular disturbances</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Microcirculation, stem cells, inflammation</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 characterise the microcirculatory disturbances associated with different inflammatory disorders in vivo and identify strategies that can confer therapeutic benefit.</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">It is widely recognized that unwanted inflammation and thrombus formation are central to the pathological mechanisms underlying many vascular diseases and cancers. For many of these diseases, no effective treatment has been identified. Collectively, these diseases consume vast amounts of limited NHS funds and time. Therefore considerable research efforts are directed at understanding both the physiology and pathophysiology of circulating leukocyte and platelet recruitment, with the ultimate view of developing treatments to prevent injury by these cells. As well as increasing scientific knowledge of these inflammatory and thrombogenic processes, these studies may provide data that would have beneficial implications for a whole host of diseases. Regenerative medicine, particularly the use of stem/progenitor cells, is currently being considered for treating a whole host of inflammatory and degenerative conditions. However, stem cell therapy is limited by the insufficient number of cells that can be isolated. Therefore, identifying factors that can enhance the recruitment of these cells to the blood vessels within sites of injury will impact positively on this treatment modality and have huge implications for the treatment of a number of</td></tr>
</table>

	degenerative diseases. Experiments are not conducted with any financial profit in mind
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years, we would expect to use no more than 6,000 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?	The majority of experiments proposed within the remit of this licence will be conducted on terminally anaesthetised mice. Inflammation will be induced in anaesthetised mice and subsequent characterisation of vascular perturbations and the therapeutic effects of stem cells will be performed under terminal anaesthesia. Hence we do not anticipate any adverse effects and so the severity level is mild. In some experiments inflammation will be induced prior to anaesthesia with all subsequent analysis conducted under terminal anaesthesia. Since these inflammatory models are well characterised and routinely used, we do not expect (nor have we experienced) any adverse effects. A mild severity limit is however given for the appropriate protocol which is required to allow for unexpected, mild severity signs for inflammatory stimuli that we are unaware of.
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 that take place within blood vessels following inflammation. Many of the cells we will be investigating rely upon their surrounding environment for cues or signals – something which cannot be mimicked in single or even multiple cell co-cultures. The presence of blood flow and the shear stresses this exerts on circulating cell recruitment cannot be reproduced accurately *in vitro* for the various different vascular beds we wish to image.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical analysis will be conducted to ensure we use the minimum number of mice per group that will be informative. Statistical analyses from previous studies (and our extensive experience) suggest groups sizes of 6-8 are required to measure significant differences. To maximise the information gained from a single experiment we aim to take samples and acquire images from multiple blood vessels and multiple

	sites in the body.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the species of choice as they are the best vertebrate model for use in *in vivo* imaging studies that look at inflammatory processes. The microcirculatory patterns in mice are very well characterised and are similar to humans. The main components of their immune system are shared by humans, which is essential when inflammatory or immune responses are being studied. Many of the responses we plan to study from a scientific viewpoint are best characterised in this species due to a widespread availability of selective murine blocking antibodies and defined transgenic and knockout mice. The models of inflammation to be used are well characterised, highly reproducible and can be induced easily in anaesthetised mice. The histological and biochemical responses obtained models the clinical disease. By using well established protocols to elicit and inflammatory response, we can mimimise the unknown effects on the mice and subsequently minimise or prevent pain, distress and suffering. During the protocols we will monitor the health status of the animal and cull mice that show physical signs of distress.

<table>
<tr><td>PROJECT 22</td><td>Regenerative capacity of skeletal and cardiac muscle</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cardiac stem cells; skeletal muscle stem cells; repair, regeneration; myocardium</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td></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 objective of the project is to obtain a better understanding of the biology and regenerative potential of stem cells, derived from adult skeletal and cardiac muscle, for repair and maintenance of muscle tissue, particularly preventing and treating a loss of muscle mass (i.e. with ageing and/or disease). Stem cells are small, immature master cells that can replicate a copy of itself and give rise to specialised mature cells, i.e. a stem cell can divide to make another stem cell, and can make a mature muscle fibre cell. If these cells are to be considered and used for clinical application in the repair and regeneration of the muscle, it is imperative that we obtain a better understanding of their biology, characteristics and regenerative potential.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>Knowledge gained from undertaking this project should be easily transferable into a better</td></tr>
</table>

project (how science could be advanced or humans or animals could benefit from the project)?	understanding of human physiology and clinical applications. The approaches used are at the forefront of current molecular and cellular biology of stem cells but despite their basic nature, the findings are directly applicable and transferrable to the treatment of cardiovascular disease and failure, and age-related muscular diseases, such as muscle wasting and loss, obtained from prolonged bed rest or sedentary living. Indeed, this project has clear and timely relevance to, and potential to influence, new strategies and interventions to improve long-term health, quality of life and prolong living independently.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats will be used. Approx. 1636 will be used over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Some animals will be given ligation of an artery in the heart, to simulate a heart attack. This is considered a substantial severity. All animals will be anaesthetised so the level of pain and suffering should be zero during this time. Animals will receive stem cells or perform exercise training to repair their heart so they should show improved cardiac function. Some animals will receive exercise training which is expected to be of enjoyment to the animals, who prefer an active lifestyle rather than a sedentary one. At the end of the protocol the animals will be killed in a humane way and their tissues will be collected for us to analyse and determine the efficiency and effectiveness of the repair and regeneration process.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Due to the type of procedure involved (i.e. induction of myocardial infarction) there is no alternative other than to use animals. The rodent heart and muscle tissue can be obtained in large whole quantities and easily sampled. Together with ethical

use non-animal alternatives	constraints for removal of human heart tissue, this amount and sort of analysis cannot be obtained with human samples. We are obtaining human samples but these are small (~200mg) and limited to the atria. The final endpoint of all research study is to a establish basis for further human experiments through transferring results of experiments carried out in animal models. In order to ascertain this, a rodent model should be used as a first step. The findings from this research are both scientifically and clinically relevant, potentially leading to the development of new medical strategies which could benefit many people.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have undertaken statistical tests to estimate the number of animals needed to obtain significant results.
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.	Rodents are commonly used in experimental myocardial infarction models and cardiac regeneration assays, which provide greater opportunities for comparing data with those from other laboratories and existing publications. Larger mammalian animals i.e. porcine, canine, could also be used but this would require larger, more specialised facilities. Lower organisms i.e. zebrafish, which are an important model in comparative biology cannot be used as their biology is not analogous to mammalian vertebrates (i.e. they are able to regenerate large areas of damaged or removed cardiac muscle). We would specifically like to use mice or rats as the heart is small and the entire atria, left ventricle and septum can be sampled in a manageable number of histological sections. Also, the availability of antibodies are more specific to mice than any other species, this makes their use more convenient. Importantly, the use of transgenic animals provides valuable information unable to be obtained and evaluated in other animals. Procedures will be carried out when the animals are anaesthetised so the level of pain and suffering

	should be zero during this time. All investigators will be adequately trained in all procedures. As the animals will be receiving stem cells or exercise training, which have been shown to give rise to new muscle and vasculature in vivo, the animals should show improved cardiac and muscle function. Animals will be administered analgesics for pain relief if needed, and euthanatized promptly if complications occur.

<table>
<tr><td>PROJECT 23</td><td>Regulation and mechanism of platelet activation by GPVI and CLEC-2</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Platelets, signal-transduction, haemostasis, 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>
</table>
</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 how platelet function is regulated by CLEC-2 and/or GPVI activation and how those receptors are assembled into macromolecular complexes in order to carry out that role.</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>Platelets are associated with thrombotic disease, i.e. unwanted activation of platelets, and with haemostatic diseases, i.e. platelets that do not function well enough. Therefore, treatment for the former leaves patients at risk from the latter, and vice versa. It is only by fully understanding the underlying mechanisms of platelet activation that we can identify new targets for drug development. A drug that can prevent inappropriate blood clotting (thrombosis) without compromising the ability of normal blood clotting (haemostasis) to occur will save lives. Platelets are also associated with infection and inflammation. Further understanding of these areas will help to develop new therapies against these disorders.</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 29,000 mice in total – 4,000 animals for scientific protocols and 25,000 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</td><td>Conditional induction of genetic modulation – we expect adverse effects of mild severity such as minor weight loss and/or asymptomatic lymphatic defects. In exceptional circumstances (e.g. CLEC-2 deletion specifically in platelets) the lymphatic defects do lead to swelling of the paws. If signs of</td></tr>
</table>

end?	pain such as tip-toed walking is observed animals will be humanely culled but this is not 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 we do not expect this to happen. *In vivo* clotting assays - mice will be under terminal anaesthesia prior to and throughout these protocols and will therefore not experience little-to-no suffering or discomfort. Vessel leakage models Reverse passive Arthus reaction - we expect adverse effects of a moderate severity such as increased pain and discomfort which will be treated with pain relief. Wound repair – we expect adverse effects of a moderate severity such as increased pain, discomfort and loss of weight. These will be treated with pain relief and hydration. Any animal with infection or weight loss ≥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 and platelet function
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. To maximise the information gained from a single animal we aim to take perform multiple *in vitro*

	analyses on each individual.
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. The majority of procedures will be under terminal anaesthesia, and therefore harms to the animals will be negligible. Mice that receive platelet modifiers, or mice with uncharacterised genetic mutations will be monitored closely and appropriate action taken if they are deemed to be suffering.

<table>
<tr><td>PROJECT 24</td><td>Cardiovascular effects of uraemia</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Kidney disease, heart failure, uraemia, 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><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>Our project has two aims, firstly to increase our knowledge about kidney disease and its effect on the heart. Deaths from heart failure are 20 times more common in people with kidney disease when compared to those with healthy kidneys and where the kidney failure is caused by diabetes, this death rate is even higher. We believe that the combination of diabetes with kidney failure creates an entirely unique disease scenario therefore requiring treatments distinctive from those given to diabetics or kidney disease patients. This is important because the incidence of diabetes and diabetes related kidney disease has risen greatly over the last twenty years and is expected to keep on rising in the future. There is therefore a need to better understand how kidney disease functions as this would inevitably result in the development of new remedies to combat it.

Our second aim is to work towards identifying new treatment approaches for these conditions in collaboration with the pharmaceutical industry in the development of novel treatments for kidney disease. We do this on a contractual basis using</td></tr>
</table>

	the same protocols that we use in our own research. Much work is being done to develop new drugs for treating diabetes, blood pressure, obesity, heart failure and transplant rejection, conditions in which the possibility of kidney injury is an ever-present risk. However the fact that the kidney is a major site of drug metabolism and elimination requires preclinical studies of an immense diversity of drugs. These factors highlight the need for close collaboration between drug companies and clinical researchers whose expertise ultimately reduces development budgets and animal volume.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In the long term these investigations are expected to benefit doctors, in particular kidney doctors, with the possibility that new disease targets may be identified, for which new drugs could be developed ultimately having a major benefit to the health of the population with renal disease. Regarding our work with the pharmaceutical industry, centralising expertise in a particular field of research is a major benefit in terms of animal welfare and drug development. If we did not offer our services to industry our clients would have to set up their own animal facilities which would be costly, time consuming, would mean greater animal usage and would involve other groups working through the process of training and model introduction in order to build up the necessary skills. Alternatively they would seek out other research establishments willing to collaborate with them perhaps in places which apply less monitoring of animal welfare. In both these cases the overall result could be greater animal suffering.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 10000 mice and 7000 rats over the 5 year course of the licence.
In the context of what you propose to do to the animals, what are the expected adverse	In our animal work we induce kidney disease or diabetes in the animals either through surgery on their kidneys or by injecting them with a substance

effects and the likely/expected level of severity? What will happen to the animals at the end?	that causes the disease. Then we administer the test agent and monitor its effect against the disease. We compare healthy and diseased animals. Diseased animals begin to display symptoms of kidney failure or diabetes (or both, depending on what we have caused), and these symptoms gradually worsen over the course of the experiment. It is not our desire that these diseases be allowed to progress so far as to kill the animal. We ensure that in each example of disease that we have generated a time limit is in place at which point the animal is humanely killed before it gets too ill and its' organs extracted for analysis in the laboratory.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The majority of our research is carried out using kidney and heart cells grown in our laboratory. These isolated examples of kidney and heart behaviour enable us to test the effects of new disease pathways or treatments on these cells thus giving us valuable clues as to what kind of therapies might work in the whole living organism. Once we are certain that we have made a discovery we test for similar results in an animal with kidney disease. This is because the response to a particular therapy observed in a single group of cells growing on a plate may be different in a complex living organism where there is the possibility of interaction between different cell types. Similarly in our collaboration with the pharmaceutical industry we only test agents which have already successfully passed the stages of initial non animal testing by the drug developer thus necessitating further investigation in live animals before administration to humans. Less evolved species eg: fish or insects are too different from humans to provide relevant information about kidney disease. Likewise the use of computer modelling to predict disease development would be impossible for this kind of

	work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All work involving animals in our laboratory is limited to a small number of highly experienced staff thus ensuring that the welfare of experimental animals is protected. Where possible we have increased our utilisation of genetically modified mice which are chosen on the basis of having the specific proteins which we are interested in modified. Thus enabling us to target our experiments more efficiently and so reducing the number of animals required. The statistical package we use allows us to achieve our experimental objectives using the minimum number of animals. In a typical example where we want to demonstrate the increase of a particular harmful protein, 11 pairs of animals will be required to demonstrate dangerous differences between diseased and healthy 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 types of kidney disease studies conducted in our laboratory are already well established and recognised by the scientific community as appropriate to human kidney disease. We choose to use mice or rats in our live experiments as these are the lowest classes of animals in which kidney disease relevant to our research can be induced. For each experiment we always first carry out pilot studies using a small number of animals to test whether the experimental design will be beneficial. We only test agents which have been proven to be non- harmful to animals.

PROJECT 25	**Systems Biology of Cardiac Biophysics**	
Key Words (max. 5 words)	Heart Rhythm, Infarction, Ablation, Fibrosis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Heart disease is the leading cause of incapacitation and death in the developed world. Current basic and clinical research is largely focussed on heart muscle cells. However – the majority of heart cells are of other types, whose relevance is largely under-appreciated. This proposal targets specifically so-called fibroblasts – cells of the 'connective tissue' that are needed for mechanical stability of the heart, yet may contribute to heart rhythm disturbances, such as after myocardial infarction. Interestingly, connective tissue can also be helpful in terminating heart rhythm disturbances, such as when catheter ablation is used to replace heart muscle to interrupt spurious electrical conduction pathways in atrial fibrillation. Recent research has identified that fibroblasts can be involved not only in mechanical but also in electrical signalling pathways in the heart. Understanding these phenomena, their mechanisms, and the means of steering them, will allow us to complement present efforts to 'make new heart muscle' by an approach that aims to make better scars'.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This programme of research proposed here is funded by the European Research Council through an Advanced Grant, aiming at prevention of post-infarction arrhythmias, and at improvement of therapeutic procedures such as ablation.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years, we expect to breed ca. 6000 animals (5000 mice, rats, 1000 rabbits) and to conduct experiments on ca.1000 of them (the discrepancy being a result of the use of genetic combinations).
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?	As with all surgical techniques, there is a moderate risk of peri-surgery respiratory distress and/or cardiac insufficiency, as well as a minimal risk of heart attack and sudden death. The risk of any of these occurring will be minimised by correct and careful dosing of anaesthetics. At the end of experiments all animals will be culled and required organs will be processed and analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have a strong track-record and continued engagement in computational modelling. We conduct in silico experiments for hypothesis formation before, and we use models to aid data interpretation after experiments. However, based on this expertise, we know the limits of biological data available. As we are moving into internationally uncharted territory, and given that cardiac disease is a complex and multifactorial entity, we need to conduct the relevant experimental work on living tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed a number of techniques to extract as much information from any individual preparation as possible. Thus ranges from pioneering novel preparations (such as live cardiac tissue slices), over improved data gathering (such as by simultaneous mapping of multiple data modalities, or by using cutting edge techniques to simultaneously obtain high spatial and temporal data), and leads through to use of computer models and advanced statistical techniques for planning and interpretation

	of experimental studies.
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 surgical models have been chosen as they mimic human disease. Surgery technique is refined to be as non-invasive as possible and analgesia combined with close monitoring of the animal (scoring sheet) is used to enable early identification of problems and subsequent early management towards well-defined end-points, thus reducing animal suffering.

PROJECT 26	**The regulation and role of protein kinases in the heart**	
Key Words (max. 5 words)	Heart failure, the failing heart, heart disease, protein kinases, phosphorylation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Heart failure is a leading cause of morbidity and mortality worldwide, It occurs when the heart cannot pump the blood properly around the body. The failure to maintain an adequate blood flow (and, therefore, oxygen) to meet the requirements of the body results in symptoms such as extreme breathlessness and tiredness, with impairment of normal activities, and potentially leads to death. The contractile cells of the adult heart (cardiomyocytes) cannot multiply. If the heart needs to work harder (e.g. because of high blood pressure) cardiomyocytes get larger to pump blood around the body more efficiently. However, they die if deprived of oxygen (e.g. during a heart attack) or exposed to toxic chemicals and, because they cannot multiply, some heart function is lost. Clearly, it would be useful to find ways of keeping cardiomyocytes alive and/or replace dead cells, whether by helping existing adult cardiomyocytes to start multiplying again or by provision of, for example,	

	stem cells. Unfortunately, the numbers and range of new drugs directed towards treating heart failure remains limited. There is a critical need for a large expansion in the range of potential new targets so that new drugs may be developed to manage and treat heart failure. Protein kinases are an extremely important group of molecules (enzymes) that regulate how the proteins in our body behave. For example, they can influence whether cells die, repair damaged cells or build muscle. Over 500 protein kinases are represented in human DNA, but not all are present in every cell and cardiomyocytes have a specific protein kinase complement. To perform their function, protein kinases stick to and modify other proteins, having a structure that allows them to select specific targets. This makes them ideal targets for small chemicals (i.e. drugs) that stick to individual protein kinases to prevent them from working or change what they do. This “targetted” approach is used successfully by pharmaceutical companies resulting in, for example, an increasing number of drugs for the treatment of cancer. The major problem in adapting the approach for heart failure is that the protein kinases that are most well-studied in heart were initially identified and studied in cancer or inflammation (e.g. arthritis) and we remain ignorant of the majority of the protein kinases that regulate cardiomyocytes. To unlock the full potential of protein kinases as drug targets to treat heart diseases, it is necessary to know which are present and establish how this may change during development and disease. We aim to (I) identify the protein kinases in cardiomyocytes and the heart, and assess how this may change during development and in disease states, and (ii) establish what the kinases do in the heart and how they are regulated.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	We will identify all of the protein kinases that are present in cardiomyocytes. Our preliminary work already shows that some protein kinases are present at high levels in the heart (and so can be assumed to

animals could benefit from the project)?	play an important role in regulating its function) but have never been properly studied in any cell; many others have never been studied in the heart. The data will provide vital insights into why and how cardiomyocytes stop dividing and will lay a solid foundation for identifying the most suitable targets to prevent and manage heart failure. Given that many cancer drugs have been and are being developed target protein kinases, a secondary benefit of this project will be an understanding of which of these drugs is likely to affect the heart. Knowing which protein kinases are present in cardiomyocytes and the heart will generate a large expansion in the range of potential drug targets. To ensure that the data can be exploited fully, we will publish the full dataset in an appropriate forum for access by other researchers, clinicians, industry and society as a whole). We will include our own interpretation and view of the most important candidates for drug development, and we will liaise with interested parties to develop these targets further. However, individuals will also be able to make their own assessments and select their own targets for future research.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 years of this project, we expect to use approximately 976 rats and 246 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?	Animals will be subjected only to terminal anaesthesia with removal of the heart immediately prior to death (i.e. non-recovery).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The contractile cells of the heart (cardiomyocytes) do not divide and there are no cell lines that are representative of these cells. It is therefore necessary to use animals for their study. For studies of

use non-animal alternatives	cardiomyocyte function within the intact heart, there are no non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For cardiomyocytes, cells are prepared under conditions that produce the greatest yield. The data from the cells are used to inform experiments with adult hearts. When necessary, or appropriate, a professional statistician will be consulted to ensure an experimental design is optimal and minimises the number of animals required, yet ensures an adequate level of precision and power, and the appropriate statistical analysis is performed. In all cases, the minimum number of experiments will be performed to detect meaningful differences in responses with sufficient power, if they occur, at an appropriate level of statistical significance.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use rodents for this study. Where possible, we will use rat models which are used widely for studies of the heart. We have worked with rats for over 20 years and have a large amount of data on protein kinases in this species on which we can base future studies.

<table>
<tr><td>Project 27</td><td>Mouse models of cardiovascular development and disease.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>heart, cardiogenesis, CHD, mouse models</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>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>We aim to investigate genetic mechanisms leading to the occurrence of congenital heart disease (CHD).

CHD is a serious genetic disorder but only 20% of the cases can find an explanation – direct inheritance from parents or detected chromosomal anomaly (like Down’s syndrome). We know that remaining 80% of the cases they must have a significant genetic component as the risk of also having CHD is increased in first-degree relatives by 2-5 fold.

Our main objective is to better understand genetic bases underlying human congenital heart disease (CHD) and we are going to address this by studying in detail embryos from mouse models with cardiovascular developmental anomalies.</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>CHD affects 5-10/1000 live births in Europe and in USA but there are presently no means to prevent its occurrence. Often the only chance for a child born with CHD is to undergo several major</td></tr>
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animals could benefit from the project)?	surgeries but still over 20% of affected children do not survive till adulthood. Every year in UK, over 600 people, half of them babies under 1 year of age, die in a consequence of congenital heart disease. In addition, over 11 thousand people die due to coronary heart disease, in this almost 140 under the age of 35. The annual health care costs of cardiovascular and coronary heart disease exceed £8 and £2 billion, respectively. By discovering the genes involved in heart development and describing the mechanisms through which they control cardiogenesis in mouse, we will significantly add to better understanding and, what follows, better prediction and treatment of human congenital heart disease (CHD). We also aim to understand how genetic mutations, often benign if present in a fetus only in a single dose, lead to generation of a disease by interacting with the environment during pregnancy. Identifying genetic bases of the CHD will help members of affected families understand the reproductive risk and the necessity of genetic testing, and will help make them aware of harmful conditions (like smoking or some medicine) that should be avoided during pregnancy. In the future, our findings may lay the foundations for gene therapies targeted at human CHD and other heart related diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice; a maximum of 15400 adults and 12000 embryos over the 5 years period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will be mostly used for breeding. We will breed and maintain only wild-type and heterozygous mice. These mice are healthy. We will routinely ear-notch the mice for the purpose of marking and collecting a DNA sample. Ear notching should involve only slight and transient pain, and no healing problems. A minority of our animals will have particular substances administered which will result in no more than transient discomfort and no lasting harm.

	Some mice will be subjected to lower oxygen levels but will be given time to acclimatise to this conditions (typically 3-4 days) so it will not be very stressful. Mice will be humanely killed at the end of the project by Schedule 1 method.
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 two main reasons: 1. Genetic analysis in humans has major limitations: ethical and practical. Although observing and analysing existing mutations in humans is acceptable, creating and breeding mutations to determine their effects and identify disease mechanisms would be unethical. From a practical point, genetic studies in humans are complicated by the variability of our genomes and a large number of genes that may be involved in CHD. Often the effect of a mutation is seen only on a particular genetic background. However, no two humans are identical (apart from homozygotic twins) and many carry various non-causative mutations which makes it very difficult to establish which mutations on what genetic background are responsible for the CHD. Using animals that are bred with a full control over their genomes and genetic alterations, allows overcome this practical problems. 2. We need to study the whole developing embryonic structure (the heart and the circulatory system) and we need to be able to observe possible developmental anomalies similar to these occurring in humans. For these reasons, our study requires using an animal model and the one resembling human cardiogenesis as closely as possible. Moreover, we need an animal that is genetically easily manipulated and whose mutations would recapitulate the cardiac malformations observed in human patients. Animal models have already successfully predicted novel genetic mechanisms and pathways in human cardiovascular developmental disorders. These include structural cardiovascular malformation and congenital heart disease.
2. Reduction	The numbers of animals will be minimised by careful planning of the experiments.

Explain how you will assure the use of minimum numbers of animals	We will also use already existing resources by investigating lines with cardiovascular defects generated by our collaborators – this will significantly reduce the production of new mice. Moreover, it will assure full usage of already generated lines.
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 because this species provides a combination of easy genetic manipulation with cardiovascular system and disease models sufficiently similar to man. As in humans, (and unlike other model organisms such as fish or fly), the mouse has a 4-chambered heart with a septated outflow tract, left-sided great arteries, and parallel pulmonary and systemic circulations. Common cardiac malformations such as septal, outflow tract and aortic or pulmonary arch defects (components o human disease) can thus be identified in mouse embryos. Mouse mutations typically recapitulate the cardiac malformations observed in patients with mutations in the same genes, and the identification of several human CHD genes has resulted directly from an initial understanding of their function in mice. These observations support the use of the mouse as a model for human CHD. Most of our animals will undergo only a mild procedure. In altered gas levels experiment, we will subject animals to the oxygen level that does not cause any harm and we will give the animals time to slowly acclimatise to new conditions (as required during climbing to high altitudes). We will also run a pilot study on a small number of animals to establish the best doses and routes of drug administration that would have no side effects on adult mice and will be the lest stressful for the animals. Animals will be kept in a high-standard animal facility where they will be monitored daily for any symptoms of ill health or discomfort.

<table>
<tr><td>Project 28</td><td>Control of early heart development in vertebrates</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart, embryo, frog</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>
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<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>Detailed molecular and cellular description of early heart development.</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>Improvements in cardiac regenerative medicine: by providing better directed differentiation protocols to supply new myocardium. Also better understanding of the mechanisms that cause congenital heart disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Xenopus laevis, 1000 adults, ≈4000 larvae

Xenopus tropicalis, 200 adults, ≈1000 larvae

Danio rerio, 500 adults, ≈3000 larvae</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td>Our routine use of adult animals in this project is to supply embryos and this requires a mild procedure.</td></tr>
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effects and the likely/expected level of severity? What will happen to the animals at the end?	Adult animals are kept and used to supply gametes. Animals that fail to produce gametes of sufficient quality and in sufficient quantity are killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research on early heart development requires embryos. Non-animal alternatives cannot yet replace this requirement.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Adult animals are used repeatedly to provide gametes after optimal recovery time. Embryos are obtained primarily by matings instead of in vitro fertilisation. Both practices act to reduce the number of animals that would have been used otherwise.
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 frog *Xenopus* and zebrafish models are the lowest complexity vertebrate models used for studying embryonic development. At the same time they are vertebrates that share many features of development with mammals, including us, so most of findings that we obtain from these non-mammalian models will be of broader significance. Minimising harms is primarily through reduction (see above).

<table>
<tr><td>Project 29</td><td colspan="3">Molecular mechanisms of development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Angiogenesis, inflammation, drug screening, molecular targets</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</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>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 colspan="3">The principal objectives of the proposed research are to screen compounds that block the formation of blood vessels (antiangiogenic) and/or the inflammatory response system, analyse their actions at multiple stages of development for effects on blood vessels, inflammation and development, isolate their molecular targets and study those targets in-vivo and in-vitro.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The production of compounds targeted to specific conditions (anti-inflammatory or antiangiogenic) without causing side-effects or adverse reactions is the ultimate goal.</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">We will carry out the project and experiments using zebrafish embryos – no more than 35000 regulated procedures over 5 years. This number has been reached from previous PPL annual returns, experience and the need for statistical relevance.</td></tr>
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	Our work will also minimise the subsequent use of mammalian species for initial drug screens as our data will have calculated the doses and time periods to apply such drugs.
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 reactions are difficult to predict, however drugs preventing blood vessel formation will likely cause developmental anomalies, through which such fish will be euthanized. Anti-inflammatory drugs, from our own work do not cause developmental anomalies. Identification of molecular targets for the drugs will allow us to produce or obtain GA fish with mutations in those genes. Such lines will be maintained as heterozygotes, so they don't exhibit the mutation and only crossed to produce the homozygote to produce embryos to study which will be complete before 6 days. All animals will be killed at the end of each respective experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aims of the proposed research are to screen compounds and study effects on angiogenesis, inflammatory system and teratogenesis, and to advance our understanding of the signalling mechanisms directing the development of the vasculature and inflammatory systems. Tissue culture approaches and gene expression studies provide an invaluable means for identifying molecules with the potential to regulate normal development. However, these approaches can not unravel the precise function and requirement of specific molecules in vivo where multiple cellular and molecular interactions occur. Determining the function of individual molecules in the context of the whole animal is critical if the mechanisms that drive normal development (and can lead to safer drugs) are to be understood.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers to be used are based on previous usage and experience, to achieve statistical significance, and reflect the numbers of animals used in my annual returns throughout the course of each of my Project Licences. The numbers of animals chosen for each experiment are

	considered scientifically relevant from over 30years of work on the zebrafish. The numbers also reflects a need to maintain several tanks of each fish line and to produce new breeding stock on a regular basis, as zebrafish are most efficiently reproductively active between 6months and 24months of age and providing fish are well cared for produce equivalent numbers of embryos at all ages. I train staff in the use of the 3R's, and encourage them to only carry out experiments with the correct number of animals to prevent repeating experiments due to unstatistical relevance.
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.	Zebrafish exhibit rapid development, high fecundity, they are optically transparent allowing live in-vivo observations to be made. Combined with the wide availability of genetically modified and/or viable mutant lines of zebrafish, and my expertise in analysing zebrafish development, this makes this the most appropriate animal model to use in these studies. Mammalian species do not allow live and in-vivo monitoring of embryos and the administration of compounds must be made to the mother, potentially causing other damage onto the embryo. Chick embryos although useful for studying the teratogenic effects of such compounds are difficult to observe effects on blood vessels and the inflammatory response. Given the zebrafish produces eggs which are fertilised outside and away from the adult fish, this means no harm comes to the adults. The zebrafish are housed in a modern aquarium. The well-being of the fish depends primarily on water quality and temperature. This is carefully controlled by a purpose built filtration and pumping system, and water quality is checked weekly and the temperature daily. Fish are fed a mixed diet regularly. Additional documents are in place to ensure the smooth running of the facility e.g. standard operating procedures and risk assessments.