Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 18

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

Project Titles and keywords

1. **Investigation of Factors Influencing Heart Failure**
    - Heart, hypertension, hormones

2. **Novel therapeutic approaches to heart disease**
    - Heart failure, energetic, metabolism, ischaemia

3. **Tissue function and metabolism in cardiac disease**
    - Heart function metabolism stem cells

4. **Transcriptional regulation of angiogenesis**
    - Angiogenesis, Cancer, Blood vessels, Transcription

5. **Switching genes on and off in haemopoiesis**
    - Gene regulation haemopoiesis

6. **Testing of novel compounds**
    - Mitochondria, heart, pharmokinetics

7. **Ion regulation and cell signalling in cardiac and skeletal muscle**
    - Exercise, heart, heart failure, skeletal muscle, sodium

8. **Protection after an acute heart attack or stroke**
    - Heart, heart attack, stroke, ischaemia, reperfusion injury

9. **Regulation/role of the ERK1/2 cascade in the heart**
    - Heart failure, cancer therapies, protein kinases, hypertension, hypertrophy

10. **Role of the cytoskeleton in cardiovascular diseases**
    - Cytoskeleton, platelet, thrombosis

11. **Novel treatments for right heart failure**
    - Pulmonary artery hypertension, right heart failure, exercise, β-blockers, phosphodiesterase 5 inhibitors

12. **Functional characterisation of novel blood genes**
    - Zebrafish, blood, stem cells, cancer

13. **Inflammation and Cardiovascular Disease**
    - Blood vessel, thrombosis, aspirin, inflammation, heart attack

14. **The long-term effects of prenatal hypoxia on cardiomyocyte function**
    - Programming, pre-natal hypoxia, cardiac, mitochondria, myocyte

15. **Developmental programming by maternal diet**
    - Maternal obesity, pregnancy, metabolic syndrome, diet, developmental programming

16. **Imaging of Cardiovascular Diseases with Novel Agents and Devices**
    - Imaging, cardiovascular disease, diagnosis, therapy

17. **Autonomic control of cardiac function and rhythm**
    - Cardiac function, arrhythmia, autonomic control

18. **Regulation of thrombus formation**
    - Clotting factors, thrombus formation

19. **Modulation of cardioprotection in pathological states**
    - Heart attack, diabetes, age, animal research

20. **Investigating normal blood development and blood cancer**
    - Haematopoiesis, blood stem cell, leukaemia, microenvironment

21. **Studies on Vascular Diseases and Repair Mechanisms**
    - Blood vessel, Restenosis, Stent, Vein Graft, Endothelium

22. **Zebrafish models of cardiovascular development & disease**
    - Cardiovascular, angiogenesis, heart, blood flow

23. **Mechanisms and therapy for limb ischaemia**
    - Limb ischaemia, angiogenesis, arteriogenesis

24. **Signalling in normal and abnormal blood cell function**
    - Blood, Cancer, clotting, bone marrow

25. **Control of adrenal development and function**
    - Adrenal, steroid, development, disease, differentiation

26. **Transverse aortic constriction (TAC) in rodents**
    - Heart failure, cardiac hypertrophy, rats, mice

27. **The role of platelets in haemostasis and thrombosis**
    - Mice, platelet, thrombosis, haemostasis

28. **Redox Mechanisms of Organ Failure in Sepsis**
    - Sepsis, Redox, Blood pressure, Multi-organ Failure, Therapy

29. **Modelling striated muscle disease in zebrafish**
    - Heart, skeletal muscle, Popeye genes

30. **Mechanisms of platelet-driven disease**
    - Platelet, cardiovascular, heart attack, thrombosis

31. **Parental nutrition and offspring development**
    - Diet, Gamete quality, Pre-implantation embryo, Offspring health, Developmental programming

32. **Regulation of normal and malignant haemopoiesis**
    - Leukaemia, haematology, development, stem cell

33. **Targeted gene delivery for haemostasis**
    - Blood coagulation, haemophilia, thrombosis

34. **Stem Cells for Blood and Tissue Repair**
    - Blood, blood vessel, repair, transplantation, regeneration

35. **Regulation of Blood Cell Generation**
    - Blood stem cells, Regenerative Medicine

36. **Zebrafish manipulation to study blood development**
    - Haematopoiesis, haematopoietic stem cells (HSCs), zebrafish

37. **Preclinical therapies for renal and cardiovascular injury**
    - Kidney injury, cardiac injury, muscle damage, blocked blood flow, therapy

Project 1	**Investigation of Factors Influencing Heart Failure**	
Key Words (max. 5 words)	heart, hypertension, hormones	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Heart failure is a serious condition in which the heart does not pump blood around the body at the usual pressure because it has become too weak or stiff. The most common causes of heart failure are a heart attack, high blood pressure (called hypertension) or diseases of the heart muscle that cause weakness (called cardiomyopathy). As we age, high blood pressure causes heart failure in more women than men suggesting that male and female hearts respond to this stress differently but the underlying reasons for the disparity are unknown. This work firstly aims to investigate the reasons for the sex difference in the response of the heart to high blood pressure. The main female sex hormones prevent or slow the development of heart failure but the way they do this is not known. Our second aim is to investigate how these hormones affect the heart and its response to high blood pressure. Our third aim is to investigate if there are sex differences in the responses of the heart to established treatments for hypertension.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The potential benefit of the project is that a better understanding of the mechanisms will influence a more tailored – and so more effective – approach to treatment strategies for heart failure that may benefit both sexes. The actions of sex hormones on improving the function of the heart may be able to be	

project)?	reproduced by designing chemically-related compounds that prevent or slow the onset of this serious condition in both sexes.
What species and approximate numbers of animals do you expect to use over what period of time?	Guinea-pig approx 80 pa over five years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The surgery detailed in the protocols produces a moderate level of severity and most animals survive with minimal adverse effects. At the end of the protocols the animals are killed by an approved ASPA method and the heart removed. We then isolate single cells or thin tissue slices from the heart tissue and this approach allows different experiments to be carried out on one heart so greatly minimising animal use. The main adverse effect is embedded in the animal model of the human condition we wish to reproduce. The changes to the heart that take place when it works against increased blood pressure produce disturbances in its contraction, electrical conduction and propagation. Some animals may die from sudden cardiac death that is probably caused by lethal arrhythmias but the exact cause of death is uncertain. Although sudden death during the progression towards heart failure is relatively rare it is unpredictable in nature because there are rarely any preceding clinical signs, akin to the human situation. Overall, the level of severity is for this programme of work is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are investigating complex inter-relationships between various biological systems i.e. blood pressure, heart responses and hormonal signalling which all contribute in the progression of disease. These inter-relationships would be too complex to be studied using computer modelling or cell culture models. We cannot use long-term cultures of adult heart muscle cells for our studies because these cells do not divide and they undergo detrimental changes to their structure when cultured. Human cells, usually obtained from valve replacement or heart transplantation surgery, can be used for these studies, however they are difficult to obtain and acquiring control tissue is fraught with obvious ethical

	and resource issues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We isolate single cells and thin tissue slices from the heart tissue and this approach allows many different experiments to be carried out on one heart so minimising the number of animals used. Rigorous statistical calculations, precise knowledge of our experimental processes and other published information, and our considerable previous experience ensures we use the least number of animals in our studies whilst maximising their power.
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.	Our animal model closely resembles the human situation. The procedure replicates the pressure-induced overload of hypertension and the animal displays many aspects of its biology that are similar to the human. Post-operative harm is minimised by surgical skill and experience and close monitoring of the animals. We use non-invasive ultra-sound techniques to characterise the structural and functional changes occurring to the heart in vivo avoiding re-use and multiple doses of anaesthesia. We isolate single cells from the heart tissue and this approach allows different experiments to be carried out on one heart and the cells to be shared by different scientists so minimising animal use.

Project 2	**Novel therapeutic approaches to heart disease**	
Key Words (max. 5 words)	Heart failure; energetics; metabolism; ischaemia	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to further our understanding of heart disease, with particular emphasis on how the heart recovers after a heart attack and the subsequent development of chronic heart failure. In the UK alone there are ~100,000 heart attacks per year and 800,000 individuals living with heart failure. Even with optimal treatment, 59% of men and 45% of women diagnosed with heart failure will be dead within 5 years, a clear indication that new and better treatments are urgently needed. One promising approach, that isn't targeted by current medication, is to improve how the heart produces and uses energy since there is abundant evidence to show that this is impaired in many types of heart disease. However, the best way of achieving this is unknown. We will perform proof-of-principle studies mostly in mouse models of heart attack and heart failure (and some rats) to test which approaches are most promising for further research and development. Approaches that work in one cause of heart disease often help in others and we will therefore also test in mouse models of heart disease due to hypertension, diabetes, and the anti-cancer drug doxorubicin, which causes heart failure as a side-effect. This type of research requires the ability to measure how well the heart is functioning in a living intact animal and we therefore have an additional objective	

	to develop new methods to achieve this, for example, by using non-invasive imaging techniques such as MRI.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will advance our basic understanding of how the heart utilises energy, how this is altered in different types of heart disease, and what approaches might be used to correct this. This will be useful to other scientists in our field and to the pharmaceutical industry by identifying potential targets for developing new drugs. A related benefit will come from the proof-of-principle studies that will test whether specific therapeutic strategies are beneficial in models of heart disease. This will directly guide future research and where to focus our resources. For example, if we show that over-expressing a particular protein in the heart protects against heart failure, then this suggests it is worthwhile looking for drug-like compounds that have the same effect. Ultimately, we hope that the knowledge generated in this project will represent the first step in developing new treatments for the clinic. Further benefits will arise from the development of new methods for studying the heart in living animals, in terms of both animal welfare and the quality of scientific information. For example, our previous work has greatly reduced the time taken to image the mouse heart using MRI and we have developed related techniques that can provide biochemical data non-invasively that was previously only available by killing the animal.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to a maximum of 11,750 mice over a period of 5 years (61% of these used for breeding purposes due to the extensive use of genetically-altered mice). In addition we will use up to 790 rats.
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?	Many of the adverse effects are related to recovery from surgical procedures used to create the disease models, but these can be readily controlled (see refinements section below). After recovering from surgery most mice will be free from symptoms for the duration of the study. However, occasionally animals will suddenly develop deep abdominal breathing, which is the first sign that they have developed heart failure. Suffering is minimised by using this as an immediate humane end-point. It is not possible to predict the affected individuals and the first sign of heart failure is often death, since it is more likely to occur overnight. These animals are likely to experience a severity level that is Severe. However,

	the majority of animals are killed humanely at the scientific endpoint without exhibiting any symptoms of heart failure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying chronic heart failure, which is a progressive disease that develops over many weeks. This represents a level of complexity that can only be fully represented by the intact animal since there is dynamic interplay between mechanical stress, haemodynamic loading, and the nervous, vascular, endocrine and inflammatory systems. Nevertheless, it is sometimes possible to use non-animal alternatives to answer specific questions. For example, we will continue to use in vitro cell culture, non-sentient phantoms for MRI technique development, and computer modelling.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The majority of animals are used for breeding, and we will take care to breed the minimum required for our experiments. We routinely use power calculations to guide our experimental design. Non-invasive imaging techniques will be used to significantly reduce the number of mice required for longitudinal 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.	We mainly use mice because this species provides the unique combination of easy genetic manipulation with a cardiovascular system and disease models sufficiently similar to man. Occasionally we will use rats, for example, where a larger heart is required. We use surgical models that mimic the major causes of human heart failure since these are the most translational and therefore considered the Gold Standard in our field. Our surgical techniques have been refined as a result of many years' experience, for example, surgery takes place using aseptic technique within a clean air environment, animals are closely monitored during recovery and provided with pain killers, fluids, heat support and access to softened food as routine practice. All cages include environmental enrichment and animals are kept in social groups whenever this is compatible with our objectives. Whenever possible we will conclude our experiments at an earlier time-point before significant adverse effects develop in order to minimise potential suffering or distress. Where this is not practicable, we apply strict humane end-points to ensure that suffering is not prolonged,

	for example, any animal developing breathing difficulties is immediately euthanized as a humane end-point.

Project 3	**Tissue function and metabolism in cardiac disease**	
Key Words (max. 5 words)	Heart function metabolism stem cells	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to learn more about the causes, detection and treatment of heart disease by a) characterising changes in heart function and tissue metabolism in models of heart disease, or disorders such as diabetes which can lead to heart disease; b) identifying potential drug targets or ways to detect heart disease; c) investigating stem cell therapy to improve function and metabolism in the failing heart.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Heart failure is a debilitating condition and heart disease is now the leading cause of mortality worldwide. In this project we aim to find out more about the link between heart disease and tissue metabolism and the best way to deliver stem cell therapy for heart failure. This will provide valuable information for the development of therapies to prevent the decline into heart failure.	
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use up to 5850 rats and 5250 mice over 5 years.	
In the context of what you propose to do to the animals, what are the	Heart failure and the pathologies that lead up to it are painful conditions and therefore investigations into causes of and therapies for heart failure cannot fully	

expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	avoid procedures which will cause pain and suffering. In some of the models we use to induce heart failure, animals undergo invasive surgery so that we can reduce blood flow in the vessels of the heart to mimic a heart attack or in the aorta to increase blood pressure. Alternatively, we use drugs to increase the heart rate over a number of days. In all cases, the animals are likely to suffer pain and discomfort, which we will reduce as much as possible with appropriate pain relief. In the majority of cases, the animals show no sign of discomfort after the first few days. However, some may experience more prolonged pain and, as a result, four of our protocols are categorised as severe. If we are not able to control the pain with analgesic the animal will be killed humanely. We have two models to induce diabetes. Type 2 diabetes is induced by a combination of a high fat diet and an injection of a drug to reduce the secretion of insulin. This mimics the human condition and we do not expect this to cause any substantial changes to the wellbeing of the animal. Where we induce type 1 diabetes, with a strong dose of the drug to kill more of the cells which produce insulin, there is a risk of the animal going into a diabetic coma. We will minimise this as much as possible by careful control of the drug administration and by giving the animals sugar water for the first few days. Animals which show signs of lethargy or severe weight loss will be killed humanely. We are investigating the effect of chemotherapy on the heart to discover why it cause cardiotoxicity in some patients and not in others. As this uses toxic compounds to we will monitor the animals and the drug doses carefully to minimise suffering. We use genetic rodent models to investigate the role of specific gene changes in heart disease and diabetes. In most cases the animals do not show any overt adverse effects, however with ageing a more severe phenotype may occur and these animals will be monitored closely. We also use animals where specific cells have a fluorescent tag and we do not anticipate that these animals will suffer any adverse effects. At the end of each study, the animals will be killed humanely and their tissue used for further studies.
Application of the 3Rs	
1. Replacement	We use cells in culture, where possible, to investigate protein and gene changes that we have observed in the

State why you need to use animals and why you cannot use non-animal alternatives	animal models. To obtain relevant measurements of cardiac function it is necessary to use an animal model with a four chambered heart like the human heart. In addition, as metabolism and contraction of the heart are inextricably linked, it is essential to study metabolism in the intact beating heart.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use MRI to measure cardiac function and metabolism on the same animal over time as the disease progresses. The MRI does not affect the animals adversely and allows us to reduce substantially the number of animals required to follow the disease progression. We can measure substrate metabolism *in vivo* in the heart and the liver in the same animal, further reducing the number of animals we require; and we have refined out ex vivo heart perfusion measurements so that we can measure two substrates in one heart where previously we would have required the use of two animals. We ensure that, where possible, researchers requiring tissue other than heart coordinate their studies with those doing heart perfusion, thereby using one animal for two 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.	We use rats and mice because the hearts are structurally and metabolically close to the human heart. We use non-invasive techniques wherever possible, for example we feed high fat diet to elevate plasma free fatty acids and give therapeutic compounds in drinking water if applicable. We use transgenic animals to help us understand genetic causes of heart disease. Appropriate medication will be used to minimize pain and distress. We seek regular advice and updates from the vets and the NC3Rs on techniques, such as anaesthesia and surgery, to ensure best practice. Our animal assessment protocols and analgesic regimes are tailored to each protocol, in consultation with the vets, to ensure that we detect early signs of pain or distress and can act accordingly. When instituting a protocol that is new to our lab we perform a pilot study using drug doses or protocols taken from the literature and consultation with collaborators, to confirm that these are appropriate for the species and strain we are studying.

Project 4	**Transcriptional regulation of angiogenesis**	
Key Words (max. 5 words)	Angiogenesis, Cancer, Blood vessels, Transcription	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of this work is to understand how genes are switched on and off during vessel development, with a particular interest in how this occurs in disease states such as tumour growth. Insights into the molecular processes controlling vessel growth, and a clear understanding of the underlying genetic programs, are essential to the development strategies to modulate vessel growth in humans, and would be applicable to diseases as diverse as macular degeneration in the eye, inflammatory disorders, cancer and heart disease	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits stemming from this work include increased understanding about how blood vessel growth in controlled. Excess blood vessel growth occurs during many human diseases, and in cancer, vessel growth is necessary for solid tumours to survive. Additionally, in some human diseases it is desirable to deliberately stimulate particular blood vessels to grow, for example after vessel damage in the arm or leg, or in the heart after a heart attack. Not all blood vessels are the same (for example, the cells that make up arterial and venous vessels express different sets of genes, as do growing blood vessels when compared to stable, quiescent vessels), so it is	

	crucial to understand both what makes vessels grow and stop growing, and also to understand what type of vessels are downstream of any particular pathway or intervention. The knowledge derived from the work described here will help development of therapies to prevent, modulate or encourage vascular growth in multiple different disease states.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and zebrafish for our work. Over five years, we expect to use 14,000 mice and 4,000 zebrafish.
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 vast majority of animal use (over 80% of mice, 100% of zebrafish) will consist solely of the maintenance and breeding of genetically modified animals followed by death and post-mortem analysis of blood vessel development. For these animals, few adverse effects are anticipated, and where adverse effects are detected, in most cases the animals will be killed immediately. For maintenance and breeding, most will only experience mild severity, with the possibility of moderate severity in genetically modified animals. To study blood vessel development, a minority of animals will be administered substances, including via mini-pumps, to alter gene expression and/or induce vessel growth. Vessel growth may also be encouraged through either the implantation of matrix or tumour cells, into which the growth of vessels, and of the tumour itself (tumour growth is dependent on effective development of new vessels) will be measured. Adverse effects may include discomfort at site of administration (e.g. inflammation, oedema, scratching) and a possibility of infection with a limit of only moderate severity. Tumours grown in mice will not be permitted to grow beyond 12.5mm wide, but may result in adverse effects with a limit of only moderate severity, including reduced body weight, reduced food and water consumption and partial piloerection. Animal numbers will be kept to the minimal required to give statistically sound results. At the end the mice will be killed.
Application of the 3Rs	
1. Replacement State why you need to use	Our work uses the study of gene enhancers. These are the regions that control the switching on and off

animals and why you cannot use non-animal alternatives	of genes. Putative enhancers require validation in animal models, as cell-based assays are unable to reliably determine the activity, nor specific expression pattern, of putative enhancer regions. However, we have replaced transgenic mice with transgenic pre-free feeding zebrafish embryos for our initial analysis of enhancers. Once identified as a potential regulatory protein, we will investigate the role of this protein in animal models where it has been mutated or deleted. Although we can and will also perturb gene expression in cells in culture, it is challenging to clearly detect the effects this has on vessel development. Endothelial cells grown in culture adopt a proliferative mode, unlike that found in mammals, and cell culture cannot accurately mimic events *in vivo*. Endothelial cells behave differently in their natural environment, where they are in tubular structures, surrounded by accessory cells and receiving intrinsic and extrinsic signals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Putative enhancer will be initially screened in transient transgenic zebrafish (before day 5 post fertilisation) in place of transgenic mice, using a three-colour reporter-gene system which will allow us to analyse three enhancers in each transgenic zebrafish. Where suitable lines exist, animals will be obtained from the relevant supplier. As the technology develops, we will also consider using genome editing technologies (e.g. Cas9/CRISPR) to generate transient gene deletions reducing the need for stable animal lines and consequently much greater number of breeding mice. To make a quantitative analysis of angiogenesis we will use the retinal angiogenesis model and Ad-VEGF tumour surrogate models, both which develop blood vessels in a known, sequential manner that reduces necessary time-points.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	Analysis of enhancer/promoters in transgenic zebrafish allows most analysis to be done before zebrafish are free feeding. Live imaging allows study of the formation of the vessel system in the same fish. Aseptic precautions will be taken to reduce the risk of infection and care will be taken to ensure the animals are properly restrained during injections.

(harms) to the animals.	Analgesia will be used when necessary to minimise welfare costs to the animal. A matrix angiogenesis assay will permit modelling of vascular growth in a healthy mouse after a minimally invasive procedure, whereas the Ad-VEGF tumour surrogate model simulates the tumour environment for blood vessel growth without subjecting the mouse to any tumour burden.

Project 5	**Switching genes on and off in haemopoiesis**	
Key Words (max. 5 words)	Gene regulation haemopoiesis	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to increase our understanding of the processes by which genes are switched on and off through early development and how differentiation into different cell types and tissues is controlled. We can then try to understand how these processes are perturbed by mutations that occur in human genetic diseases. Studying human conditions in which acquired mutations occur has already identified many genes affecting blood cell production. However, curative treatments are not yet available. Genetic blood diseases, such as thalassaemia and sickle cell disease, are among the most common single gene disorders in the world with 340000 children being diagnosed every year leading to a global population of millions of sufferers. Although their molecular bases are understood, treatments are unsatisfactory as many are blood transfusion dependent and this too leads complications such as iron overload which, in turn, can lead to multiple organ failure, poor quality of life and shortened life expectancy. The only other treatment is bone marrow transplant but there is a shortage of suitable donors and it is a treatment which is not available in many developing where these diseases are prevalent.	

	The X-linked alpha thalassaemia mental retardation (ATRX). syndrome is a severe, non progressive form of mental retardation that is frequently associated with multiple congenital abnormalities and with a mild form of alpha thalassaemia. It is one of the commonest single causes of syndromic mental retardation in boys. It is caused by mutations in the ATRX protein. This protein interacts with others and acquired mutations in these proteins have been found in a growing number of cancers. It appears that ATRX plays an important role as a tumour suppressor but it is currently unknown how mutations in ATRX give rise to human disease. We will concentrate our studies on how the alpha globin genes are affected by the 2 different isoforms of the ATRX protein but Making our new animal models available to, or setting up collaborations with, researchers with expertise in the cancer field will be of benefit to the wider research community. Another disease, CDA-I is clinically characterised by a particular type of moderate to severe anaemia which arises as a result of mutations, which affect blood cell production. Currently there is only one treatment for CDA however, this is not effective in all cases and one third of patients treated with this drug develop side effects which include depression. To improve treatments of all of these acquired and inherited disorders we need to understand more fully how the genes responsible for these diseases are normally regulated.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Disorders of genes which control haemoglobin production comprise the commonest monogenic disorders worldwide with 340000 children diagnosed each year. These diseases are responsible for a huge burden of morbidity and mortality. Although life expectancy has increased over the years from patients living to their teens and twenties to now where some live into their fifties and sixties their quality of life can be poor from complications of current treatments. The most common causes of alpha thalassaemia are deletions of the alpha globin genes. The most common of these leaves the embryonically-expressed alpha globin gene intact and available for reactivation. The only potentially curative therapies currently available for severe alpha thalassaemia are bone marrow transplantation and gene therapy, both of which have significant limitations for widespread use. Several lines of

	evidence suggest that alpha globin can functionally substitute for alpha globin and ameliorate severe alpha thalassaemia, however, very little is currently known about the regulation of this gene. In the work included here we will gain the first insight into how alpha globin may be repressed. This is likely to identify novel therapeutic targets which may be developed in the longer-term to ameliorate the disease burden and improve the quality of life for patients with severe alpha thalassaemia. Developing animal models of the ATRX syndrome will further help to define their functions and the pathological consequences of perturbing these proteins and how the alpha globin genes are affected. Studying these animal models and making them available to collaborators with expertise in the cancer field will help them to determine how these cancers arise and facilitate the development of novel therapies. In most cases the cause of the anaemia can be identified using simple diagnostic tests (eg for sickle cell anaemia). However, in a substantial minority of cases (estimated at 40-50 cases per year in the UK), the cause cannot be identified. This often leads to lifelong chronic anaemia and/or transfusion-dependence with attendant complications of iron overload and failure to thrive. For families with affected children there is also the uncertainty over the risk to future children, the long-term prognosis and the best form of treatment. In addition, repeated investigation to try to identify the cause is expensive and optimising treatment and/or development of new treatments is extremely difficult. One group of conditions for which a molecular diagnosis is usually very difficult are the Congenital Dyserythropoietic Anaemias (CDA). The focus of our work on CDA is to characterise pathogenic mechanism underlying the disease in an animal model and use this information to improve the diagnosis and ultimately to develop novel therapeutic agents for human patients. Other benefits of this work will be in the area of carrier-testing and improved genetic counselling and risk estimation. This is very important for patients with undiagnosed anaemias, as new advice on disease management and diagnostic services develop.
What species and approximate numbers of	We estimate that we will use in the region of 7700 mice over the 5 year life span of this PPL based on

animals do you expect to use over what period of time?	our previous experience.
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?	Harmful adverse effects are rare and unpredictable in the production of new GM lines. Any animal showing a phenotype which causes more than a temporary deviation from normal behaviour that is not of scientific interest will be humanely killed by a schedule one method. The majority of mice will be used for breeding and may experience no more than transient discomfort from 2 or 3 injections prior to being humanely killed for tissue analysis. We do not expect to see any adverse affects as a result of any drugs administered in the doses and timescales to be used. Only a small number of mice administered drugs may undergo surgery for the implantation of a mini pump to administer drugs which would otherwise require repeated injections. Good aseptic surgical technique will minimise rare complications that arise following surgery. Any mice having undergone such surgery this will be monitored closely post operatively and will be administered analgesia as required. Any mouse failing to fully recover from this surgery within 48 hours will be humanely killed by a schedule 1 method. We expect some mice to be mildly anaemic but do not expect to see overt clinical signs, physical signs of anaemia may be difficult to access in pigmented mice but checking the colour of the footpads for pallor or paleness will give an indication of anaemia when compared to wild type littermates. We do not expect these animals to show any deviation from normal behaviour. One line of mice, P53 Knock out, that we propose to breed with our ATRX show no visible phenotype but it has been reported that some homozygotes may develop tumours at 3-6 months of age. Heterozygous mice may develop tumours at about 10 months of age. Our previous experience has shown this to be rare. We would however, only keep these mice for the minimum time to produce the combination of genotypes that we require. These mice will be monitored regularly for any deviation from normal behaviour and /or physical signs of tumour development. Any mice developing tumours would be immediately culled 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	Although Zebrafish and lower vertebrates may be appropriate model systems for studying many developmental processes particularly at the early stages in of reseaerch, a mammalian model still remains necessary in order to fully understand the effects of many human genes and their disease-associated mutants and other complex physiological systems that mammals share. In order to fully understand the effects of human genes and their disease-associated mutants, there is no suitable substitute for a mammalian model. This will allow the analysis of the effect of a gene at early developmental stages in different tissues. The study of interactions between factors requires a whole animal model. There are no suitable non animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will only breed mice as required. We will cryopreserve by Sperm freezing wherever possible will be encouraged over embryo freezing. This will reduce the number of mice required to cryopreserve a line by eliminating the need to superovulate large numbers of female donors and maintain stud males to produce fertilised embryos for freezing. All steps in every process will be carefully monitored to minimise numbers. Experimental procedures will be updated as appropriate and new technologies will be introduced as they develop to minimise mouse numbers. Blood and tissue samples will be shared across several groups working on this project.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mouse is the species of choice for genetic modifications modelling human disease because of the availability and ease of manipulation of mouse ES cells. Best practice will be used for all procedures and staff will keep up to date with new methodologies and implement new procedures as they arise. Wherever possible constructs and/or manipulated ES cells will be produced and tested in the bioengineering facility in an *in vitro* system before

	going on to produce new GM lines. Good aseptic surgical technique will minimise rare complications that arise following surgery. Mice having undergone surgery for the implantation of an osmotic mini pump will be monitored closely post operatively and will be administered analgesia. Any mouse failing to fully recover after 48 hours of surgery will be humanely killed. Analgesia will be used wherever appropriate.

Project 6	**Testing of novel compounds**	
Key Words (max. 5 words)	Mitochondria, heart, pharmokinetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	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)	We have several novel compounds which are designed to either protect the heart against various diseases (e.g. heart attack and heart failure) or which can be used as diagnostic tools in these diseases. The aim of this project is to test these compounds in vivo to generate data for drug registration for human clinical trial and clinical use.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our intention is to bring these compounds forward for human use in patients with heart disease. The information gathered from this project will allow us to see how these drugs react in the heart and also the whole body and what doses and treatment regimes are most likely to be effective.	
What species and approximate numbers of animals do you expect to use over what period of time?	380 mice over a period of up to 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	The severity of the procedures will be mild at most. Most of the procedures are routine procedures used in patients, such as blood sampling, imaging of the heart, exercise testing, of blood pressure measurements. Nevertheless, we will also use genetically altered	

end?	animals bred under a different license which might show moderate signs of their underlying disease (e.g. diabetes). If their clinical signs exceed a moderate limit they will not transferred to this license and not used under this protocol. The specific adverse effects can be seen under the relevant section in the corresponding breeding licenses. It is expected that 60-70 % of animals are entering this protocol will not show any clinical signs and 20- 30 % of animals will show mild to moderate clinical signs. Less than 5 % are expected to exceed moderate severity and will be killed. At the end of the experiments, all animals will be killed but some may undergo blood pressure and cardiac measurements under terminal anaesthesia before they are killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All our novel compounds will be pretested in non-regulated experiments using immortal cell lines, primary cells, and isolated organ models. However information about the distribution of the drugs, effects on the heart, blood pressure and exercise tolerance, cannot be answered using cell-based models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use state-of-the-art imaging techniques, such as positron emission tomography (PET) and magnetic resonance imaging (MRI) which allow us to detect the function of the heart in a non-invasive, very well- tolerated manner over a period of time.
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 the smallest animals that can be used to model cardiac function and to provide meaningful data to support licensing of compounds for human use. All experiments will be undertaken by well- trained staff with the use of modern equipment which is specifically designed to reduce animal suffering to an absolute minimum.

<table>
<tr><td>Project 7</td><td>Ion regulation and cell signalling in cardiac and skeletal muscle</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Exercise, heart, heart failure, skeletal muscle, sodium</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Hearts grow bigger when stressed. This process is called hypertrophy. In disease this process is detrimental and can lead to heart failure while, in athletic training, hypertrophy is normal and benign. Patients with heart failure also show increased skeletal muscle fatigue. This project aims to compare hypertrophy in diseased (failing) hearts and hearts of exercised mice and rats. We will also examine skeletal muscle from these two states. The hypothesis is that disease-induced hypertrophy will lead to defects in the control of sodium movements into and out of both heart and skeletal muscle cells and these changes will be absent in exercise-induced hypertrophy.</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 objective is to learn more about the disease process and to develop new ways to treat heart failure and skeletal muscle fatigue. We propose to test new drugs designed to treat both heart failure and the skeletal muscle fatigue that so often accompanies the later stages of heart failure.</td></tr>
<tr><td>What species and approximate numbers of</td><td>We propose to use rats (<1,000) and mice (<4,000)</td></tr>
</table>

animals do you expect to use over what period of time?	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?	Under general anaesthetic with recovery, animals will be subjected to a variety of techniques to simulate heart failure. They will be allowed to recover and then compared to 'athletic' animals that have been trained to run or swim predetermined distances or times. In this way we can compare disease-induced heart growth with exercise-induced heart growth. While the exercise is not severe, the induction of heart failure is and these animals will show all the signs and symptoms of human patients with heart failure – ie breathlessness, fatigue, listlessness etc. During the study, animals may be imaged using techniques such as echocardiography or MRI (similar to those used in people). At the end of the experiment all animals will be humanely killed and their tissue taken for further study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Heart failure is a complex disease involving the whole body. Hormones, the central nervous system, the kidneys, etc all actively influence the disease progression. Athletic training also alters the whole-body – it changes our circulation, metabolism, fat, blood composition, insulin secretion, nervous system controls, hormones etc. It is impossible to simulate these complex scenarios in cells in culture or in computer models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use sophisticated monitoring and serial non-invasive imaging systems to maximise the data that is generated from a single animal. Studies that 25 years ago would have taken tens of animals can now be done in a single animal. All experiments are designed in advanced using statistical power analysis to ensure that the minimum number of animals will be used that is compatible with proving our scientific hypotheses.
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	Many years ago such experiments used larger animals (often dogs). Recently, however, miniaturisation has allowed us to use smaller animals in particular mice and rats. The mouse has become the preferred animal of choice for many studies as it is cost effective to house in large numbers, it has a short gestation period and, in captivity, breeds readily and repeatedly just as it does in the wild. This makes

measures you will take to minimise welfare costs (harms) to the animals.	it the species of choice for manipulating its genome (that is adding, deleting or mutating its genes) to test the role of specific genes or proteins. Mice also love to run! So they are a good species in which to study exercise physiology. Given a running wheel, a mouse will voluntarily run for 4-8 km/night – our record is 17 km in one night – the equivalent of a human running to Australia and back! Running wheels and environmental enrichment enhance the environment for the animals. We are refining the swimming protocols so that the animals acclimatise to the procedure and stress is kept to a minimum. All surgical experiments are done using anaesthesia and analgesia (pain killers) as would be provided to humans and post-operative care will be designed to minimise stress and suffering. Any animal found to be suffering outside the expected limits of this application will be humanely killed.

Project 8	**Protection after an acute heart attack or stroke**	
Key Words (max. 5 words)	Heart, heart attack, stroke, ischaemia, reperfusion injury	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The present project will try to understand how heart tissue can be saved in the event of an acute heart attack or stroke. A heart attack or stroke occurs by the occlusion of an artery which supplies either the heart muscle or the brain with oxygen and nutrients. If such an occlusion occurs, it inevitably leads to death of the dependent tissue which can lead to severe long-term disability and increased mortality. In order to develop ways to salvage heart or brain tissue in the event of an acute heart attack or stroke, the mechanisms leading to such a detrimental event need to be mimicked in a suitable model.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our intention is to bring novel protective drugs forward for human use in patients with heart disease and stroke. The information gathered from this project will allow us to see how these drugs react in the heart or brain and what doses and treatment regimes are most likely to be most effective.	

What species and approximate numbers of animals do you expect to use over what period of time?	3900 mice and 800 rats over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The severity category of the breeding of genetically modified animals will be moderate. The adverse effects of the various genetically modifications are due to the development of diseases, such as diabetes, high blood cholesterol or obesity, and include lethargy and weight loss or gain. All surgical procedures under this license will be nonrecovery which means, performed under terminal anaesthesia. All animals will be killed at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will first test these protective interventions in non-regulated experiments using immortal cell lines, primary cells, and isolated organ models. Heart and brain function is a highly regulated mechanism and many processes cannot be studied in isolated cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Wherever possible, we will use non-invasive technology to assess heart and brain function such as magnetic resonance imaging, positron emission tomography or hyperpolarised carbon 13 scanning. Furthermore, we will try to assess as many parameters in a single animal as possible and reduce the numbers in one treatment group to an absolute minimum that the statistical analysis will allow us.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats are the most useful species from which it is possible to obtain relevant and meaningful physiological and pathophysiological information. Especially non-invasive imaging such as MRI and PET cannot be performed in smaller species. Furthermore, mice allow genetic manipulations in order to more specifically study the underlying mechanisms. Animal suffering will be minimised through the use of analgesics and anaesthesia when appropriate.

<table>
<tr><td>Project 9</td><td>Regulation/role of the ERK1/2 cascade in the heart</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart failure, cancer therapies, protein kinases, hypertension, hypertrophy</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>Cancer and heart diseases are leading causes of death and illness. Many cancer treatments target enzymes required for the heart to function properly. One group of enzymes forms the ERK1/2 pathway which protects the heart from damage and is required for the contractile heart cells to grow. This is necessary for the heart to increase its ability to pump blood around the body in "healthy" situations (e.g. regular exercise or pregnancy) or in pathological conditions (e.g. high blood pressure). However, the ERK1/2 pathway is important in cancer, and drugs targeting these enzymes are being used as cancer therapies. We aim to determine how the ERK1/2 pathway is regulated in the heart and establish how it elicits its effects. A second goal is to determine how cancer drugs that inhibit the pathway affect healthy hearts or hearts that are dysfunctional as a result of high blood pressure.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science</td><td>The study will increase our understanding of the regulation/role of the ERK1/2 pathway in the heart. This will enable us to identify ways in which the</td></tr>
</table>

could be advanced or humans or animals could benefit from the project)?	pathway may be manipulated to protect the heart (e.g. in patients with high blood pressure) or promote "healthy" growth (as in exercise or pregnancy) rather than pathological growth that leads to heart failure. The study will also aid in understanding whether/how cancer drugs that inhibit the ERK1/2 pathway cause cardiac dysfunction. This may help target cancer therapies to avoid cardiac problems, and may identify patient populations who should or should not be treated with these drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 1200 rats and 550 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?	Approximately 50% of the animals will be subjected only to terminal anaesthesia with removal of the heart immediately prior to death (i.e. non-recovery). In all *in vivo* studies, we aim to study early stages of heart failure as heart function becomes impaired and before overt clinical signs of the disease develop. This will be achieved using echocardiography to monitor the hearts of live animals (as in patients) over time. Pilot studies will be conducted to determine the earliest end-point at which we can reliably detect functional changes in the heart. Thus, although the predicted level of severity for protocols 2-4 is moderate, the actual level of severity should be restricted to mild in most cases. The rat model of high blood pressure develops an enlarged heart over ~6-8 weeks and heart failure over 11-12 weeks. The experiments will be conducted for a maximum of 8 weeks to permit study of early stages of heart failure, but restricting the level of severity as far as possible to mild. We will use genetically-modified rodents that have the potential for heart problems and heart failure to develop, particularly over prolonged periods (several months). We will use the latest technology to ensure, as far as possible, that the highest level of severity is likely to be mild. However, unexpected cardiac complications could still develop and the level of severity may become moderate. Animals will be exposed to drugs that induce

	changes in the heart (and are used to treat heart failure in humans), and/or drugs that are used to treat cancer in humans. To minimise adverse effects, concentrations of drugs will be based on previous work in rats/mice or use amounts that do not exceed those that are appropriate for humans. The duration of drug delivery will be minimised to restrict the severity to mild, as far as possible. Animals that show signs of developing moderate heart failure or distress will be killed immediately. At the end of the experiments all animals will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 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 cardiomyocyte experiments, 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, if they occur, at an appropriate level of statistical significance. *In vivo* studies will require pilot studies to determine the numbers of animals required. These will be informed by published data where possible. Otherwise, they will be informed by data from cultured cells and *ex vivo* perfused hearts. The pilot studies will be initiated with small numbers of animals. Power calculations will be performed using the pilot data to determine the minimum numbers to detect meaningful differences in responses, if they occur, at an appropriate level of statistical significance.
3. Refinement	We will use rodents. Where possible, we will use rat

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.	models which have been used widely for studies of the heart and which represent the most suitable model for studies of, for example, hypertension. We have worked with rats for over 20 years and have a large body of data on ERK1/2 signalling in this species. For studies of hypertension, we will use pre-existing models with their appropriate controls since these have been well characterized over ~50 years. For genetically-modified animals, we will use pre-existing mouse or rat models where possible. Where possible, the modification will be targetted to the cardiomyocytes for postnatal expression and we will use a drug-inducible system. Using these approaches, we can determine the effects of the ERK1/2 pathway and/or inhibition of the pathway in properly developed cardiomyocytes, thus avoiding confounding effects of, for example, kidney dysfunction or developmental defects. Echocardiography will be used to monitor cardiac function longitudinally in individual animals throughout the course of *in vivo* experiments. This reduces the number of animals required, brings end-point of the study forward (restricting heart failure development), and improves the quality of the data.

<table>
<tr><td>Project 10</td><td colspan="3">Role of the cytoskeleton in cardiovascular diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cytoskeleton, platelet, thrombosis</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>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">Abnormal platelet function causes thrombosis, a pathological process that underlies cardiovascular diseases (CVD) such as heart attacks and stroke. CVD) constitute the leading cause of death worldwide. In the UK CVD causes more than a quarter of all deaths, accounting for more than 161,000 people each year.CVD inflicts a significant health and financial burden on the UK in terms of premature death, lost productivity, hospital treatment and prescriptions that is estimated at £19 billion.

Our aim is to understand the roles of specific proteins in the regulation of the function of blood platelets, and the implications for haemostasis and thrombosis. The proteins of relevance include cytoskeletal components (actin associated proteins) and signalling proteins implicated in the regulation of actin dynamics</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or</td><td colspan="3">This project will help us identify the specific roles of the proteins that regulate platelet function and allow us to evaluate their potential as targets for the development of new antiplatelet drugs that</td></tr>
</table>

humans or animals could benefit from the project)?	could reduce/prevent heart attacks and strokes.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 4000
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?	Studies of bleeding time following a small wound to the tail will be carried out under general anaesthesia from which the animal will not be allowed to recover. Some animals will be humanely killed so that their platelets can be assayed in laboratory tests. No adverse effects are expected when breeding the transgenic lines therefore a mild severity limit is anticipated. The animals will be humanely killed using an approved schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-sentient animals cannot be used for our studies since they lack recognisable platelets. Platelets are not amenable to genetic manipulation because they lack a nucleus. Transfection studies are therefore not possible. For the same reason platelets cannot be cultivated and *in vitro* tissue culture models for the generation of platelets from primary megakaryocytes or cell lines have not been established yet.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations have been used to ascertain the minimal number of animals required to use in a single experiment based on previous studies in this area. Where required, additional expert statistical advice will be sought to assist with experimental design.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives.	Mice are the lowest vertebrate group amenable to genetic modifications on which well characterised models for the study of platelet function are established. We will use tissue biopsy by ear punching as it is considered the least stressful and painful

Explain the general measures you will take to minimise welfare costs (harms) to the animals.	procedure. Procedures that require living animals will be conducted under general anaesthesia.

Project 11	**Novel treatments for right heart failure**	
Key Words (max. 5 words)	Pulmonary artery hypertension, right heart failure, exercise, β-blockers, phosphodiesterase 5 inhibitors,	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Pulmonary artery hypertension (PAH) has no cure and new treatments are needed. Constriction of the pulmonary blood vessels leads to increased pressure that causes the right ventricle to fail. We wish to test the beneficial potential of novel treatments for PAH, these are, voluntary exercise and the simultaneous dual targeting of blood vessels and the right ventricle with existing drugs.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	To provide animal data that supports the use of novel treatment paradigms for right heart failure in PAH. Proposed treatments are cheap (exercise) and/or already used in other contexts (phosphodiesterase 5 inhibitors and β-blockers) increasing their translational potential.	
What species and approximate numbers of animals do you expect to use over what period of time?	Rats ,706, 5 years	
In the context of what you propose to do to the animals, what are the expected adverse	Rats in heart failure groups are given a single injection of a chemical that promotes PAH and right heart failure within 3-4 weeks. These animals	

effects and the likely/expected level of severity? What will happen to the animals at the end?	are killed when they show external signs of heart failure. Control animals are given a single injection of saline. In each group some animals will be given free access to a running wheel and others treated with non-harmful pre-existing drugs to try and prevent the development of heart failure. The level of severity will vary from mild (animals given a single injection of saline) to potentially severe, though usually moderate (heart failure animals with ECG implants). All animals are killed at the designated experimental end point.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are interested in understanding changes in the electrical and mechanical remodelling of the heart in heart failure. There are no none-animal alternatives for whole hearts or single adult cardiac myocytes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are designed so that one animal/heart can be used in multiple types of experiment. Our animal data is being used to generate a computer model of the disease which in the future will reduce and replace some animal 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.	We have several years of experience using the rat model we have chosen. It is a rapid (5 weeks max.) non-surgical model induced by a single injection. We successfully use small (designed for mice) ECG implants in rats to reduce their impact. We are promoting the use of voluntary exercise regimes rather than stressful enforced regimes. Our designated end points minimise suffering by removing animals when heart failure signs appear rather than waiting for these signs to progress to severe heart failure.

<table>
<tr><td>Project 12</td><td colspan="2">Functional characterisation of novel blood genes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Zebrafish, blood, stem cells, cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>x</td><td>Basic research</td></tr>
<tr><td></td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td></td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">The number and characteristisation of blood cells are frequently used in clinical practice and deviations outside normal ranges can be indicative of a wide array of blood pathologies. It is becoming increasingly clear that genetic changes acquired throughout life play an important role in the initiation of blood disorders including blood cancers.

In the last few years, DNA sequencing technologies have been developed that allow the identification of every genetic change in a given cancer sample, promising the discovery of new cancer genes. However, many identified genes were not previously implicated in blood formation and there is a real need to investigate the biology and potential therapeutic aspects of these genes. The proposed project will bridge this knowledge gap by providing a method of using a relevant model organism (zebrafish, Danio rerio) that will allow the determination of the role of the cancer genes which have a hitherto unknown functions, This will be achieved by pursuing two main objectives:

1. First, a high-throughput screen in zebrafish will determine the functional role of novel cancer genes implicated in blood cancers. This effort will define</td></tr>
</table>

	the function of about 50 novel cancer genes in blood cell formation by using zebrafish. 2. This early objective will be followed by a far more ambitious long- term one, which will extend beyond the end of the project. The objective will be based on finding the functions of genes that carry the genetic code for proteins involved in modifying RNA and how changes in the order of bases in genes change their functions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The research I propose here will provide an example of the usefulness of zebrafish for the efficient translation of genome wide association study (GWAS) findings in humans and next generation sequencing technologies into relevant biological information. If successful, this project will be the major first step towards finding the functions of newly identified genes implicated in normal blood formation and blood malignancies in a relevant living model system and will explore how acquired changes in genes change their functions. The identification of genetic changes will allow us to further describe some of the function of each gene we study. Ultimately this basic functional information will be used to understand how the affected genes control blood formation and how they contribute to disease processes. Additionally, we will model human diseases in zebrafish. These fish models of human disease can be used to better understand the disease process, to identify other components of the process and be used as an assay for potential therapeutic agents.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use zebrafish and we expect to use approximately 12,000 fish/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 majority of fish will experience no adverse effects, some fish are likely to suffer mild transient adverse effects and only a minority of fish are likely to suffer moderate adverse effect. For example some signs of stress are failure to swim upright, failure to feed and thrive or persistent swimming at. either the surface or bottom of the tank. Distressed or unhealthy animals will be humanely culled immediately.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to understand how the interaction of different cell types in an intact animal control developmental decisions. As we are intending to understand the function of genes, it is essential to have every cell-type of the whole animal present to detect this.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals kept under regulated procedures we will maintain the fewest number necessary to carry out a particular experiment, we will also preserve lines that are not currently being used by sperm freezing, thus only maintaining actively used animals in the facility and giving us the ability to share animal models with other researchers around the world, We will also re-use fish where permissible. Finally, we will use appropriate statistical methods to design experiments which use the minimum 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.	Zebrafish studies in the past two decades have made major contributions to our understanding of how blood cells are made and associated disorders. The zebrafish has proven to be a valuable organism for studies in this area owing to its amenability to large-scale genetic and chemical screening. In addition, the externally fertilized and transparent embryos allow convenient genetic manipulation and live imaging of normal and altered blood cell production in the body. The wellbeing of the animals is paramount and we will provide optimal husbandry, housing and breeding conditions to satisfy the needs of the zebrafish.

<table>
<tr><td>Project 13</td><td colspan="2">Inflammation and Cardiovascular Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Blood vessel, thrombosis, aspirin, inflammation, heart attack</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td>X</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>X</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">We want understand the link between inflammation and cardiovascular disease, This is because cardiovascular disease remains the commonest cause of death in the world and because regular anti-inflammatory drugs like ibuprofen are used all over the world to treat inflammation but have serious cardiovascular side effects including causing heart attacks and strokes. This is made worse by the fact that people with chronic inflammatory diseases, like arthritis have a higher risk of cardiovascular disease than the general population. We know that cardiovascular disease and its link with

inflammation involves hormones made in blood vessels and small cells in the blood called platelets. However, we don’t know enough about how inflammation contributes to cardiovascular disease and we have no way of knowing which people will have heart attacks or strokes whilst taking drugs like ibuprofen or why in some people blood thinning drugs don’t work to protect them. We do know that people with arthritis, infections, who smoke or who have high blood pressure are more likely to have heart attacks or strokes. This is because of inflammation in their blood vessels</td></tr>
</table>

	together with, in some people, problems in their kidneys. Our project will look closely at these problems to work out simple tests to be able to find people most at risk and to work out how to treat them. Our work will also identify how better. to use existing drugs as well as ways of making new more effective drugs with fewer side effects.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work has the potential to impact on the health of millions of people in the UK and all over the world. As people are living longer there is more arthritis and the use of anti-inflammatory drugs like ibuprofen or blood thinning drugs like aspirin and clopidogrel are increasingly important. These drugs are useful but have serious limitations and don't work in everyone. The worry about anti-inflammatory drugs causing heart attacks is also stopping new drugs in this class being made. New work shows that anti-inflammatory drugs like ibuprofen are good at preventing cancer, but because of the side effects in the cardiovascular system they cannot be used in people until we work out how this happens. The benefits of this project are that we will be able to make simple tests that will find those people that are mere at risk of heart attacks and treat them with other drugs. Using these tests we will also be able to find out when and why blood-thinning drugs stop working in some people and have new ideas of how to treat them too. This will mean that reassurance can be given for those majority of people who can take commonly used drugs and that drug companies will have the confidence and scientific information to make new and better drugs to treat inflammation and cardiovascular disease.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mainly mice and rats. Numbers will be kept to a minimum and in general be less than 1000 animals per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For most of our experiments animals will be killed humanely, with no procedures performed, and samples of their organs and blood taken for study. This means that for a large majority of our work there will be minimal suffering to animals. Where experiments are to be performed on the animals they will, where possible, be fully anesthetised and killed at the end of the experiment, suffering no pain. We have worked hard to design the best

	possible experiments but using procedures with mild or moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible we will use cells in culture as well as experiments with computers where mechanisms of disease can be simulated. However, because we know from our work that for some aspects of inflammation and cardiovascular disease the mechanisms involve processes in different organs and that one organ (like the kidney) releases hormones that cause inflammation in other organs (like the heart), for some of our questions we will need to use whole animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have planned our experiments very carefully and will work as much as possible without using whole animals. Where we do need to use animals we will use both male and female animals, this reduces the number that we need to breed. We will also take tissue and cells from the animals, which means that we can answer more than one question using the same animal.
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.	Most of our work will be conducted in mice because mice are the commonest species for genetic modification and refined models of inflammation and cardiovascular disease have been established in them. In all experiments we will look to use non-invasive measurements to gather data, minimise the duration of the experiment and level of model disease severity. Where possible, terminal procedures will be used to prevent any post-operative suffering and complications under appropriate general anaesthesia.

Project 14	**The long-term effects of prenatal hypoxia on cardiomyocyte function**	
Key Words (max. 5 words)	Programming, pre-natal hypoxia, cardiac, mitochondria, myocyte	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall objective is to assess the long-term effects of low oxygen levels (hypoxia) during development on mouse heart function. This goal will be realized by addressing the following specific objectives in fetal, juvenile and adult mice previously exposed to hypoxia during development: 1. To measure heart cell contractile force and calcium regulation 2. To assess mitochondrial function in heart cells 3. To characterize gene expression and modification of key proteins involved in heart cell function	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The main benefit of the study is the advancement of current understanding of the cellular and molecular mechanisms underlying the developmental origin of cardiovascular disease. We hope to identify cellular targets for drug intervention to protect people from developing cardiovascular diseases later in life. All of the findings will be published in peer reviewed leading scientific and clinical journals as appropriate to ensure wide dissemination of the research	

	findings. The information is of direct benefit to basic scientists, physiologists and clinical cardiologists and will provide key information enabling better management of cardiovascular disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Wild type mice, approximately 850 animals over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	**Adverse effects:** **1**. Maternal reduced food intake, activity and weight: • Severity band, mild • Dams exposed to hypoxic environments are known to exhibit a decrease in food intake (up to 40%) and a substantial decrease in physical activity, leading to a decrease (~20%) in maternal body weight. 2. Maternal preeclampsia-like symptoms: • Severity band, moderate Hypoxia during pregnancy can cause maternal preeclampsia-like symptoms such as hypertension, proteinuria and kidney pathology. 3. Intrauterine growth retardation (IUGR) and physiological and morphological defects associated with prenatal hypoxia • Severity band: Moderate. • Prenatal hypoxia causes IUGR and a host of physiological and morphological defects, some of which persist into adulthood. 4. Disease susceptibility in offspring. • Severity band: Moderate. • Although we are not specifically inducing this, it is possible that offspring exposed to prenatal hypoxia will experience disease susceptibility in association with aging (i.e. cardiovascular diseases, such as heart failure) later in life. All animals will be sacrificed according to Schedule

	1 at the end of the protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	*Cell lines and culture:* Adult cardiac myocytes are terminally differentiated and cannot be maintained in tissue culture conditions. There are no suitable cell lines that can be used to fill these purposes. Moreover, we will be studying the long-term effects of prenatal hypoxia on cardiomyocyte function, which cannot be reproduced using cell culture techniques nor can they be suitably modelled using computer simulations given the lack of understanding of the fundamental processes. *Human volunteers* Human tissue is; i) of limited availability, ii) rarely not already diseased and iii) nearly always subject to pharmacological interventions. *Alternative species* Since we wish our findings to be clinically relevant and translational to human diseases of the heart, the use of other less sentient species, such as lower vertebrates (reptiles, fish and amphibians), is not appropriate as the structure and function of lower vertebrate hearts differ significantly from mammalian hearts and mammalian hearts are known to be significantly more sensitive to hypoxia than lower vertebrates.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental design has been discussed with, and approved by, our statistical advisor. In order to minimise the number of animals required, sample size has been estimated for each experiment based on existing published data and the use of power analysis (desired power of 0.8, $\alpha = 0.05$). These estimates will be updated and recalculated throughout the project as we generate new data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	We are committed to using the most translationally relevant model. We have chosen the mouse as our experimental species for several important reasons: 1) Mice have a short generation time and an accelerated lifespan (2 years) which allows the long-term effects of prenatal hypoxia to be studied within a reasonable timeframe.

(harms) to the animals.	2) Our ability to directly manipulate the mouse genome provides an incredibly powerful tool to identify and confirm molecular targets for drug intervention. 3) Due to their small size and short generation time, maintaining mice requires less resources and space, and the time required to perform research is manageable. 4) The mouse has large litter sizes which allows the generation of multiple, identically reared progeny. **Steps to minimise welfare costs to animals:** 1) Basic requirements for good rodent housing and husbandry will used at all times. 2) It is not possible to house pregnant mice in groups, but once pups have been weaned, mice will be housed in stable, compatible groups. 3) The following parameters will be measured during the protocol to ensure animals remain within the outlined severity limits: Body weight, body condition scoring (BCS), food and water intake and cardiovascular status. Control animals not subjected to any procedures will be used as a benchmark for normal changes in these parameters. 4) Oxygen levels will never be reduced lower than 9% 5) When animals are first put into the chamber, oxygen levels will be normal (21%) for 24 hours and then reduced slowly (over another 24 hour period) to avoid shock.

<table>
<tr><td>Project 15</td><td>Developmental programming by maternal diet</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Maternal obesity, pregnancy, metabolic syndrome, diet, developmental programming</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</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 programme of work described in this project licence, is focused on study of the influence of maternal obesity in pregnancy on the developing offspring, notably the persistent effects on the heart and blood pressure (cardiovascular), and the development of obesity and diabetes in the next generation and beyond. The programme addresses the developmental origins of disease and has implications for the current epidemic of obesity and related disorders, through the ‘transgenerational acceleration of obesity’ from mother to baby. The programme also addresses the long-term implications of in utero growth restriction, in which babies are born small with increased risk of cardiovascular disease, including diseases of the kidney, and a reduced life expectancy.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This field has generated huge interest in the gene-environment interactions which give rise to altered disease risk profiles and is increasingly providing novel and valuable information on the initiation and progression of cardiovascular diseases, which account for the majority of mortality and morbidity Worldwide. A greater understanding of the aberrant</td></tr>
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	developmental processes in early life that give rise to subsequent cardiovascular disease provides an opportunity for early intervention in individuals at risk and will inform public health strategies to stem the growing tide of obesity and cardiovascular disease.
What species and approximate numbers of animals do you expect to use over what period of time?	The programme employs rats and mice including genetically altered mice, which will be fed high fat and sugar diets typical of western diets to study the effects of obesity in pregnancy. Approximately 5000 rats and mice will be used over the course of the 5 year project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Dietary manipulations give rise to animals that are obese in pregnancy, and offspring which are themselves at greater risk of obesity, diabetes and high blood pressure. The expected level of severity associated with dietary studies is therefore mild. Some procedures require the surgical implantation of probes or drug delivery systems for the measurement and treatment of high blood pressure for example, for which there are moderate risks associated with anaesthesia and surgery and similarly with occasional blood sampling. Adverse reaction to the administration of drugs used, clinically for the treatment of diabetes or blood pressure is extremely rare as these drugs are well described in rodents. All animals will be killed humanely 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	Non-sentient alternatives shall be used wherever possible to avoid the use of live animals. However, the developmental processes involved in pregnancy and the plasticity of the developing fetus towards environmental influences are currently too complex to model accurately without recourse to the study of experimental animals employing a life course approach to investigate the physiological systems and pathological processes involved in the early origins of cardiovascular disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Minimum numbers of animals is ensured by efficient experimental design. Animal numbers are calculated for the minimum number required to provide sufficient statistical ‘power’ to detect a biologically significant effect. Moreover, good laboratory practice should prevent, or at least minimise, the introduction of variability and bias into the experiments minimising

	the numbers 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.	As mammals, rodents show close homology with human physiology and their relatively quick life cycle allows investigation of the consequences of maternal nutritional and hormonal influence on the subsequent adult offspring characteristics within a meaningful timeframe. Although the basic cardiovascular biology and cerebral architecture is comparable, there are obviously species differences for example in stage of development at birth. Nevertheless, rodents are a suitable model species for this exploratory pre-clinical investigation. There is no reason that experimental animals should experience undue pain and suffering as a result of any of the procedures in this licence pain and suffering will kept to an absolute minimum through good laboratory practice and there will be appropriate use of anaesthetic and analgesic drug regimen.

Project 16	**Imaging of Cardiovascular Diseases with Novel Agents and Devices**	
Key Words (max. 5 words)	Imaging, cardiovascular disease, diagnosis, therapy.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Heart and circulatory diseases remain the most common cause of death in the UK and are responsible for approximately 240,000 deaths per year. Coronary heart disease is the most common single cause of death affecting about 117,000 people per year. Heart attacks frequently occur without preceding clinical symptoms but suddenly can lead to life threatening complications and approximately half of patients affected by a heart attack die immediately often before reaching hospital. The purpose of this project is to develop experimental animal models of cardiovascular disease to be used in proof-of-concept and validation studies of novel imaging methods, contrast agents and devices for earlier and more accurate diagnosis of heart disease, to better guide interventional treatment and to monitor how patients respond to therapy. If heart disease can be detected earlier with more understanding of the underlying pathology, this may lead to better medical and interventional treatment of patients and eventually to a reduction in the disabling effects of heart disease and improved survival.	
What are the potential benefits likely to derive from this	The project outlined will allow us, with the help of disease models, to develop novel imaging	

project (how science could be advanced or humans or animals could benefit from the project)?	techniques and devices for image guided intervention that will help to better diagnose and quantify the severity of atherosclerosis (plaque build up in arteries) and myocardial ischaemia (lack of oxygen to heart muscle due to blockage of blood vessels) and to improve current interventional procedures for the treatment of cardiac arrhythmias, congenital heart disease and heart failure. It thereby may allow us to more efficiently treat patients with coronary heart disease and help to assess how patients respond to treatment. This knowledge will help us in identifying patients that are at high risk of a heart attack and provide the appropriate treatment to patients based on more quantitative and objective measures. Ultimately this study aims to minimise the number of heart attacks by early detection and interventional therapies and improve the efficacy of interventional treatments to cure atria(fibrillation (irregular heart bear) and heart failure.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (9750), rats (3800) and rabbits (700) will be used over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No adverse events are expected to be caused by the imaging methods used. Any adverse events expected are related to induction and maintenance of anaesthesia (animals may die from respiratory depression <1 -2 % and/or hypothermia). Mild discomfort may ensue from injection of substances (however, where possible, this is generally done under anaesthesia) or withholding of food prior to imaging (as done in the clinic) but efforts are being made to optimise anaesthesia, administration of substances and avoid unexpected adverse effects &/or deaths. Adverse events may occur related to surgery designed to induce a particular aspect of cardiovascular disease e.g. blockage of vessel in heart to induce heart attack but these can be minimised with good aseptic surgical techniques, good monitoring measures, and painkillers. Symptoms of disease which develop over time in genetically altered animals or due to disease induction by chemical or pharmaceutical intervention, may occur but again careful monitoring will minimise as much as possible, any pain and suffering and distress. All animals will be humanely killed at the end of the experiments.

	However, if at any point during the studies the animals reach a predetermined end-point at which pain, suffering and distress can be avoided or minimised, then these animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals have to be used because 1. Data generated from this body of work may be used to inform whether to go forward to human clinical 2. To validate mode of action of novel compounds, experiments are required which cannot be conducted in humans for ethical and scientific reasons. 3. Bio-distribution in whole organisms (i.e. tracking the injected agents route! accumulation and excretion through the body), with intact biological barriers and excretion mechanisms, is key to clinical use. Non-animal alternatives cannot replace the complexities of the interactions of these probes in whole body systems or with realistic models of cardiovascular disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary *in vitro* screening (i.e. cells or tissues) will eliminate unsuitable candidates which will not progress to *in vivo* studies, thus reducing the numbers of animals. The use of imaging to determine bio-distribution of novel contrast agents rather than conventional killing at sequential time points, with removal of tissues for analysis is a major contributor to reduction of numbers. Imaging allows repeated observations/measurements over a period of time (longitudinal study) on the same animal, with humane killing only at the last time-point. Thus, if a longitudinal study involves six time-points, the numbers of animals are reduced to one sixth by use of repeated imaging. Since each animal serves as its own control to compare different time-points, the data obtained are statistically more robust (reduction), requiring fewer animals. Moreover, distribution of contrast agent within organs, not just between organs, is obtained, and unexpected uptakes that may not be detected by conventional methods can be found by whole body scanning. All these attributes of imaging contribute to a greatly improved benefit: cost ratio (benefit = data quality and quantity, cost = animal numbers, procedures and their severity).

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.	Species: Mice, rats and rabbits are the species of lowest neurophysiological sensitivity that provide the necessary size compatible with the scale of resolution or movement associated with the techniques being studied. Resolution of the whole body imaging techniques is of the order of 0.5 - 1 mm. Distribution within smaller animals will be beyond these limits. Pilot studies are small experimental groups which help us to decide quickly how best to design a statistically and scientifically valid experiment. Thereby helping develop better larger study design and reduce possible suffering. Generally, inhalation anaesthesia will be used to minimise any transient pain or distress and where possible, used for blood sampling, contrast injection, weighing and combined with imaging techniques where it is mainly used for restraint. In addition, there would be full and complete recovery between periods of anaesthesia and/or food withdrawal; rehydrating of animals during long imaging sessions; monitoring of respiration and/or cardiac function and maintaining body temperature during imaging. These steps will all be conducive to the animal's wellbeing. However, induction or development of cardiovascular disease will inherently make an animal more susceptible to pain and suffering and therefore careful monitoring of animal wellbeing, pain relief and analgesia will be used when required, together with veterinarian advice, to minimise any pain and suffering.

Project 17	**Autonomic control of cardiac function and rhythm**	
Key Words (max. 5 words)	Cardiac function, arrhythmia, autonomic control	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cardiac diseases such as when the heart does not pump very well (chronic heart failure; HF) and following heart attacks (myocardial infarction; MI) have been shown to be associated with abnormal control by nerves that supply the heart. Patients with HF and MI frequently die from an irregular heart rhythm called ventricular fibrillation (VF) that leads to sudden cardiac death (SCD). SCD affects over 50,000 people per year in the UK but medical treatments to prevent this are poor. We know that SCD is linked to the nerves that supply the heart but we do not understand the mechanisms enough to develop good treatments. The main objective of our PPL is to understand how autonomic nerves and important molecules that are released in the heart work in health and in disease. Hearts from normal animals will be used and compared to hearts from animals given a heart attack, which is essential to produce an animal model of heart failure that is of direct relevance to the human condition. The effects of nerve stimulation on the heart will be studied to understand how nerves affect these disturbances in heart rhythm and function. Important molecules released from nerves will be studied which may	

	help develop new treatments. In addition, data from our work will be used to improve current mathematical models that may eventually help replace and / or reduce the need for animals in this type of research.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This licence is essential to advance the understanding of why sudden cardiac death occurs and the role that the nervous system plays. The experiments are designed to identify key signalling pathways that will further scientific knowledge, which we hope will one day improve current therapies and develop new ones. A new emphasis of this PPL is that data collected will be combined with mathematical modelling to improve and advance what is currently available which will incorporate up to date information on structure and physiology of nerves which plug into the heart.
What species and approximate numbers of animals do you expect to use over what period of time?	5 years, approximately 1000 Rabbits, 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?	For two protocols detailed in this licence that are necessary to remove the heart, we do not anticipate any significant adverse effects, as they are performed under terminal anaesthesia. To develop the heart failure model we perform an open-chest surgical procedure that is rated at moderate severity. Premature death is the most significant adverse effect that we try to keep to a minimum through sound aseptic surgical techniques and rigorous post-op monitoring with rapid reactive treatments if symptoms develop. Rabbits do not commonly display symptoms of heart failure like humans, but the most common adverse harm is pain, which is controlled with good pain medication. At the end of procedure, animals are anaesthetised without recovery and following confirmation that the animal is dead; the heart is removed for use in the laboratory for scientific study.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is impossible to investigate modulation of ventricular fibrillation in humans due to its lethal nature. Hence animal models are essential for scientific advancement relating to understanding the mechanisms involved. The use of whole animals and *in vivo* experiments are not ideal for studying pure effects from direct nerve stimulation on the heart due to substances in the blood and brain mediated reflexes that are difficult to control. Mathematical modelling is imperfect and still being developed to a point that would be acceptable to replace procedures requiring animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental variability will be kept to minimum to ensure the lowest numbers of animals are used. Staff well trained in surgical skills and experimental procedures will also follow good laboratory practice. Experiments will be well designed and study plans followed with rigorous experimental data appraisal to ensure quality and low failure rates.
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.	Rabbits are preferred as the structure and physiology of the heart is more similar to humans, than rats or mice. We use guinea pigs in some protocols where new drugs that affect the heart and in early stages of development have data in this species, where data in the rabbit is not yet available. Hearts are used and removed from the animals for study because things that circulate in the blood and autonomic reflexes are difficult to control. The heart failure model is only performed on adult animals that are easier to operate on, by well trained individuals with robust monitoring and rapidly responding treatments if symptoms develop.

Project 18	**Regulation of thrombus formation**	
Key Words (max. 5 words)	clotting factors, thrombus formation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project are to find ways in which formation of blood clots (thrombus formation) can be manipulated in order to help develop new drugs for the treatment of diseases in which blood clots have a part to play, e.g. stroke, heart attack. Although advances in the treatment of clot formation have improved greatly, there are still many areas of its involvement in life threatening response that are unknown.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will give us greater understanding of how blood clots are formed and what cells and molecules are involved. Improving our understanding and treatment of these diseases including one of the most common causes of death, heart disease, will enable much better drugs to be developed in the future. The ultimate aim is to help develop new treatments for patients suffering from fatal diseases in order to help save their lives.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse models designed to copy what happens in human thrombus formation are used in order to investigate the effects of manipulating molecules thought to be involved in these disease processes. We expect to breed approximately 900 mice over 5 years. We also expect over 5 years to use 950 mice in experiments and a further 200 mice for the supply of blood components.	

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?	Mouse blood will be obtained from animals under terminal anaesthesia (i.e. they will not wake up from the beginning to the end of the procedure). Experimental procedures to induce thrombus formation will mostly be carried out also with the animals under terminal anaesthesia; we expect to use approximately 150 mice in experiments where the animals will recover from anaesthesia. Some of the treatments we give may make the animals more prone to bleed more if they get injured so we will monitor them closely and take the necessary precautions to avoid this happening. Very rarely (1 mouse in every hundred) carotid injury may cause mild stroke-like symptoms. This can be recognised by a one-sided weakness. At the end of the experiments mice will be given an anaesthetic overdose.
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A large amount of work will be carried out using cells and tissues in order to give us some idea of the molecules we should be looking at. In this way we will minimise the number of animals used for this programme of work. However, the body is a complex system and looking at cells and tissues outside of this (in vitro/ex vivo) can often give us results that do not reflect what really happens inside the body (in vivo).
2. Reduction Explain how you will assure the use of minimum numbers of animals	If the it-i vitro data leads us to believe that a molecule may be important only then will we advance to investigating what happens when we manipulate the way in which that molecule works.
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 will be used in this programme of work due to the availability of genetically modified mice ‘and inhibitors relevant to the inflammatory process. The amounts of the compounds of interest that we need to use will be checked in vitro/ex vivo before they are used in animals to refine the way in which the animal experiments are carried out. Most of the experimental procedures (approximately 80%) will be carried out under terminal anaesthesia.

Project 19	**Modulation of cardioprotection in pathological states**	
Key Words (max. 5 words)	heart attack, diabetes, age, animal research	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Heart disease is one of the leading causes of death and disability worldwide. Coronary artery disease, the most common type of heart disease, causes blockage of one or more blood vessels supplying blood to the heart muscles leading to heart attack. When this occurs, the heart does not get enough oxygen and a part of the heart muscle wall that is supplied by the affected blood vessel becomes damaged. A large number of cells in the affected area die and since new cells cannot replace the dead cells in the heart, the damaged area will not be able to function normally. This increases the workload on the rest of the heart, which will further worsen the heart function with time leading to what is called heart failure and eventually death. The first obvious step to reduce the extent of injury to the heart is to restore blood flow by opening the blocked vessels using drugs and surgery. However, this sudden return of blood flow (called reperfusion) is known to make the injury worse. A large part of current research is aimed at protecting the heart from injury caused by the return of blood flow. Although different treatment methods are being used, the extent of success has been somewhat variable between patient groups. It is recognised that to achieve better results, we need to understand in greater detail	

	how heart cells die during a heart attack and during restoration of blood flow. The treatments that appear promising will also have to be tested for their long-term effects on heart function, to confirm that the benefits seen in the short term will contribute to improved survival of patients in the long term. A typical patient presenting with heart disease may be aged, obese, and/or have other diseases such as diabetes. Advance age and several diseases that the patients may have can affect the extent of injury to the heart, the way heart responds to treatment and the long term quality of life after surviving the heart attack. In order to develop and improve treatments that are effective in such a varied patient population, there is a need for conducting research on animal models with disease conditions similar to those in humans. Studies by us and others have helped to identify several promising drugs and mechanical methods to protect the heart. This project aims to improve our understanding of the basic process of cell death and protection of heart against such damage by developing and/or testing newer treatments, and to improve available treatment methods with the goal of making them effective in the target patient population.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our work aims to improve current methods/drugs and find new ways of reducing injury in patients who experience a heart attack. Since those who survive a heart attack frequently develop heart failure due to the damage to the heart, improving available treatment methods and developing new techniques will help patients to survive longer and have a better quality of life. A decrease in the occurrence of heart failure following heart attacks would also provide substantial cost benefits to the public health service. Using animal models that have disease conditions similar to humans (e.g., diabetes) we also plan to validate the success of treatment strategies in patients with other diseases. This is particularly important since different diseases can increase the risk of developing heart disease. For example, patients with diabetes mellitus (DM) are 2-4 times more likely to develop cardiovascular disease, which is the main cause for death in ~65% of patients with DM. In 2010/11, diabetes cost NHS about £9.8 billion, of which around £3 billion was spent on heart disease and associated complications. With diabetes projected to become the 7th leading cause of death worldwide by 2030, improving efficacy of treatment/management of heart disease in diabetic

	patients is essential to reduce the healthcare expenses. More generally, the knowledge gained from this research can contribute to the scientific understanding of reperfusion injury which occurs in other organs such as brain (stroke), kidney and liver, and during procedures such as cardiac surgery and transplantation. In the current Project licence application, some of the protocols (for e.g., recovery after cardiac surgery) have the potential to cause severe distress in animals. However, necessary steps will be taken to reduce pain and suffering at all instances. These procedures are essential for our work for the following reasons - damage to the heart occurs within a short time span after the heart attack and needs rapid clinical attention. In addition, there will always be long term consequences of this injury, which may lead to heart failure and reduced function and/or damage to other vital organs. These long-term effects and their resolution by treatments/drugs can only be assessed in a model where heart attack is induced, blood flow is restored and the animal is allowed to recover for a period of time during which it will be studied for how the disease progresses.
What species and approximate numbers of animals do you expect to use over what period of time?	Species: Rats – 5000 to 5500 over 5 years Mice –5000 to 5500 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be used in experiments deemed absolutely necessary, based on initial data from non-animal models. In a few experiments animals may be treated for a defined period of time with drugs which are already being used in patients, or in preclinical/clinical trials. Hence the safety, dose, and the methods of treating animals with these drugs are well-documented. This will help design experiments taking into account possible non-desirable side effects and effective management of the same. Although most of the drugs used are not expected to cause adverse reactions at the concentrations intended, the animals will be closely monitored and necessary steps taken to ensure their wellbeing. The severity limit set for the different procedures ranges from ‘Non-recovery’ to ‘Severe’. All the animals will be observed regularly for food and water intake, general

	features of discomfort (e.g. starey (puffed-up) coat, hunched position, lethargy, reluctance to move, isolation from the group, self-harm, changed nesting behaviour) and weight loss (maximum 20%). If these symptoms develop, the animals will be humanely killed. Experiments using animals in this project involves administration of drugs and monitoring their effects on body tissues. These can be broadly classified into two: - Non recovery – experiments carried out under general anaesthesia; deep anaesthesia will be maintained throughout the duration of experiment followed by a final overdose of anaesthetic to kill the animal without waking it up from the experiment. This method is not expected to cause any suffering as the entire protocol is carried out under deep anaesthesia. - Recovery – experiments carried out under general anaesthesia; deep anaesthesia will be maintained throughout the duration of experiment. Upon completion of the experiment, animals will be allowed to recover from anaesthesia. These animals will be under continuous observation until they become fully mobile and start to feed and drink; which normally happens in the first 3hrs after completion of procedure. In animals undergoing heart surgery, wherein the chest cavity is opened, surgery performed and the wounds closed surgically before recovering the animals from anaesthesia, effective pain relief by medication will be provided to ensure that the animals are not in pain while recovering from the procedure and in the days thereafter. The animals will be killed by humane methods after the experiments and tissues of interest collected for further studies, making maximum possible use of the animals. At all points in time either during maintenance of the animals or during experiments or afterwards all possible steps will be taken so that animals do not suffer unnecessarily. Where the pain/suffering is not transient (occurring within the duration of recovery as a result of the procedure undergone) and cannot be resolved by pain-relief medications, humane methods of killing will be used to end suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why	This project aims to help the heart survive better after a heart attack thereby improving the quality of life in the long term in these patients. Our initial ‘proof of concept’ experiments will be carried out on experimental models

you cannot use non-animal alternatives	of non-animal origin. These models include cell lines - cells available commercially and grown in the laboratory, and also cells and tissue samples from human volunteers. Based on this pilot data, experiments that show promising results will have to be confirmed in more complex biological systems similar to the human heart and whole body before the treatment can be tested in patients. Since these studies cannot be carried out in humans until more information on the safety and effectiveness of the treatment is available, experimental models of animal origin have to be used. Among animal-based work, we initially use the isolated heart and cell models (which are of lower severity), and expand to the whole body and recovery models (higher severity) only if positive results are obtained in the initial animal experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals used we plan to do the following: - Choosing alternative methods that do not rely on use of animals wherever possible - Using pilot data from non-animal studies to assess the need of extending the investigations to animal models - Careful experimental planning to include the minimum required number of animals in experiments - Regular monitoring of the experimental activity in the lab by the project licence holder and experienced senior scientists to ensure high quality of research and optimum use of animals - Use of experimental techniques that will give maximum amount of data from least possible 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.	• Rats and mice will be used in this project as it is easy to measure extent of damage to the heart and experiments can be performed in a controlled manner • Rats and mice with diseases similar to human conditions (for e.g. diabetes) will be used in the project for studying heart disease in a setting similar to human diseases • Planning studies on intact animals on the basis of initial data obtained from experiments on non-animal models will help reduce the number of animals used in the project • Refining experimental skills: in addition to the training undertaken for obtaining personal licence, new members of the research team will be trained,

	supervised and guided by more experienced investigators in all aspects of animal research including designing experiments, handling animals, recognising signs of pain, suffering, lasting harm and distress; and sacrificing animals by humane methods when necessary. • Researchers will be assessed for competency before being allowed to carry out experiments on animals. • At all instances special care will be taken to prevent and reduce animal suffering caused by the experiments. • We will work closely with the Named Veterinary Surgeon (NVS) and the Biological Services to help us refine our procedures and also for advice while planning new procedures.

Project 20	**Investigating normal blood development and blood cancer**	
Key Words (max. 5 words)	haematopoiesis, blood stem cell, leukaemia, microenvironment	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Understanding how blood cells, especially blood stem cells, are formed is vital for producing enough blood cells for transplants and transfusions and for treating blood cancers. A lot can be learned from studying how blood (stem) cells are first generated during foetal development as this reveals the basic mechanism of their emergence, expansion, differentiation and migration. Furthermore, many of these early developmental processes are reactivated during cancer development and therefore offer insights into the origins of cancer and how it may be treated. Yet, little is known about how blood cell formation in the foetus is regulated on a molecular level. Key questions that remain largely unanswered include the precursor cells from which blood cells are derived, the cellular composition of the microenvironment or niche in which this occurs, the intrinsic and extrinsic factors involved and how the subsequent migration to the adult bone marrow is regulated. The aim of our project is therefore to identify new regulators that control blood cell formation and to define the microenvironment in which this occurs.	

	We will also investigate how these processes become corrupted during 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 research is likely to provide new insights into how blood is generated during foetal development. The knowledge gained from these studies will assist in developing protocols for the production of blood (stem) cells in defined culture conditions for clinical applications, such as cell replacement therapies. It will highlight ways for keeping these cells alive and for generating them from more immature cell types, such as induced pluripotent cells. Identification of the cell of origin of cancer and the processes that become dysregulated during disease development will also reveal new targets for drug development. The mouse cancer models developed as part of this study may also allow potential drugs to be tested directly.
What species and approximate numbers of animals do you expect to use over what period of time?	The laboratory mouse is the animal of choice for these studies. The similarities between the mouse and the human blood system make the mouse an ideal model. Over the course of this project, which is expected to last 5 years, we will use a maximum number of 33,200 animals.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the generation of new gene-manipulated mouse lines, female mice will be implanted with embryos, a surgical procedure of moderate severity that will be performed under anaesthetic and from which the animals are expected to make a full recovery. The different mouse lines will be bred and maintained over the course of the project, with the majority experiencing no or mild adverse effects. In some rare cases, the gene manipulation may result in the animal becoming unwell including development of leukaemia The mice will be monitored for any signs of ill-health, in which case a veterinary surgeon will be consulted and the animal euthanised should the severity reach moderate levels. To test the ability of a blood stem cell to regenerate the haematopoietic system, mice will have to be irradiated and transplanted with blood-forming cells. In some rare cases, mice may suffer adverse effects as a result of the irradiation; however, these will rarely reach moderate levels at which point the mice will be

	euthanised. The mice may also experience adverse effects as a result of the administration of substances. As these will have been tested previously, it is unlikely that the adverse effects will exceed moderate severity levels. The mice will be closely monitored for signs of ill- health. At the end of the experiments or if the severity of adverse effects exceeds moderate levels, animals will be euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Blood development is a complex system that requires the cooperation between different cell and tissue types. This cannot currently be achieved in culture conditions. Furthermore, the only way a true blood stem cell can currently be identified is by its ability to regenerate the entire blood system upon transplantation. Many steps in the development of cancer are still unknown, but it is appreciated that it involves the accumulation of mutations over time and the interaction of the tumour cells with the local microenvironment and the immune system. These complex processes cannot currently be modelled in culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Information and preliminary data on the role of new candidate genes and cells will be initially obtained from the literature and experiments that do not involve animals. Only those candidates that have shown potential in these culture assays will be further investigated in animal models. Similarly, drug doses and responses will first be tested in cultures before administering them to animals. Wherever possible, statistical analyses will be performed prior to the experiment to determine optimal numbers of animals to be used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to	The mouse is the animal of choice for these studies as its blood development and blood system is highly similar to the human system. It is also the species in which gene manipulation can most reliably be performed. All of the procedures we will use are very well established. A lot of data on the mouse blood system and cancer development in mice is already available which

minimise welfare costs (harms) to the animals.	will allow us to put our findings into context. Gene manipulations may be performed in such a way that they can be restricted to a specific tissue and to a specific time point, thus minimising the adverse effects on the animals. Mice will also be irradiated in two separate doses to minimise adverse effects and allow for a quicker recovery.

Project 21	**Studies on Vascular Diseases and Repair Mechanisms**	
Key Words (max. 5 words)	Blood vessel, Restenosis, Stent, Vein Graft, Endothelium	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Current cardiological techniques such as stenting and vein grafting are not perfect and in some patients they are not so effective. This project seeks to understand the processes which cause vein grafts to fail and arteries to renarrow after a stent is implanted. We will also be trying to optimise the delivery of novel drugs from stents into the artery wall which may overcome some of the problems seen with the currently used drug-eluting stents.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project will increase our understanding of how blood vessels repair themselves after a procedure such as stenting of vein grafting. This will include studying the natural processes which occur over time. This can sometimes help to identify the healing mechanisms which can be targeted with drugs in order to improve the clinical outcome.	
What species and approximate numbers of animals do you expect to use	We intend to use primarily pigs and mice. We anticipate that over the 5 year life of the project up to 150 pigs may be used. The numbers of mice will be higher but many of the number will be derived from breeding colonies of genetically	

over what period of time?	modified mice used to produce mice for experiments. We anticipate a total of 500mice/year (2500 over the life of the project). Some rabbits and rats may be used but numbers would be small (~250 over 5 years).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All procedures on this licence fall into the moderate or mild classification. Breeding- which will be by far the biggest use of animals is mild and no adverse effects are anticipated. For pigs and mice on procedures involving recovery, some mild discomfort post-operatively may be experienced. This will be treated with pain-killing drugs and animals closely observed. In some procedures, the animal will be terminally anaesthetised and so will not recover at the end of the procedure and instead will be humanely killed. Animals which are recovered will be kept for up to 28 days (or 90 days in some cases for pigs), then killed humanely using an approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We derive much of our preliminary data from experiments which do not use live animals and this helps us to decide on doses of drugs and other conditions for studies which do use animals. It also helps to cut down the risks of unforeseen problems when we do use live animals. However, when studying repair of blood vessels there is no adequate substitute for using a live animal with circulating blood and a similar cardiovascular system to humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise animal numbers, we use a statistical test called a power calculation which helps us decide the lowest number of animals we can use for a study. In the pig studies we often utilise more than one of the coronary arteries which overall will reduce the number of animals we use. Once we have used an animal we maximise the use of all organs/tissues to gain as much experimental data from that animal as 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	We use normal, large white or landrace pigs for several reasons. Their size and the closeness to humans- particularly in their heart and circulatory systems mean that pigs are the best species for pre-clinical cardiovascular research. The pig model (and the small animal models of blood

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	vessel injury or vein grafting) are accepted by the scientific community and we have, through years of experience, refined our techniques to make each of the proposed procedures have a high success rate. In all experiments, suffering to the animals is minimised by careful experimental technique and judicious use of analgesics and anti-inflammatory agents at dosages recommended by the veterinary surgeon. Close supervision of the animal during recovery also ensures that any complications can quickly be identified and dealt with to minimise suffering.

Project 22	**Zebrafish models of cardiovascular development & disease**	
Key Words (max. 5 words)	Cardiovascular, angiogenesis, heart, blood flow	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	1) to observe the behaviour of cells during formation of the cardiovascular system in living animals 2) To alter the function of certain genes and examine the effect on formation and function of the cardiovascular system 3) To identify new drugs that might be used to treat cardiovascular diseases.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will give the most detailed understanding of the behaviour of individual cells during formation of the cardiovascular system, and how certain genes control this process. This will greatly advance our scientific understanding, and may give insights into conditions where the cardiovascular system forms abnormally in human babies. If this work does discover new drugs that alter cardiovascular development then these may represent new treatments for cardiovascular diseases.	
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish; 38,100	
In the context of what you	In most of my studies, I will simply observe cell	

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?	behaviour in developing embryos and juvenile zebrafish. This is not likely to induce adverse effects. I will maintain large colonies of breeding adult zebrafish using established and expert husbandry, and this causes few if any adverse effects except occasional fighting between fish. In some of my studies I will manipulate genes and assess the effect on cardiovascular development. This may cause problems such as reduced heart function or abnormal blood vessel development. This has the potential to cause adverse effects, such as swelling, inability to swim properly, and death. These animals will be very closely monitored. All animals will be humanely killed at the end of my studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The cardiovascular system develops from multiple different cell types and requires a complex three dimensional environment with blood flow and other important contributors. This makes it impossible to study without animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	I am using cutting edge microscopy that will gather more information from each animal than any previous studies, and timecourse studies where the same animal is studied over time, rather than using separate 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 zebrafish is the animal of lowest neurophysiological sensitivity that can be used for any studies of cardiovascular development. We will minimise harms by; expert husbandry and handling of our animals and by close monitoring of animals that have undergone any intervention.

Project 23	**Mechanisms and therapy for limb ischaemia**	
Key Words (max. 5 words)	limb ischaemia, angiogenesis, arteriogenesis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The formation of fatty deposits (plaques) in the arteries of the limbs (peripheral arterial disease), can give rise to blockage of these arteries, leading to a poor blood supply (ischaemia) of the limbs. This condition affects 20% of individuals over 75years of age and is associated with pain on walking. If the condition progresses it can severely restrict blood flow causing intractable pain and gangrene, called critical limb ischaemia (CLI). Over 30% of patients with CLI cannot be treated successfully by conventional surgery and will eventually require an amputation. The purpose of the studies outlined in this project license is to help us identify molecules and cells that have the capacity to promote the growth of new blood vessels (angiogenesis) in order that we can develop a treatment for such patients. The utility of treatments with blood vessel growth promoting factors is confounded by the complex nature of vessel development, which undoubtedly involves more than one factor. To overcome this, the use of cells with angiogenic properties has been advocated, but no treatment for clinical use has resulted from these studies. This is likely because of our poor understanding of the precise cell types involved, and their fate and function in inducing stable	

	blood vessel growth in limbs with poor blood supply.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We have shown, using the hind limb ischaemia model, that defined populations of white blood cells (monocytes) and stem cells have the capacity to generate new and robust blood vessels. We aim to use both studies in the laboratory (in vitro) and in the animal (in vivo) to: i) elucidate the mechanisms that these cells use to promote the growth of blood vessels ii) determine which cells or combination of cells are the most effective iii) determine the best way of delivering the cells and their behaviour and fate in the complex and dynamic environment that exists in ischaemic limbs in vivo. The in vivo model will also allow us to develop imaging methods to study blood perfusion that can be used to measure limb perfusion in man. This work will help us to develop novel cell-based therapies for the treatment of patients with critical limb ischaemia who have no option other than amputation, and novel imaging methods that can measure blood perfusion and inform therapy and the effects of therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats We expect to use approximately 1200 animals per year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The rodent hind limb model is a commonly used model for studying ischaemia that is well tolerated by the animal, as there is an inherent recovery of blood vessel function over time. Animals may experience temporary lameness in the affected limb, While the new blood supply is established which occurs reasonably quickly (within 7-10 days). This does not appear to affect the wellbeing of the animals as evidenced by their normal eating, drinking, grooming and general activity after surgery. The angiogenesis model is also well established (only involving the introduction of a small implant or gel plug beneath the skin) and has little effect on the wellbeing of the animal. Cell donors are needed to provide a variety of cells such as bone marrow stem cells, inflammatory cells

	etc., that we believe may be important in helping the process of new blood vessel growth. The procedure for obtaining the cells will be carried out under terminal anaesthesia and the animal will therefore feel no pain. All the other protocols involved in this project are for breeding of genetically modified animals. The protocols or genetic modifications would not be expected to cause complications that would affect the general wellbeing of these animals. All animals will be humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not ethical to study the effect of injecting substances and cells into human patients with peripheral vascular disease without preliminary evidence that this form of intervention may be of benefit. The model of hindlimb ischaemia in the rodent that we propose to use is a recognised model of severe peripheral vascular disease. In vitro studies will initially be carried out to assess the effectiveness of these cells and their combinations as well as their potential mode of action before using them in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our hind limb model of ischaemia uses only one limb with the opposite limb acting as a control. This reduces the need for extra control animals. We use imaging techniques that enable us to assess blood perfusion in the same limb over time. This imaging approach removes the need to sequentially kill animals at each time point in order to assess blood vessel growth in treated limbs, and dramatically reduces the numbers of animals for experimentation. We will keep animal numbers to a minimum by carrying out statistical calculations that will tell us how many animals are needed for each experiment. These will be based on our knowledge of the variability in various parameters that we wish to measure in the model.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	A simple, well tolerated model of blood vessel growth, the ‘angiogenesis gel plug model’, which has minimal effect on the wellbeing of the animal, will be used to initially evaluate any potential blood vessel growth-

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	promoting treatment. Rats and mice are the lowest mammalian group in which models of hind limb ischaemia have been successfully characterised and are therefore the two species most often used in the study of limb ischaemia. In this model, new blood vessels develop spontaneously in the affected limb through processes similar to those seen in man. Rodents tolerate surgery well; good recovery rate and low post-operative infection is achieved through good aseptic surgical technique and post operative care including the use of pain relief and appropriate methods of keeping the animals comfortable. As a result of the ischaemia induced, animals may have a temporary mild disuse of the affected limb. This may persist up to one week after surgery during which time the new blood vessels spontaneously re-establish and blood flow is restored. Our aim is to see whether our treatments enhance this natural process. The techniques to evaluate blood vessel growth in this model such as angiography, Laser Doppler Perfusion Imaging (LDPI) and analysis of muscle by specific staining of blood vessels in sections taken through the muscle (histology) are well characterised and internationally accepted. The mouse model allows the use of animals with a modified immune system that facilitates investigation of cells of human origin without rejection. They also facilitate investigation of the importance of specific genes on blood vessel growth through genetic modification in this species. All surgical procedures will carried out under general anaesthesia with pain relief during and after the operation. Any animals showing signs of ill health or disturbance of wellbeing (e.g. persistent lameness) will be humanely killed to minimise any suffering that would otherwise be experienced.

Project 24	**Signalling in normal and abnormal blood cell function**	
Key Words (max. 5 words)	Blood, Cancer, clotting, bone marrow	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	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)	Blood cell production is an extremely complex process and needs to be tightly controlled to maintain normal blood cell numbers. If this process is disturbed, through a genetic mutation in certain blood cells, the effects on humans can be extremely severe. In some cases, blood cancers can develop, where too many blood cells are made. Alternatively, certain conditions can lead to too few blood cells being made. In this project, we will study how a special blood protein supports the production of blood cells, how this protein can lead to specific blood cancers and why blood cancers cause clotting abnormalities in humans. While this blood protein is important in blood cell production, it is not currently known how it works. We have shown that this blood protein is also important in blood cell production. However, it is not currently known how this works. When this protein is not present blood cancer will not develop. We now want to try and develop a new drug that can reduce levels of this protein to see if it can be used to treat blood cancers. Finally, although we know that people with blood cancers often die from forming blood clots that can lead to heart attacks and stroke, it is not known why this happens. We have found that a mutant	

	protein expressed in the cells that line blood vessels prevent them from working properly. This work will try and find out what this protein does, so in the future, it might be possible to develop new treatments to prevent clotting in these patients.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We hope to try and better understand how blood cells are made and apply these findings to blood cancer, anaemia (lack of blood) and bleeding problems. It is possible that these findings may contribute to the development of new drugs to treat blood cancers and blood clotting in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	The research will use approximately 250 mice per year for five years. Most of the mice will be monitored for about 4-12 months, to see whether they develop a blood disease. Some will receive bone marrow transplants to study how blood cells develop and which cells contribute to clotting.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Some of the mice will develop a mild form of blood cancer but they are far more resilient to these conditions than humans and they don't show any ill-effects. If the disease starts to develop too severely, humane endpoints will be used. Some of the mice will be injected with cells or drugs that can cause a little bit of pain (similar to getting an injection). Bone marrow transplants can occasionally make mice liable to infection, but prevent this by giving the mice antibiotics and housing them in an extremely clean environment. If the mice appear to be suffering, humane endpoints are used.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not currently possible to study how blood cells are produced or how blood diseases develop using test tube approaches. These processes are complicated and require interactions between lots of different cell types and tissues, it is still necessary for us to study these conditions in a whole organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce the number of mice by using controlled disease models which vary very little from each other. This makes sure we use the minimum number of mice to get significant data. We also test drugs and the roles of certain genes or proteins in test tube experiments first, to reduce the number of mice in each experiment.
3. Refinement	Blood cell production and the development of blood

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.	diseases are very similar in our mouse models compared to humans. This allows us to make important patient-relevant findings using these mice. We constantly monitor the welfare of these mice, not only by monitoring their general behaviour, but also by looking at changes in the number of blood cells. If it looks like they might start developing a more severe condition, we will use a humane endpoint.

Project 25	**Control of adrenal development and function**	
Key Words (max. 5 words)	Adrenal, steroid, development, disease, differentiation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The adrenal gland controls very important bodily functions such as controlling our ability to fight infection, create energy and control blood pressure. We do not know how the adrenal gland develops and how it controls the output of steroids which control these important processes.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	When the adrenal gland does not work properly the consequences are poor, and can often be fatal. Investigating the mechanisms controlling these should give us much more information about how this is done and hopefully lead to the development of medicines which can be used to treat patients suffering from under or overproduction of adrenal steroids. This may be particularly useful to treat hypertension, which is one of the major health risks in the world and is largely caused by overactivity of the adrenal gland.	
What species and approximate numbers of animals do you expect	Rats and mice (both wild type and genetically modified) will be used. We anticipate using up to a maximum of 1000 rats and 4000 mice in	

to use over what period of time?	the next five years but the numbers could well be a great deal lower.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the procedures we intend to do, for example breeding of genetically modified mouse strains and surgical procedures, are known to be a maximum level of severity of moderate, with many providing little or no discomfort. Introduction of the agonist/antagonists and manipulation of pathways that impact upon adrenal function may by themselves or in combination with these surgical approaches have adverse effects. This is unknown at present although thought to be unlikely. If this does occur, such that an animal is showing pain, by hunching or writhing, or distress (lack of appetite, not grooming) it will be terminated by a schedule 1 method following advice from the NVS or NACWO.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	These experiments are designed to investigate the growth, differentiation and function of the adrenal gland which is a three dimensional organ comprised of cells of different types of cells which respond to signals from each other and from other organs in the body. Organ culture is not yet possible for the adrenal gland and the few available cell lines, whilst useful to provide background information, are not capable of providing the description of complex organ biology sought in these objectives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will perform initial small scale experiments to determine the effect size of our procedures which will then let us calculate the minimum number of animals required. Using too few animals would be wasteful because we would not get a meaningful result and these calculations will allow us to limit the number of animals we use. These calculations will be performed by a statistician experienced in these sorts of studies.
3. Refinement Explain the choice of species and why the animal model(s) you will	Rats will be used because most of the background data on adrenal physiology collected over the last eighty years or so has come from rats. Genetically altered mice will

use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	be used when necessary to observe effects of the removal of certain genes in the pathways we are interested in or be able to genetically label cells for the study of their behaviour which is otherwise impossible. We will also attempt of grow cells from these animals in the laboratory, and if successful this will allow us to use many fewer animals. If any of our proposed experiments prove to be harmful to the animals we will discontinue them, however over the short period of our experiments the effects on the adrenal are expected to be well tolerated.

Project 26	**Transverse aortic constriction (TAC) in rodents**	
Key Words (max. 5 words)	Heart failure, cardiac hypertrophy, rats, mice	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The incidence of chronic heart failure in the UK is increasing, however the underlying causes are not fully understood and more effective treatments are necessary. The purpose of this project is to better understand the molecular processes underlying heart failure development and the identification of novel therapeutic targets.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	During this project we will generate reproducible models of heart failure in rats and mice in order to mimic the equivalent human disease. We will examine the mechanisms of heart disease progression in control animals and in animals which have been genetically modified to alter specific genes or proteins that may play an important role in the disease process. These studies will lead to improved understanding of the heart failure process through identification of the genes and proteins contributing to the disease. Investigation of these new therapeutic targets will ultimately lead to better treatment and prevention of human heart disease.	
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate that we will use approximately 100 rats and 100 mice per year over a 5 year period.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The transverse aortic constriction (TAC) surgical procedure (i.e. permanent constriction of the major blood vessel from the heart) will initially cause an increase in blood pressure and heart size, which are not expected to result in any adverse clinical signs. However, over time the increased load on the heart (as a result of continued elevated blood pressure) will become detrimental and the heart will begin to lose its ability to function normally. All animals undergoing TAC surgery will be carefully monitored on a daily basis and any animal displaying signs of heart failure will be promptly and humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The nature and complexity of the heart disease process makes finding alternatives to live animal models extremely difficult. However, wherever possible we will use non-animal alternatives (e.g. cell-based assays) for our investigations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We obtain expert statistical advice before commencing any new studies and perform power calculations which allow us to identify the lowest appropriate group sizes for each procedure. In addition, the use of techniques such as MRI and echocardiography allow non-invasive serial measurements in the same animal thereby significantly reducing the number of animals required for most 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 rat and mouse models used in these studies have been carefully selected based on their unique genetic profiles. These animal models will allow us to determine the direct contribution of specific genes and proteins to the progression of heart disease and heart failure. Our *in vivo* protocols have been designed to provide the maximum detailed characterisation of the cardiovascular system whilst at the same time ensuring that the animals under investigation experience the least pain, suffering, distress or lasting harm. For example analgesia is given for all surgical procedures and disease development is monitored by non-invasive imaging that allows early endpoints to be instigated prior to animals showing significant clinical signs.

Project 27	**The role of platelets in haemostasis and thrombosis**	
Key Words (max. 5 words)	Mice, platelet, thrombosis, haemostasis	
Expected duration of the project (yrs)	Five years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The inappropriate activation of platelets inside blood vessels leads to formation of blood clots or thrombi, which are associated with the propagation of heart attacks and strokes. Our objectives are to 1. Identify proteins critical for the control of platelet function. 2. Identify proteins that propagate uncontrolled platelet function and thrombosis. 3. Determine how metabolic disturbances influence the actions of proteins identified in objectives 1 and 2	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Thrombotic disease associated with heart attacks, stroke and cancer is the leading cause of death in the UK. With epidemiological studies pointing to increased levels of obesity and cancer it is likely that thrombotic disorders will continue to rise. While current anti-platelet medication is widely used and has been successful in reducing disease burden, the death rates are still unacceptably high. This project is designed to allow the role of new potential therapeutic targets to be identified and evaluated under the complex conditions that resemble human disease.	

What species and approximate numbers of animals do you expect to use over what period of time?	4000 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?	Blood platelets are a group of cells that clump together to form blood clots, ensuring that we stop bleeding after injury. However, in cardiovascular disease, platelets also form blood clots inside the blood vessels, which may lead to thrombosis, heart attacks and strokes. In this study we will use mice that have had some of their genes altered, so that their role in platelet function can be established. In some cases we may also feed them a high calorie diet, to examine how this influences clotting. We will perform two types of experiments. Firstly, we will remove blood from mice that have been killed humanely to isolate and study the platelets. Secondly, some mice will undergo procedures that assess blood clotting, which will be performed under anaesthetic from which they will not recover The level of severity of all the experiments is classified as mild or non-recovery, with all animals being humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are two major reasons for the proposed use of animals. 1. Blood platelets cannot be grown in culture and therefore must be harvested for each individual experiment. 2. Blood platelets lack a nucleus and therefore are not amenable to standard molecular biology approaches for the manipulation of specific proteins. Therefore the only realistic method for examination of the role of individual proteins in platelet function is to use genetically modified mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We used statistical procedures such as power calculations to ascertain the minimum number of animals required to use in a single experiment. Even so, we will only progress to changing the diet of the mice or examining blood clotting when experimentation with isolated platelets suggests strongly that the mice may be prone to thrombosis. Therefore relatively few mice will be subjected to the full series of experiments. For invasive experimentation we have now moved to a procedure

	that allows us to assess clotting in multiple blood vessels. This refinement of procedure will reduce the overall numbers of animals these procedures.
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 blood clots in a very similar to that of humans making mouse platelets an attractive model to use. However, there are number of areas where we aim to continuing improving our approach to these experiments. For example, all invasive ones are completed under anaesthesia from which the animal will not recover. Moreover, should methods become available for the culture of blood platelets to maintain cell populations in vitro or the generation of platelets in vitro, we will move our studies in this direction.

Project 28	**Redox Mechanisms of Organ Failure in Sepsis**	
Key Words (max. 5 words)	Sepsis, Redox, Blood pressure, Multi-organ Failure, Therapy.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	☑	Basic research
	☑	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	☑	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Sepsis (or blood poisoning) is a disease caused by excessive bacterial growth inside the body. During sepsis blood vessels abnormally widen their lumen resulting in a very low blood pressure, under-perfusion of main body organs, their malfunction and often death. Sepsis presents an enormous burden on healthcare systems and society. Annual cost of severe sepsis for NHS is over £2.5 billion. Even for septic survivors, life is difficult due to developing mental problems, sleep disturbances, chronic pain and/or loss of digits or limbs. Current therapies of administering antibiotics or drugs which constrict blood vessels are often ineffective, with a 25% mortality rate even in straight forward cases. In recent years much attention has been paid to the idea that septic injury is driven by over-production of gas nitric oxide which detrimentally widens blood vessels and lowers blood pressure by activating protein kinase G (PKG). However, clinical trials preventing nitric oxide production during sepsis were ineffective and in fact increased mortality. Clearly our current understanding of the disease is lacking. By better understanding the mechanisms that underlie the dysfunction that occurs in sepsis we improve our chances of implementing or designing rational therapeutic strategies. Our	

	previous work identified an important novel way of activating PKG that is independent of its classical activation mode. This new molecular mechanism is only active when excess of oxidants is present, like in the human sepsis scenario. Overall this important new mechanism that we have identified, mediates organ injury and malfunction during sepsis. Here we propose studies that may lead to new drug therapies that interact with that mechanism and help to treat this major disease burden.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary benefit of this project is obtaining new knowledge about the redox mechanisms of organ dysfunction and failure in sepsis, as well as possibly identifying potential therapies that will help to treat this major healthcare problem. The groups of patients are likely to benefit from these new treatments are older population (who are more susceptible to sepsis), younger adults and septic babies in a clinical settings, as well as public health sector, healthcare professionals treating septic patients and society. The data obtain will be of potential benefit to biochemists, structural biologists, physiologists, medical chemists, pharmacologists of the worldwide scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use no more than 3000 mice (including genetically altered) and 500 rats over the period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	As with humans, sepsis has a substantial negative impact on animals' well-being, i.e. pain, low blood pressure, malfunctions of heart and main organs, low body temperature and decreased locomotor activity. However this will be minimised by animals being closely monitored and applying a scoring system to identify when the pre-determined end point of the experiment has been reached at which point the animals will be humanely killed. Inhalational anaesthesia will be the primary method of general anaesthesia and appropriate analgesic (to control pain) will be given peri-operatively. Aseptic precautions will be taken to prevent non-sepsis related infections. We will induce sepsis by surgical means or by administration of substances that promote sepsis development. We have substantial hands-on experience of such studies and any surgical techniques will be performed by experienced

	researchers who maintain best practice of perioperative care and consult regularly with veterinary surgeons to ensure any discomfort or distress is minimised. If any animal reaches a predetermined endpoint prior to the end of the study it will be humanely killed. Some imaging will be performed under recovery anaesthesia. An animal is only re-anaesthetised if it is considered fit and has recovered from the previous anaesthetic. When we anaesthetise an animal for the final time from which it will not recover ('terminal anaesthesia') we will maximise the amount of data collected by obtaining both non-invasive imaging data and by harvesting blood/tissue for further analysis post mortem. All animals are humanely killed at the end of experiment.
Application of the 3Rs	
1. **Replacement** State why you need to use animals and why you cannot use non-animal alternatives	Sepsis is a complex multi-factorial multi-organ disease, in which the underlying pathogenesis develops over a period of several hours to several days. We cannot employ cell models of sepsis as they fail to provide information that is useful in terms of how therapies may translate to use in humans. Animal models are required to understand sepsis development and its effects on body organs, and to test any potential therapeutic strategies in the intact animal. Studies of human post-mortem tissues are not ideal as not only is tissue difficult to obtain, available samples are limited to diseased surgical tissue from heterogeneous uncontrolled populations, which interfere with accurate studies. It is not currently possible to use computational models, although our findings may guide future programs, In the future, artificially constructed protein networks might help to predict the likely course of a disease and its response to treatment. We will utilise state-of-the-art imaging tools whenever possible, to enable us to track progression within an individual animal and thus maximise the data collected from each animal used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All efforts will be made to restrict animal numbers. Dynamic telemetry readings and repeated non-invasive imaging permit animals to serve as their own controls. The data obtained from terminal procedures (without causing any additional harm to the animals) will maximise data yield from each subject, and inform further studies. Thus data is maximised whilst

	numbers are minimised. The use of power analysis will be employed to ensure meaningful and relevant statistical analysis and care is taken to minimize animal usage wherever possible. We will define the number of animals required by calculating sample size according to statistical principles.
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 refine the usage of animals by surgically placing very small probes (known as telemetry probes) into an animal's blood vasculature, to give a continuous reading of the pressure in these vessels in the recovered animal as it undertakes its normal activity. When surgery is completed, this technique does not require restraint or, sedation as required by other approaches. Monitoring is entirely remote, removing the stress of human contact. Any surgical techniques will be performed by experienced licensees who will keep best practice of peri-operative care and consult regularly with veterinary surgeons and experienced technicians to minimise any discomfort and distress. We will follow good laboratory practice guidelines to ensure accurate and reproducible data are obtained.

Project 29	**Modelling striated muscle disease in zebrafish**	
Key Words (max. 5 words)	heart, skeletal muscle, Popeye genes,	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The POPDC genes encode a family of membrane proteins, which mediates adrenergic signalling in the heart. Recently a number of patients were found, which develop heart and muscle disease and harbour point mutations in POPDC genes. The fundamental functions of PODPC genes and the disease mechanisms associated with this gene mutations need to be unravelled.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Defining the gene networks in which POPDC proteins are acting will provide fundamental insight into the processes maintaining structure and function of striated muscle. Insight into the disease mechanisms associated with mutations in POPDC genes causing striated muscle disease will be important for the development of novel therapies.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using zebrafish to model heart and skeletal muscle disease and introduce mutations in the zebrafish genome that are also found in patients with heart and muscle disease. Over the period of 5 years a total of 10.000 embryos and 13,000 adults will be used.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Breeding of genetically altered zebrafish. Most genetic alterations have little or no impact on their welfare. Some however may have an effect on skeletal muscle or heart with more serious, adverse effects. In addition, heart and skeletal muscle regeneration will be studied. Animals subjected to cardiac lesioning may die or rapidly recover. Because of the risk of death it is a severe procedure. Skeletal muscle regeneration does not normally result in death but causes a short-duration impairment of the animal's swimming ability before the animal fully recovers. Therefore this procedure is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cardiac and striated muscle disease can only partially be modelled using cell culture models. Wherever possible we will be using cell cultures or using computer modelling. However these experiments cannot substitute research in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We shall only use a minimum number of animals in our experiments. Breeding strategies are performed by qualified personnel and aimed to avoid unnecessary animal generation.
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 zebrafish model is an excellent model organism, which due to its transparency of the embryo, its high fecundity, its rapid development and the availability of sophisticated genetic tools provides vast opportunities to model the genetic basis of cardiovascular and skeletal muscle disease. We will be using the zebrafish to introduce point mutations found in patients to unravel the pathomechanisms of Popdc gene mutations. The phenotypes often become first apparent during embryonic development and thus suffering of the animals will be minimised due to the short period until the cardiovascular and muscle pathologies becomes apparent.

Project 30	**Mechanisms of platelet-driven disease**	
Key Words (max. 5 words)	Platelet, cardiovascular, heart attack, thrombosis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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)	We aim to identify substances to which the body is exposed through natural biological production, the environment or due to medical treatment. Specifically, we will investigate the role of substances produced by blood vessels to protect against heart attack and determine how exposure to small particles of pollution and medicine used to combat HIV infection may increase the risk of heart attack. As part of this project we will work with refined animal models previously developed in our group and shall introduce further refinements to implement into our own project and for use by other research teams.	
What 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 help us to understand how blood functions during normal health in order to protect us from heart attack. We shall also identify disturbances which lead to heart attack either through the loss of normal biological function or exposure to elements of pollution or medicines. By understanding blood function, we shall be able to identify new ways of treating disease. Identification of exposure risks will influence political decision makers and medical prescribing practises in order to reduce the incidence of heart attack in the population. The development and uptake of refined techniques for assessing clot formation in animals as an	

	alternative to currently used models which use death as an end point will benefit animals by reducing the use of techniques that inflict pain and suffering because our procedures are conducted entirely under general anaesthesia.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. 5000 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?	The vast majority of our experiments are conducted at a mild severity limit. The most likely side effects are infection or damage following administration of substances by injection or into the airways of mice. These adverse side effects are extremely unlikely to occur because our scientists are highly trained and we use sterile techniques to avoid infection. Occasionally, we treat mice with drugs to remove platelets from their blood. These are the cells which drive blood clotting and which cause heart attack and we wish to replace the circulating platelets with a different population of platelets that are genetically modified or are from human donors. This treatment can sometimes cause weakness in mice and in this instance the severity limit can rise to moderate. When we induce blood clotting in mice to mimic heart attack, this is conducted under general anaesthesia so that severe procedures are avoided. At the end of our procedures animals are humanely killed and sometimes organs are collected for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible we work with platelets from human blood donors rather than mice. These platelets can only be analysed outside of the human body which means that factors external to the blood such as those generated by blood vessels cannot be taken into account. In addition, when working with pollutants, the pollutant must be delivered into the airways of an animal to mimic inhalation by humans. The use of clinical drugs also requires animal experiments since many of the drugs are in an inactive form until processed by the liver. For ethical and practical reasons much of our work cannot be conducted with human volunteers and so we must work with animals in order to acquire meaningful information.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The responses that we measure in mice are reversible which makes it possible to record more than one response in an individual animal (whilst remaining under anaesthesia). This reduces animal use. We also conduct a statistical analysis based on the variability of our data and the differences between treatment groups that we wish to detect to determine the minimum number of animals required for our 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 standard model for the experiments that we wish to conduct involves the injection of clotting agents into groups of mice to cause death by pulmonary embolism. The ability of drugs to reduce or increase death is then assessed. This approach inflicts considerable pain and suffering since the animals experience a pulmonary embolism and often hind limb paralysis without any use of anaesthesia or pain relief, uses large numbers of mice and will not be used in this project. Instead, we shall use a technique developed by us whereby clotting agents are induced at a lower dose and in anaesthetised animals so that animals only experience a mild severity level and painful procedures are avoided. Rather than induce death, we record clot formation using radioactive imaging technology. We also plan to develop simple techniques for sampling small amounts of blood during the clotting response Our entire procedure is conducted under general anaesthesia and far fewer animals are used.

Project 31	**Parental nutrition and offspring development**	
Key Words (max. 5 words)	Diet; Gamete quality; Pre-implantation embryo; Offspring health; Developmental programming	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The number of adults afflicted by heart disease, obesity and diabetes has grown rapidly in recent decades, affecting up to one quarter of the world's population. Typically, these diseases are associated with lifestyle factors including lack of exercise and smoking. However, a large number of studies conducted in both humans and animals have shown that a mother's nutrition during pregnancy can affect the growth of her unborn baby, increasing its risk for heart disease, obesity and diabetes in adult life. Whilst the connection between a mother's diet and the long-term health of her offspring has been studied in great detail, our understanding of whether offspring health might be affected by a father's diet remains poor. In addition, the interaction between sperm and eggs from parents of poor nutrition is unknown. Therefore, the objectives of these experiments are:- (i) to investigate the underlying biological mechanisms affecting offspring heart and metabolic ill-health in response to low protein diet	

	of the father (ii) to understand how a father's low protein diet affects the quality and function of his sperm and the development of the early embryo it generates. (iii) to determine the interaction of sperm and eggs from parents both fed a low protein diet prior to conception.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	With the increased global prevalence of heart disease, obesity and diabetes, a greater understanding of how adult ill-health is determined has never been of greater importance. This novel study will provide new insight into the role of a father's diet for the long-term health and development of his offspring, will identify the underlying biological mechanisms regulating heart disease, obesity and diabetes, and will provide scope for the development of new interventions and treatments to alleviate their effects and benefit human health. In addition, this study will identify the interaction between sperm and eggs, both from parents fed a poor diet, to determine their combined effect on offspring development.
What species and approximate numbers of animals do you expect to use over what period of time?	This study will use approximately 1300 mice in total over a period of 5 years. This total number reflects a series of different used including:- (i) approximately 300 adult male and female mice used for the collection of sperm, eggs, embryos and for mating for the generation of offspring. (ii) the generation of approximately 500 male and female offspring for analysis of adult health. (iii) the hormonal stimulation of approximately 200 female mice for the precise timing of ovulation and mating in the generation of embryos and offspring. (iv) approximately 100 males for vasectomising and use in sterile mating and analysis of uterine responses to seminal fluid (v) approximately 200 females required as recipients of embryos to allow their development to be analysed
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	It is expected that the severity of all experiments within this study will be mild-to-moderate. All surgical procedures (embryo transfer and vasectomising of males) are associated with potential complications, infections and risk of death. However, these will be

happen to the animals at the end?	minimised through the use of the most appropriate techniques. Analyses of offspring health will require the sampling of blood from the tail as well as the restraining of animals which may cause distress and discomfort. At the end of the study, all animals will be culled humanely and quickly. During the course of the study, any animal showing significant signs of injury, infection or ill-health will also be culled at the earliest time possible.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The manipulation of parental nutrition, gamete quality, embryonic development and long-term offspring health would be considered un-ethical in humans. As such, animal models are essential for obtaining a greater understanding of the mechanisms linking parental diet and offspring well-being. Mice are essential to this project as they will maintain experimental continuity with my existing studies, their development mirrors closely that of humans and our detailed knowledge of their genetics makes them a powerful tool for determining the genetic factors involved. These essential studies will however, identify targets for analysis in human tissue samples and patients resulting in the replacements of mice with humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure the minimal number of mice are used, statistical approaches have been employed to accurately determine how many mice are required. In addition, through the collection of a range of organs from each mouse used, a bank of tissues will be collected for future studies, minimising the need to use/generate more mice in future studies. Finally, this study will ensure that where possible, each mouse is used to its maximal capacity (i.e. male mice mated several times) and that as much biological information is obtained from each animal reducing the overall number required and maximising the amount of data generated.

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 extensive use of mice for scientific studies means detailed and optimised protocols for the experiments used within this study are widely available. In addition, equipment and devices are available to minimise the necessity for surgical procedures. As such, this study will employ the most up-to-date protocols and procedures available, whilst providing extra information for other researchers. All surgical procedures (embryo transfer studies and implantation of blood pressure recording devices) will be conducted under general anaesthesia with constant and regular monitoring during and after surgery and provision of pain relief. Any animal which shows persistent or marked health problems will be killed humanely and immediately.

Project 32	**Regulation of normal and malignant haemopoiesis**	
Key Words (max. 5 words)	Leukaemia; haematology; development; stem cells;	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand how normal blood cell biology is subverted to drive cancers. It focuses on two themes: 1) the creation and maintenance of blood cells during development and throughout adulthood and 2) understanding disease development to devise new therapeutic strategies to treat blood cancers. The process of how a single cell develops into a cancer still remains unclear.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	It is currently impossible to study the complex role of the microenvironment of normal and malignant stem and progenitor cells in vitro since the complete set of important factors have not yet been identified. Also, to study stem cell function, the cell must be shown to possess the ability to sustain lifelong blood cell production and this cannot currently be assayed outside the body. Finally, to assess the function of normal and patient derived human stem and progenitor cells, the xenograft model (where human blood cells are transplanted into mice with	

	compromised immune systems to avoid rejection) is currently the only system capable of determining long-term multi-lineage capabilities.
What species and approximate numbers of animals do you expect to use over what period of time?	Species: Mouse We expect to use approximately 4568 animals per annum over 5 years. (i.e. —22840 animals during this project.)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Since this project will explore the role of previously unstudied genes in the blood system, it is possible that disease will develop in these animals including leukaemia development. Animals will be very closely monitored for signs of disease and killed by a schedule I method should the clinical signs necessitate intervention. The majority of mice under this licence will show no signs of adverse effects that impact materially on their general health. It is estimated that more than 60% of animals will not exceed a sub-threshold severity category. Approximately, a further 25% may not exceed mild and —10% may reach a moderate severity category. Adverse effects may rarely be such that the humane end points are reached (less than 5%). These animals may develop haematopoietic malignancies causing abdominal distension, weight gain or loss, anaemia or erythrocytosis, laboured respiration, inactivity or inappetence, combined with signs of hunched posture or piloerection. Animals showing any of these clinical signs will be immediately 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	It is currently impossible to study the complex role of the microenvironment of normal and malignant stem and progenitor cells in vitro since the complete set of important factors have not yet been identified. Also, to study stem cell function, the cell must be shown to possess the ability to sustain lifelong blood cell production and this cannot currently be assayed outside the body. Finally, to assess the function of normal and patient derived human stem and progenitor cells, the xenograft model (where human blood cells are transplanted into mice with

	compromised immune systems to avoid rejection) is currently the only system capable of determining long-term multi-lineage capabilities.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Colony sizes will be carefully managed to ensure that supply matches the demand, and any surplus mice are used for other scientific purposes and tissues shared over multiple experiments. When designing experiments we perform statistical analysis (e.g., power calculations) to ensure that we use the minimum number of mice per group that will be informative. Finally, we will use human cell lines (including patient derived cell lines) of particular mutations (e.g., JAK2 V617F; CALR) to study the biochemistry of the mutations.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the most appropriate and most widely used model for studying blood cells and cancer. The techniques are therefore very well established and findings can easily be integrated with other groups' data. The mouse is also the species in which reliable gene delivery systems are best established. For our studies that involve blood cell transplantation, we have recently introduced a recipient mouse model that permits much lower irradiation does and we have also removed techniques that are no longer required from our licence.

<table>
<tr><td>Project 33</td><td>Targeted gene delivery for haemostasis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Blood coagulation, haemophilia, thrombosis</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>
</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 aims of this project are to improve the technology underlying gene therapy to develop treatments for inherited blood coagulation disorders. In addition, it will further our understanding of the role of blood coagulation factors in inflammation and determine the feasibility of developing novel treatments.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This project will aid the development of treatments for severe inherited diseases. It may also open up the possibility of alternative therapies for the treatment of disease(s) where blood coagulation factors play an important role in the response to injury.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will use mice (approximately 3000 over the five years)</td></tr>
</table>

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?	Every attempt has been taken in designing the experimental protocols to minimise adverse effects. However, it is possible that adverse events may occur due to anaesthesia, surgery, bone marrow transplantation, obesity following a high fat diet and in the sepsis model due to differences in potency between batches of agonist (LPS). We aim to be vigilant for signs of distress/adverse effects and to treat them appropriately. The incidences of adverse effects associated with anaesthesia are connected with the use of injectable rather than inhaled anaesthetic agents. Where possible inhalation anaesthetics will be used minimising the incidence of adverse effects, such as hypothermia, respiratory and cardiac depression, due to general anaesthesia. To further improve anaesthesia vital signs will be monitored. Adverse effects of surgery will be minimised using appropriate aseptic techniques, analgesia and appropriate post-surgery observation and care. The potential adverse effect of bone marrow surgery is the failure of the graft due to technical failure to deliver the donor cells appropriately. The operator will receive appropriate training to minimise the risk of such events. The expected level of severity is moderate. Mice are culled at different time points throughout the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The disease states we are studying involve multiple cell types throughout the organism. The molecules of interest synthesised by one organ/cell type play an important role in injury incurred at other sites in the body. The viral gene therapy vectors will be validated by in vitro assays before use in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Based on previous experimental results and consultation with a statistician we will ensure the use of the minimum numbers of animals to achieve a statistically valid result. The mice have a homogeneous genetic background which is more likely to give statistically valid data while minimising the number of mice used. Throughout the

	programme of work we will monitor the breeding programme and maintain the minimum number of animals required. In addition when animals are killed by schedule 1 all tissues will be harvested and stored for further analysis by other researchers maximising output from each animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We are using mice, in particular transgenic mice where a defined phenotype has been generated by mutagenesis to create a mouse model resembling the human disease(s). Appropriate use of anaesthetics and analgesics will minimise harm to the animals. Initial experiments to achieve objective 1 of the experimental plan will be carried out using cells/tissues from animals killed by schedule 1 to minimise harm. Finally, where appropriate pilot studies will be performed to refine the experimental design. Other refinements will be a specific environmental enrichment programme for these animals and strains where all the items will be carefully evaluated to avoid any minimal bleeding (for ex. Wood blocks might be eliminated to avoid the risk of bleeding following microlesions in the mouth, we will provide cardboard rolls instead of hard plastic tubes etc). Animals can be handled using tubes or cupping hands –both after habituation- to reduce stress, decrease physical restraining and possible injury that it can cause. Habituation of animals will itself reduce stress in the mice during normal husbandry operations and procedures and therefore the likelihood of bleeding/injuries due to sudden/unexpected movements/jumps stress related.

Project 34	**Stem Cells for Blood and Tissue Repair**	
Key Words (max. 5 words)	Blood, blood vessel, repair, transplantation, regeneration	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	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)	Blood stem cells are critical to life. These stem cells reside in the bone marrow where they can generate all blood cells over a lifetime. Disorders of these stem cells (e.g. leukaemias; sickle cell disease) reduce patients' quality of life and survival. Transplantation of blood stem cells, with or without replacing defective genes, is potentially curative. While 2013 marked the one millionth such transplant, this therapy is associated with significant morbidity/mortality resulting from chemo-/radio-therapy or graft quality. Our first aim is to increase the quality of blood stem cells in the graft ex vivo to improve their take in the bone marrow and hence reduce associated morbidity and mortality. This will be accomplished i) by expanding stem cell numbers with specific factors in vitro before transplantation studies in animals, ii) by repairing damaged to bone marrow to enhance blood cell production, and iii) to eradicate malignant disease or repair the defective blood stem cells. In this way, we will provide better treatments and	

	hopefully cures for blood disorders. Many patients die from organ failure or from debilitating organ diseases. Since most organs require a blood supply for their survival and function, our second aim is to regenerate a blood supply in damaged tissues from stem cells using knowledge acquired in aim 1 in order to reduce the need for organ transplantation.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Blood cancers are the 5th most common cancer in the UK, and severe anaemias the most common inherited diseases worldwide. Over 1 million patients have received bone marrow transplants to treat these diseases, but, although successful, there is significant mortality and morbidity. Many millions of individuals suffer from diseases affecting the blood supply to organs (e.g. cardiovascular disease, stroke, chronic skin ulcers). The latter for example represent a silent epidemic affecting 1% of EU individuals. For diabetic patients, this can lead to limb amputation. In the UK chronic skin ulcers cost 3% of the NHS budget (>£3bn p.a.). Our aims are to further improve stem cell therapies for severe blood and organ disorders. While we will perform as many experiments as possible ex vivo, longer term stem cell therapies can only be tested for their efficacy in in vivo models ahead of clinical trials in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We would use a variety of mouse strains, particularly those that lack a functional immune system so that human cells are not rejected. The mice may alternatively be deficient in particular genes so that we can assess the function of these genes and their contribution to blood formation and tissue revascularisation and repair. Over a 5 year period, we would expect to use up to 3000 mice for breeding, a proportion of which would be used in assessing blood and tissue repair and regeneration, together with an additional 1500 for blood stem cell transplants, 500 for humanised niche studies and 500 for wound repair.
In the context of what you propose to do to the animals,	1. Animals produced by crossbreeding may have an unexpected phenotype that could have an impact on

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	animal welfare (e.g. less resistance to infections). 2. Myeloablation is used in human patients to reduce disease burden (e.g. in leukaemic patients) and/or to allow blood stem cell transplantation to occur. Any irradiated animals or animals receiving chemotherapeutic agents for myeloablation may show signs of adverse effects to radiation/chemotherapeutic agent or signs of delayed blood cell recovery (particularly up to 21 days post-transplant) such as lack of appetite, weight loss, listlessness and increased susceptibility to infection. 3. Genetically altered animals may display less tolerance to radiation or chemotherapy than wild type animals. 4. Animals administered with leukaemic/diseased cells may show signs of disease burden, and this will be closely monitored and controlled for. Treatments (e.g. analgesia, antibiotics, anaesthesia, heat therapy) will be administered where appropriate to minimise adverse effects. Monitoring procedures are designed to ensure that adverse effects are picked up early and appropriate actions taken to care for and ensure animal welfare. The level of severity is expected to be mild to moderate. At the end of the experiments, animals used on breeding or experimental protocols will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The repair using stem cells or their products of the damaged bone marrow, the long term generation of blood cells and the generation of a functional blood supply which contributes to the repair of organs or tissues can only be assessed by in vivo studies because interactions in different systems are needed to accurately predict successful clinical translation of new therapies or other.
2. Reduction Explain how you will assure the use of minimum numbers	We will minimise animal numbers by careful cell/animal selection, by identifying statistically significant changes in cell functions in in vitro experiments, through careful experimental design,

of animals	and by the use of human cell sources wherever possible before testing *in vivo.* Small pilot in vivo studies may be conducted first (e.g. to assess cell transplant numbers, to develop a humanised niche, to test irradiation protocols). To avoid culling animals at suboptimal time points, we may i) take small blood samples regularly to monitor stem cell take and ii) use live animal imaging to monitor outcomes in the same animal over time. Previous experiments or published data will be used to define animal numbers likely to give statistical significance, otherwise power calculations will be performed. By consulting a statistician before/during the experimentation, we will minimise/optimise animal numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have the lowest neurophysiologic sensitivity to produce scientifically robust results and enable comparison with established outcomes. Suitable immunocompromised mouse strains are needed in experiments to test and prevent rejection of human materials as this increases the translatability of results. Enhanced human blood cell formation has been achieved with improved animal models (e.g. NSG mice), and we will use these or newer strains where possible. Radiation dosages depend on mouse strain used and its method of application. The lowest doses for effective transplantation of specific mouse strains will be used and administered as a split dose to reduce welfare impacts. We will use the husbandry method of adding baby milk power/mash to the cage and providing antibiotics before and following irradiation and house our animals in a ‘quiet room’ with minimal disturbance following transplantation to improve overall conditions for the animals and support the animals’ recovery and minimise harms. Surgical techniques for transplanting cells in scaffolds and live imaging will be carried out under general

	anaesthesia. Mice will receive pre-emptive analgesia to prevent pain. Where possible, we will use mice which do not have abnormalities which could compromise welfare when housed correctly. Wounds will be dressed to prevent damage by self-grooming and infection.

Project 35	**Regulation of Blood Cell Generation**	
Key Words (max. 5 words)	Blood stem cells, Regenerative Medicine	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The process by which blood stem cells are generated during embryonic development is poorly understood. To understand this process in sufficient detail to be able to manipulate it for therapeutic purposes in e.g. transplantation therapies for leukemia, we need to study this process as and when it occurs in the embryo and apply the lessons learned to develop new culture systems to generate and amplify blood stem cells in the laboratory.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	These studies will generate a better understanding of how blood stem cells are made. As factors that are already known to play a role in this are frequently deregulated in blood cancer, a detailed insight into the birth of blood stem cells will serve as a benchmark to assess what cellular processes go awry in blood cancer. Worldwide there are approximately 350,000 new cases of blood cancer per year. The insights obtained from our studies will aid the future development of protocols for blood stem and progenitor cell generation in vitro, and the	

	improvement of intervention and stem cell-based therapies in leukemia.
What species and approximate numbers of animals do you expect to use over what period of time?	These studies will be preformed in the mouse. We expect to use up to 12000 mice over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will breed genetically modified mice, and use embryo tissues for analysis (expected severity level: mild). The majority of our studies are done ex vivo and in vitro. Three procedures on living mice are needed, (1) irradiation of adult mice and injection of donor cells (transplantation assay; expected severity level moderate), (2) administration of a substance to mark dividing cells (expected severity level mild) and (3) administration of transgene-inducing or deleting substances (expected severity limit moderate). Mice undergoing these procedures are closely monitored for adverse effects (e.g. dehydration as a result of the irradiation), and killed if adverse effects arise, though these are rarely seen. The in vivo assays involve momentarily discomfort from the injection, but no other adverse effects are expected. Based on previous experience, most of the animals we use will actually experience a sub-threshold severity level (breeding of up to 9000 mice over 5 years).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Blood stem cell generation in the embryo is a complex process which currently cannot be replicated in cell culture. We use the mouse as a model organism as it is the lowest mammalian species with clear similarities to human blood cell generation. It is not feasible to use human material due to the limited and irregular availability of human embryo material.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are carefully planned to avoid wastage, and the size of the mouse colony is continuously tailored to meet experimental needs, without creating surplus. We freeze embryos/sperm to archive lines not in use.
3. Refinement	Studies are preformed in the mouse, as in this

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.	species markers are available to identify and isolate the cells of interest. Such markers are not available for human cells, and this, together with the limited availability of human embryonic tissue, precludes the use of human cells for our studies. The mouse is a well-established model for this type of studies, and many similarities exist between mouse and human stem cell generation. In addition, the multiparity of the mouse makes it feasible to obtain sufficient material for study.

Project 36	**Zebrafish manipulation to study blood development**	
Key Words (max. 5 words)	Haematopoiesis, haematopoietic stem cells (HSCs), zebrafish	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Blood stem cells (BSCs) are immature cells of our bone marrow (BM) that generate trillions of new blood cells every day. BSCs can also restart the blood system in organisms that have lost the ability to make blood cells, for example patients that have undergone chemotherapy. This ability has turned BSC transplantations into the most common type of stem cell therapy. Yet, problems remain. Incomplete matching of donor and recipient blood cells can lead to transplant rejection and graft-versus-host (GvH) disease. While the former leads to BM failure, the latter causes side effects that can result in organ failure. A third of all patients do not to have a matched BM donor. Even those patients who receive a matched BM transplant, frequently suffer from GvH disease (80% of all patients). Another unresolved issue concerns the number of transplanted BSCs. BSCs are rare and their number is limited in transplants. The smaller their number, the longer it takes for the patient's bone marrow to restart blood formation, leaving patients vulnerable to infectious diseases. Despite enormous efforts, we have not	

	managed to maintain BSCs in culture, let alone increase their number. To overcome these problems, scientists are trying to generate BSCs in culture from other, more abundant cell types. Two approaches are currently investigated. One reprograms cells into BSCs by forcing the expression of BSC transcription factors (TFs). The correct combination of BSC TFs will drive all BSC-specific genes and turn the cells into proper BSCs. The other approach tries to gently coach the cells by giving them signals. The right signals given in the correct order and at the appropriate time will guide the cells in a process that mimics embryonic BSC development. Progress has been made using both approaches, but remaining obstacles can only be overcome if we learn more about the signals and the TFs that control BSC development in the vertebrate embryo. To test whether particular genes are involved in BSC formation we need to be able to (a) manipulate their expression and/or control the activity of their gene product, and (b) unambiguously identify BSCs in a model organism. This licence will allow us to generate genetically modified zebrafish lines. This involves the injection of DNA/RNA/protein into pre-free-feeding stage embryos which will then be raised to adulthood. This licence will also allow us to follow blood progenitor cells in the adult. Such experiments make use of a genetic trick that involves the recombination of a reporter transgene by a recombinase that is expressed in these cells. Recombinase activity is induced by 4-hydroxy-tamoxifen. The reporter expression is under the control of a heat-shock promoter. Thus, fish need to be injected with the drug and heat-shocked to achieve reporter gene expression.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from	Being able to manipulate gene expression in zebrafish embryos will allow us to investigate the roles that particular genes play in the molecular programming of BSC development and BSC maintenance in a vertebrate model organism. Genetically modified lines will also help us follow the fate of labelled cells and allow us to trace

the project)?	back the cells' origin at early embryonic stages. The knowledge gained will improve our chances to generate BSCs from more abundant cell types in culture, and open up the possibility to provide patients with patient-matched or patient-specific stem cells for transplantation therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish Danio rerio; our experience tells us that we will need to use about 2,150 animals in 5 years to generate new genetically modified lines. We estimate that about 250 embryos and 100 adult fish will be used to perform lineage studies.
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?	Manipulations performed on embryos (including those that lead to the generation of genetically modified animals) may cause behavioural and physical abnormalities (the frequency will depend on the kind of manipulation). Manipulated animals will be checked on day 4 or 5 and only mildly affected animals are allowed to grow beyond free-feeding. These will then be used for breeding to see whether they pass on the genetic modification to their offspring. In rare cases, adverse effects may also occur as a consequence of general anaesthesia (<5%) and heat shock (<1%). Fin clipping for genotyping (<5%) and intra-peritoneal adult injections (<1%) may cause persistent infections. Fish will be checked daily and only mildly affected animals will be maintained – those where clinical concerns are noted that may exceed the Mild severity limit of this project will be humanely killed using an overdose of anaesthetic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	BSCs can neither be generated nor maintained in culture outside the body. Thus, there is no non-animal experimental system that would allow us to study BSC formation.
2. Reduction Explain how you will assure the use of	Many of our experiments examine success or failure to achieve a desired goal. The number of embryos injected and grown up to generate lines with novel genetic

minimum numbers of animals	modifications (transgene insertions and mutations) can only be estimated from experience. In cases where we compare treated with untreated fish, appropriate statistical tests will be used to ensure that the lowest number of animals is used that gives us statistically significant data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In haematological research, zebrafish has the potential to replace the mouse in a number of experimental contexts. The optical transparency of its embryo and of the Casper mutant adult allows cell imaging in live animals in experiments that cannot be performed in any other organism. The ease with which compound mutants can be generated allows us to answer questions in zebrafish that cannot easily be addressed in the mouse. We apply the most recent refinements in zebrafish transgenesis and genetic targeting to combine experimental success with reductions in (a) the number of animals used and (b) the suffering that these animals experience. Animals treated before free-feeding are checked on days 4 or 5 to ensure that only mildly affected animals are allowed to grow beyond free-feeding. Treated adults are checked at least daily to ensure that more severely affected animals are spotted early and are given appropriate treatment in consultation with the named veterinary surgeon. In cases of persistent infections, animals will be killed to keep animal suffering to a minimum.

Project 37	**Preclinical therapies for renal and cardiovascular injury**	
Key Words (max. 5 words)	Kidney injury, cardiac injury, muscle damage, blocked blood flow, therapy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to develop therapies that could treat patients with injuries to their kidneys and their heart, and with blocked blood flow in their legs. The therapies are either substances or cells that may help heal the injuries. We will use mice in which we induce injuries to the kidneys or heart, or block blood flow to their leg, to test the therapies.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our project may help develop therapies for the treatment of kidney or heart injuries or blocked blood flow. In our project we will test what effect the therapies have on the healing and function of the injured organs (kidney, heart, leg). As we will use cells as therapies, we will follow the cells after we give them to the animal. We will use specialised labels and imaging techniques to follow the cells inside the animal. This is important to assure that the cells do not start forming tumours inside the animals.	
What species and	We will use mice. We may use up to 3000 animals	

approximate numbers of animals do you expect to use over what period of time?	during 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 of the injuries are caused by surgeries. The injuries may cause about 10-20% of the animals to transiently have pain, transiently lose weight, develop scarring on their skin and/or develop tumours. At the end of each experiment, the animals will be killed humanely without pain.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We cannot develop these therapies in humans as they may carry risks for the health of the human patients. We need to understand how the therapies work in the whole animal, as there may be effects to our therapies that cannot be studied in isolation, in a non-animal situation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before the studies we will consult with biostatisticians about the minimum number of animals to achieve our goals.
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 perform our experiments over periods of up to 6 months where we will follow cells inside the animals using imaging techniques. This means that less animals will need to be used. We will find out the optimal conditions for the treatments using small groups of animals before starting a large experiment. The methods we will use have all be well described previously by other scientists and involve accepted models for the diseases.