Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 7

Projects with a primary purpose of: Translational and applied research – Human Cardiovascular Disorders

Project Titles and keywords

1. **Mechanisms of heart regeneration and development**
    - Heart disease, regeneration and development

2. **Analysis of blood clotting in mice**
    - Platelet, thrombosis, cardiovascular, blood clot, NOX

3. **Pathology and prevention of vascular diseases**
    - Stroke, cerebral ischemia, diabetes, myocardial ischemia, thrombosis

4. **Molecular analysis of blood cell development**
    - Blood cell production

5. **Cardiovascular studies in the zebrafish**
    - Cardiovascular, zebrafish

6. **Novel Targets for Pulmonary Hypertension**
    - Pulmonary hypertension, artery smooth muscle

7. **Therapies for heart failure with preserved ejection fraction**
    - Hypertension, heart failure, nitric oxide, NSAIDs

8. **New treatments for peripheral arterial disease**
    - Atherosclerosis, diabetes, poor circulation, ulcer

9. **Models of aortic aneurysm and dissection**
    - Aortic aneurysm, aortic dissection, inflammation, biomarkers

Project 1	**Mechanisms of heart regeneration and development**	
Key Words (max. 5 words)	Heart disease, regeneration and development	
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)	Heart attacks are still one of the world's biggest killers. During an attack, part of the heart dies, which can never be replaced by new heart muscle. Finding a treatment that can repair damaged heart muscle would be of immense value to human health. In contrast to humans, some fish can repair their heart muscle after damage. The Astyanax mexicanus is an extraordinary species of fish living in rivers and caves in Mexico. The fish living in the rivers can quickly repair their hearts after damage. About 100.000 years ago, some of the river fish started living in caves. The lack of light in the caves caused them to go blind and lose all their pigment. With their eye sight, they also lost the ability for heart repair. Comparing the hearts and DNA of the river fish that can repair and the cavefish that cannot repair their hearts will tell us what special mechanism is required for heart repair. This will help explain why fish can and human cannot repair their hearts after damage and to find a treatment for human patients. While the heart beats on its own, nerves are important for changing its beating frequency and pumping force	

	when needed, during exercise for instance. Abnormalities of the nerves result in abnormal heart rhythms, which can lead to sudden cardiac death. This is for example one of the problems after a heart attack, when part of the heart muscle has died and is replaced by scar tissue. Nerves will abnormally grow back into the scar area where they will stimulate the wrong cells to start beating, resulting in repeated abnormal, possibly lethal, heart rhythms. There is not much known about why nerves decide to enter a certain part of the heart during disease, but also during normal embryonic development. Here we will investigate the role of the Slit-Robo signalling pathway during nerve guidance in the developing heart. The role of this cellular pathway during brain development suggests that it plays a role in sending signals to nerves to either enter or avoid a certain region of the heart.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The first project is likely to produce novel information that will be useful in designing therapeutic strategies for heart repair. The results from the second study will help understand how nerve guidance decisions are made in the heart and why nerves wrongly enter a region during heart disease. This will contribute to the prevention and treatment of abnormal heart rhythms in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use Astyanax mexicanus, zebrafish and mouse models. The expected usage of animals, in the procedures outlined, during this project is approximately 600 adult mice and 1200 adult zebrafish per annum (not including breeding and maintenance) over the 5-year lifetime of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In part of our project we need to study heart regeneration in living animals. At the end of the study, animals will be humanely killed and tissues removed for further study and analysis. We have chosen two models of cardiac injury to further investigate heart regeneration, resection and cryo injury, and a wound model. Following the heart surgeries recovery rates are high, 80-90% for resection and >90% for cryoinjury. These surgeries can be combined with pretreatments and imaging. The fish will be carefully monitored and any fish showing

	changes in behaviour such as reduced activity, lower frequency of swimming and/or altered respiration rates exceeding mild discomfort will be humanely killed by Schedule 1 procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to mimic heart regeneration or the effects of a heart attack in in vitro models, e.g. cell culture models. However, whenever possible, we will use in vitro models. Furthermore, a large part of this research will be performed with bioinformatics tools using computers.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce numbers of animals, all experiments are first tested by statistical formulas to determine if the experiment is feasible before it is started and to make sure the minimum number of animals is used to reach significant conclusions. We have expertise in our laboratory with the used protocols as well as extensive expertise in the department.
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 there is no other model known that has a difference in regeneration capacity within one single species and because of its unique characteristics, the Astyanax mexicanus is the most refined model for the intended purpose. Furthermore, its close relation to zebrafish allows us to further explore the results obtained from Astyanax in the zebrafish. Zebrafish are the most suitable model for detailed analyses of the results obtained from the Astyanax studies. The main advantage of the fish models is their ability for adult heart regeneration, which is not possible in mammalian models. The mechanisms that we can learn from them will be crucial for treating patients after a heart attack. To investigate the development of the cardiac innervation, we have chosen for the mouse for its close resemblance to the human heart in combination with the extensive possibilities in genetic modifications.

<table>
<tr><td>Project 2</td><td colspan="2">Analysis of blood clotting in mice</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Platelet, thrombosis, cardiovascular, blood clot, NOX</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>✓</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>
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<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 overall scientific unknown addressed is the regulation of platelets in human vascular diseases. In this project, we will study the role of an enzyme called NOX1, which was recently identified in human platelets. The specific objectives will be:

1) To understand how blood clotting is regulated by NOX1
2) To identify novel molecules that can reduce blood clotting by blocking NOX1</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="2">The information from this project will open new avenues for the treatment of diseases caused by excessive blood clotting, such as heart attacks, strokes and limb thrombosis.

In addition to new knowledge, this project will also generate new molecules able to inhibit blood clotting, which may become new drugs for heart attacks, strokes and limb thrombosis.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td colspan="2">Both normal and genetically modified mice will be used for this research. 1845 mice will be needed in 5</td></tr>
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over what period of time?	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?	Very limited adverse effects can be expected from the procedures required for this project. The genetic modifications required for this project have been shown to have very minor adverse effects (i.e. marginally lower blood pressure and partially reduced immune response to pathogens). The testing of novel molecules will be strictly monitored for the appearance of adverse effects and is unlikely to have negative consequences for animal health. Other procedures used to test blood clotting in the animals will be performed under terminal anaesthesia. This eliminates the risk of discomfort for the animals. Therefore the overall severity level of the project will be MILD.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We minimise the use of animals by using platelets from human volunteers. However, the experimental approaches that we can use with human platelets are limited. This is because platelets do not contain genetic material (i.e. DNA). This means that they cannot be grown in culture and cannot be modified through standard genetic techniques used in other cell types. Mouse gene knockouts provide a powerful approach to understand the role of a specific gene in platelet function. This cannot be replicated with human platelets. Another limitation of experiments using human platelets *in vitro* is that we can only observe the physiology of platelets in isolation, whereas animal experiments offer the possibility of observing complex pathophysiological phenomena involving different cell types, such as blood clotting and blood vessel occlusion.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We keep up with developments in the field to avoid duplication of experiments and use knowledge derived from complementary technologies (including our own *in vitro* studies). Apply statistical analysis routinely to estimate

	minimum numbers of animals required for valid comparisons. Power calculations/curves are used to project numbers, and are required by all major funding bodies. Collect multiple samples from the same animals, where possible in longitudinal studies and/or after animals have been humanely killed. Pool data from different time-points (when appropriate) for statistical analysis. The experimental design described here will allow publication of results according to ARRIVE guidelines.
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 work is specific to mammals, including understanding the regulation of blood clotting and blood vessel occlusion. Therefore, the work cannot be carried out in non-mammalian animals without significantly increasing the risk that observations are not relevant for human health. Mice are the species of choice for the proposed investigations because they are a good mammalian model with a well-characterised microcirculatory patterns and in particular platelet-endothelial interactions that are similar to humans. Studying thrombus formation in mice therefore provides valuable information that will further our understanding of human biology and diseases. In addition, molecular biology and genetic modification of mice have become very successful. A large variety of genetically modified mice has become available, which allows the investigation of the physio-pathological role of a large variety of genes. This is the case of NOX1 and NOX2 in this project.

Project 3	**Pathology and prevention of vascular diseases**	
Key Words (max. 5 words)	Stroke, cerebral ischemia, diabetes, myocardial ischemia, 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
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
	X	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 objectives of the project are to understand the underlying mechanisms of vascular and degenerative diseases to identify novel therapeutic targets. The project will also investigate the effects of potential therapeutic drugs and develop diagnostic tools for early detection of these diseases.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This data gathered from these studies will provide a basis for the development of future therapies to combat these life-threatening diseases. The experiments will also involve testing of potential new drugs for their therapeutic efficacy and identify potential markers, which will aid the development of new diagnostic tools and therapeutic strategy.	
What species and approximate numbers of animals do you expect to use over what period of time?	The project will primarily use mouse but for some studies will involve rats as some surgery can be performed easily in these species without increased risk of mortality and morbidity.	
In the context of what you	All procedures, except non-invasive imaging, feeding	

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?	high fat diet or behavioural studies, will be performed under appropriate anaesthesia as detailed in the project. No adverse effects are expected on recovery. Any animal showing adverse signs of discomfort will be promptly and humanely killed. After the experimental procedures animals will be humanely killed at the designated establishment under appropriate anaesthesia
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The studies described in this project require preparations in which the cardiovascular and the nervous system remain functionally intact. This cannot be achieved using cultured cells. In order to simulate the characteristics of human vascular and degenerative diseases these conditions must be replicated as closely as possible in the whole mammals. Rats and mice have been chosen for this work, since we have extensive experience of experimental work using these species, and we can build on the large body of current knowledge obtained from previous work undertaken in these species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In the biological experiments we will maximise the usage of tissue by preparing it in a manner suitable for analysis by a range of different techniques. This will reduce the total number of animals to be used The optimum experimental conditions have already been determined in earlier studies and as a result the number of animals required can be determined with reasonable accuracy. The total number of animals required has been calculated by using appropriate statistical analysis.
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.	All procedures will be performed under short term anaesthesia and the procedure length will be as short as possible (expected duration <45 minutes) and a heating pad will be used to minimise loss of body heat. All animals will be observed regularly. Any animal showing adverse signs of discomfort will be promptly and humanely killed. We have also refined our techniques, which have resulted in a better success rate and consequently the need for a lesser number of experiments.

Project 4	**Molecular analysis of blood cell development**	
Key Words (max. 5 words)	Blood cell production	
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 understand the molecular processes involved in blood cell development because this will help us to make blood cells in the laboratory that could be used to treat patients with blood disorders and cancers. To this end we are studying the role of specific proteins during blood cell development by creating transgenic animals that lack these proteins and/or have these proteins tagged with markers that allow us to track their progeny. It is currently possible to make some blood cells in the laboratory from pluripotent stem cells but the cells that are produced in this way at the moment are not identical to the blood cells produced in the body. We aim to understand the key differences between cells made in the body and those made in the laboratory as this will help us to improve the efficiency and quality of blood cells that we can make to treat patients in the future. Cells generated in the laboratory must first be tested in an animal model before they could be used in patients. We have	

	chosen to use the immunodeficient mouse as a model for in vivo studies because it is the most appropriate established model available.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A better knowledge about the molecular processes involved in blood cell development would greatly improve our understanding of blood disorders and thus aid in the development of new treatments. Furthermore if we could generate fully functional blood cells in the laboratory with high efficiency we could use these cells to treat patients with blood cell disorders or cancer. At the moment these patients rely on donated bone marrow or blood transfusion. A source of blood cells that is not dependent on donors would have a significant benefit in developing countries where there is no blood transfusion service due to high viral infection rates/
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 18,000 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will generate mice that carry mutations in genes that are involved in blood cell development. These animals may display defects in blood cell production such as anaemia or immunodeficiencies and as a result they may be more susceptible to infection. We also plan to use immune-deficient and/or irradiated animals as recipients to assess the function and survival of blood cells that we have made in the laboratory from pluripotent stem cells. Cells may be injected into blood vessels, bone marrow, and abdominal organs including by surgery in some cases. Adverse effects of these procedures might include an increased risk of infection of recipients and possible morbidity. In some experiments previous experience indicates a small number of mice (~1%) may be found dead without previous clinical signs. We will investigate the causes of any such cases closely during this project but because of this the protocol is classified as Severe. To alleviate the risk of adverse effects we will house all animals in

	individually-ventilated cages and all recipient animals will be carefully monitored to assess their health status. All animals will be culled at the end-point of the experiment, which is typically after 24 weeks, but we will cull any animal sooner if they display signs of sickness where recovery is not expected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We aim to produce blood stem cells and mature blood cells (including red blood cells) in the laboratory. We will carry out many in vitro tests to test the function of the cells we produce but before clinical trials can take place it is essential to carry out some animal testing to assess whether these cells can function appropriately in vivo. We will also use these in vitro culture assays (included those that we have developed from pluripotent stem cells) to assess the function of specific genes. However, there are no culture assays that can assess blood stem cells. The only way to test whether a cell is a true blood stem cell is to test its ability to reconstitute the blood system in a living animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of in vitro systems to generate blood cells in vitro has already led to a significant reduction in the number of animals that my laboratory has used in research over the last few years. These culture systems have already been used as a model for haematopoietic development. However we are now at a point where the function of these cells must be assessed in vivo so there is no alternative but to use animals in these studies. Animals will be bred to achieve a colony size that is sufficient to meet our research needs. The availability of inbred mouse strains permits the study of a gene mutation on a defined genetic background that reduced variability and thus numbers of animals required to reach statistical significance. There is considerable experience in cell transplantation in our centre and, coupled with statistical methods, we will ensure that an appropriate number of animals are used gain a meaningful data sets.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most accessible mammals for studies on stem cells due to the ease and isolation of stem cells. Mouse and human blood stem and progenitor cells share conserved regulatory pathways at various stages of blood cell production. Other advantages of the mouse model include the availability of antibodies for the identification and purification of different classes of stem cells and mature blood cells and the availability of assays that can be done in cell culture (proliferation, colony-forming cell assays) that tests the function of blood cells without the need for animal experimentation. However, ultimately it is necessary to test the function of our cells in an animal model in vivo before they can be used in the clinic. The mouse is the best model because there are well-established protocols to replace the blood system in mice with "test" cells. Also, immune-compromised mouse strains are available so we can test the function of culture-generated human cells without rejection. Another advantage is that the mouse genome is more similar to human than other model organisms (eg fish and frogs) that are used to study blood cell development. The availability of complete genomic sequence and extensive chromosomal synteny, makes the identification of human genes relatively easy. The availability of inbred mouse strains permits the study of a gene mutation on a defined genetic background that reduced variability. To ensure technical competence, the staff performing the experiments will be fully trained and supervised by experienced staff. To minimise infections of immunocompromised animals they will be housed in barrier caging under sterile conditions and handled in a Class 2 cabinet. If mice are in pain, analgesic will be given as directed by the Named Veterinary Surgeon. Transgenic animals exhibiting any unexpected harmful phenotype will be humanely culled under Schedule 1, or in the case of individual animals of particular scientific interest, advice will promptly be sought from the local Home Office Inspector and the Named Veterinary Surgeon. For protocols involving transplantation we have developed and successfully used a stringent scoring

	system which allows an immediate identification of mice with adverse effects. This system will allow us to efficiently minimise animal suffering.

Project 5	**CARDIOVASCULAR STUDIES IN THE ZEBRAFISH**	
Key Words (max. 5 words)	CARDIOVASCULAR ZEBRAFISH	
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)	To describe, understand the molecular and genetic mechanisms of cardiovascular development, growth and disease in zebrafish models. The work to be undertaken under this project license has two main themes which will explore the growth and development of the heart under the influence of genetic and environmental factors that are well recognised to be linked to human disease. This first theme addresses early life programming of molecular pathways by adverse environmental conditions. This phenomenon is well described in mammals, including humans, and is linked with increased risk of heart disease in adulthood. This project will assess whether the zebrafish can be used to further elucidate the mechanisms involved in this process. In addition we will including assessment of whether it affects the regenerative capacity of the zebrafish and will explore whether this has relevance to humanheart disease. The second theme is that of inherited conditions that affect heart function and which can lead to cardiomyopathy. We will use novel models of this condition which will allow us to test new drugs which have the potential to improve outcomes. Novel	

	imaging techniques including magnetic resonance and ultrasound will be used to assess the recovery of injured hearts in live fish. Histological and molecular studies will be performed to assess the types of cells and the molecular processes that influence recovery from injury. All surgery will be performed under general anaesthesia. The number of fish used will be minimised by ensuring accurate measurement using high resolution ultrasound techniques and accurate molecular studies of particular genes and proteins of interest. The benefits of this research are that it will elucidate mechanisms and pathways that could be used in the future for development of new drugs. It may also highlight new drugs that could lead to treatments for 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)?	Better understanding of human cardiovascular disease and obtain novel mechanistic insights which permit the discovery and development of novel therapeutic strategies. The benefits of this research are that it will elucidate mechanisms and pathways that could be used in the future for development of new drugs. It may also highlight new drugs that could lead to treatments for heart disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish, 3000 per year of which about 60% is for breeding and maintaining fish with genetic alterations.
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 animals used for these studies will experience Mild or no significant distress. In some cases, for example during surgery on the heart there may be suffering which we have graded as severe. At all times we will strive at to ensure that fish are handled in a humane and ethical manner. We will ensure this by using appropriate anaesthetic where this is required, we will minimize the number of fish used in any experiment and we will humanely kill any fish showing any perceived signs of suffering or distress immediately upon identification.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whole organisms with the genetic resources now available for this species make it highly suited to the proposed work. Cell culture will be used for some aspects of the work where appropriate.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful power calculation for each experiment where appropriate. Use of highly accurate imaging methods which reduce the number required to observe differences between groups.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The zebrafish is an excellent model in which to study cardiovascular disease during both early development and in adult life. A unique feature of the zebrafish heart is its remarkable capacity to regenerate after injury at virtually all stages of its life cycle. Its pliable genome provides an excellent opportunity to model a number of human cardiomyopathies and to explore fundamental molecular pathways that may modify or reverse the effects of the disease. Animal suffering will be minimized by using embryos wherever possible. A monitoring and scoring system has been established for fish that undergo procedures. Using these scoring systems fish will be euthanased at earlier time points before reaching adulthood. .

Project 6	**Novel Targets for Pulmonary Hypertension**	
Key Words (max. 5 words)	Pulmonary hypertension, artery smooth muscle	
Expected duration of the project (yrs)	5 yrs	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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 overall objective of our research is to advance the understanding of pulmonary arterial hypertension. Pulmonary arterial hypertension, which is high blood pressure in the lung, is a devastating condition that leads to heart failure and death. There is currently no cure for pulmonary arterial hypertension. Pulmonary arterial hypertension is associated with the increased proliferation of cells, in particular pulmonary artery smooth muscle cells, in the walls of the blood vessels in the lung. Excess pulmonary artery smooth muscle cells contribute to the narrowing of the blood vessels and restrict blood flow. We will investigate the mechanisms that maintain normal blood flow and cell growth in the lung, how these are altered in pulmonary arterial hypertension and if remodelling of the lung can be reversed. We believe that by understanding these processes we will uncover new drug targets for pulmonary arterial hypertension.	
What are the potential benefits likely to derive from this	The expected benefits of this project are to provide new knowledge about the pathophysiology of	

project (how science could be advanced or humans or animals could benefit from the project)?	pulmonary hypertension. It is predicted that this project will identify new molecular targets for new drugs for pulmonary hypertension.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (300) and rats (400) 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 work will involve animals developing pulmonary hypertension (using either a hypoxia chamber similar to living at high altitude or by injection) , remodelling of the pulmonary artery and right ventricular hypertrophy. In 20% of cases clinical signs of pulmonary or cardiac failure may be observed but he animals will be killed as soon as they show any clinical signs, hence the level is classified as mild or moderate All animals will be closely observed. All animals will be euthanized either as soon as they show clinical signs of disease or at the end of each study and the tissues used
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible, our laboratory performs experiments using cultured human pulmonary artery smooth muscle cells isolated from patients with pulmonary arterial hypertension to help dissect the molecular mechanisms that contribute to the development of the disease. All novel targets and drugs will be validated in cultured pulmonary artery smooth muscle cells before commencing animal studies. However, since pulmonary hypertension is a complex multifactorial and multicellular disease it cannot be fully modelled using isolated pulmonary artery smooth muscle cells. By consulting FRAME and the Journal of Alternatives to Animal Experimentation no other viable alternative models are currently available for pulmonary arterial hypertension.
2. Reduction Explain how you will assure the use of minimum numbers	All animal studies are based on cell culture experiments using human cells, which validate the target and provide predictive insight into responses of the animal models. Animal numbers will be

of animals	determined using power calculations so as the minimal number of animals will be used to produce meaningful data. Pilot studies will be performed in such a way that any data generated can be added to any further study.
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 experimental protocols used to model pulmonary arterial hypertension are based upon well-established methods optimized to minimize animal suffering. Mice and rats have been shown to be highly effective model organisms to study pulmonary arterial hypertension and have validated the use of now approved drugs and helped uncover novels finding regarding the pathophysiology of the disease. Appropriate observational monitoring protocols are implemented to detect any signs of heart failure as soon as it occurs. Any animals showing signs of heart failure will be immediately humanely killed.

Project 7	**Therapies for heart failure with preserved ejection fraction**	
Key Words (max. 5 words)	Hypertension, heart failure, nitric oxide, NSAIDs.	
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)	Heart failure is a serious medical condition where the heart doesn't pump blood around the body as well as it should. There are two types of heart failure, one with preserved ejection fraction (HFpEF; EF being the percentage of blood pumped by the heart every heart beat) where the heart is unable to relax well enough to accommodate the blood coming back to the heart; and the other type is characterized by reduced ejection fraction (HFrEF), where the heart muscle is too weak to pump the blood efficiently. HFpEF is a common cause of morbidity and mortality, and there is a lack therapies for this condition. There is also controversy on whether or not HFpEF is a preceding stage eventually leading to HFrEF, or a separate entity altogether. The objectives of this programme of research are: 1. To test whether drugs that activate important regulatory systems within the cells of the heart, such as nitric oxide (NO), will prevent the development of HFpEF using a rat model of HFpEF secondary to induction of hypertension (high blood pressure). 2. Given that use of non-steroidal anti-inflammatory drugs (NSAIDs) appear to predispose to HFrEF in susceptible populations (e.g. hypertensive	

	patients), we will evaluate whether the use of NSAIDs in a HFpEF rat model will result in HFrEF as opposed to HFpEF.
What 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 public health problem since it is a common cause of morbidity and mortality. It has been estimated that *1-2%* of the population in developed countries are affected. At least 10 million patients in Europe have symptomatic heart failure. The incidence of heart failure tends to increase in older populations with estimates of up to 10% in patients older than 85 years. This study will assess whether treatment with drugs that activate regulatory systems such as NO, during the phase of cardiac hypertrophy (increased heart size due to hypertension) in hypertensive rats, could prevent the development of HFpEF. This will help the development of novel/preventive therapies for HFpEF, and also contribute to the understanding of the mechanisms o this disease. We will also investigate whether HFpEF and HFrEF are different entities in the heart failure spectrum, this will help us to better understand these cardiovascular pathologies and the predisposing factors leading to one or another heart failure type, and at the same time guide individualised therapies according to the underlying mechanisms of disease progression. These investigations will further our understanding of heart failure, and also in the long term improve the prognosis of patients affected as well as reduce the social and economic costs related to the disease.
What species and approximate numbers of animals do you expect to use over what period of time?	For objectives I and 2, we estimate that -80 rats will be necessary 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?	Induction of hypertension to the rats by high salt diet is unlikely to cause adverse effects since hypertensive rats will remain asymptomatic. However, once heart failure develops at ~19, animals may exhibit moderate signs of congestion (e.g. laboured respiration, weight gain), as well as reduced motility and reduced appetite (severity moderate), therefore animals will be monitored twice a day from week -17 and careful consideration will be made on stopping the protocol if these signs worsen (e.g. loss of motility or appetite) suggesting that we are reaching an endpoint. Very low mortality is expected during the induction of HFpEF up to -19 weeks of age. The Dahi

	salt sensitive rats exhibit normal characteristics whilst maintained on a low salt diet. Isosorbide dinitrate/hydralazyne and sGC activators/stimulators may reduce blood pressure, but doses will be optimised to maximise their benefit on the myocardium without the risk of causing hypotension.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Alternative methods to the use of animal models for cardiovascular research include primary cardiomyocytes (the contractile cells of the heart) cell cultures. Even though we have good experience with these cultures, the proposed work can't be completed using this system as the heart function cannot be evaluated in cell cultures and therefore has obvious limitations for the study of pathologies such as HFpEF.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical advice is provided by staff from our University with expertise in biostatistics, and an appropriate experimental design and sample size calculation has been performed which translates in a REDUCTION in the number of experimental animals. Historic age matched controls will be studied and used in both objectives of the study to REDUCE the number of experimental animals. We will perform longitudinal studies (several observations of the same parameters over a period of time in the same subjects) of cardiac function in the same animal, thus REDUCING the number of animals required for this programme of work.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The model chosen is refined and has been well documented to reproducibly induce HFpEF in a timely fashion, upon implementation of high salt diet. Animal suffering will be minimised by use of appropriate perioperative anaesthesia and analgesia in the several steps proposed for each series of experimental studies. The use of osmotic minipumps is another refinement, since one minor operation under short term general anaesthesia, and with postoperative analgesia, replaces up to 36 days of two to three times daily injections.

Project 8	**New treatments for peripheral arterial disease**	
Key Words (max. 5 words)	Atherosclerosis, diabetes, poor circulation, ulcer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The project aims to improve our knowledge and understanding of peripheral arterial disease (PAD) and diabetic foot ulceration. In PAD, disease in the leg arteries lead to poor blood supply resulting in pain, gangrene and limb loss. People with diabetes suffer from more aggressive PAD and diabetic foot ulcers (DFU) are difficult to treat. Current treatment options have limited success and many patients face major leg amputations. This project will study the pathology of the diseases and evaluate new treatment options for these conditions.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By improving our understanding of the development and progression of PAD and DFU, new treatments will be developed. These will then be evaluated within this project to provide proof of concept, with the aim of moving towards clinical trials for patient benefit in the near future.	
What species and	Mouse (approximately 900) over 5 years.	

approximate numbers of animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The models used in this study will result in some temporary pain and discomfort and possible limping in some animals following surgery and animals will be given analgesia during this period. A small proportion of animals may develop toe discolouration and beginnings of changes that may lead to toe gangrene. These are usually temporary lasting a few days. Animals who do no recover from these effects will be killed humanely. Animals in whom diabetes have been induced may feel unwell with some weight loss initially. Most will recover but continue to show effects of diabetes such as increased drinking and urination and poor wound healing, which enables the effects of diabetes to be studied. All animals will be placed under terminal anaesthesia at the end of the study or terminated immediately if found to be in significant distress or pain.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Laboratory experiments using cells and analyses of tissue samples from patients have identified areas that require further investigation. PAD and DFU involve many complex and overlapping processes which cannot be adequately modelled in the laboratory. The benefits and problems of potential new treatments also cannot be completed assessed in the laboratory.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A significant part of the project is carried out in the laboratory using non-animal models and animal studies are used only when important areas have been identified for further study. By using validated models that are as relevant to the human disease as possible and by clearly defining the study endpoints, the number of animals required are kept as low as possible.
3. Refinement Explain the choice of species	The models which will be used have been shown to be applicable in rats and mice, the lowest mammalian

and why the animal model(s) you will use 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 in which pharmacological studies are usually predictive of the situation in man. Genetically altered mice which mimic the overall disease that occur in patients provide more relevant information and mice where relevant genes are altered enable the study of specific areas which may form the basis of new treatments. Pain killers will be used pre-emptively and as required to minimise discomfort and distress. Where possible, topical application of slow release test agents will be used to reduce the use of invasive techniques and to reduce the dosing frequency required.

Project 9	**Models of aortic aneurysm and dissection**	
Key Words (max. 5 words)	Aortic aneurysm, aortic dissection, inflammation, biomarkers,iniging	
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)	Aortic dissecting aortic aneurysm is a condition involving a tear in the inner lining of the main artery of the body (the aorta) followed by separation of the layers of aortic wall due to blood seeping into the vessel wall. This can block arteries coming from the aorta leading to loss of circulation to vital end-organs or complete tear of the aorta causing massive bleeding. The mortality rate in the first 24 hours is more than 1%/hour with an incidence of approximately 6/1 00,000 in the UK. Approximately 6,000 patients die from this condition each year in England and in Wales. Diagnosis and treatment of the condition while being improved in recent years is still fodused on post-onset management. Identification of a precursory' condition before onset might allow for new strategies aimed at preventative treatment of the condition and revolutionize our approach and management of the condition. The aim of this project is to identify predictive markers of aortic dissection using imaging techniques [e.g. magnetic resonance	

	imaging (MRI), computed tomography (CT), ultrasound] and blood based methods [e.g. flow cytometry analysis (FACS)] to identify localized inflammation of the aorta that is seen prior to onset. Through such efforts, we aim to make identification of precursory conditions and preventative treatment of aortic dissection possible.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We aim to determine predictive markers and therapeutic targets for aortic dissection. The clinical translation of the markers and therapeutic targets will benefit patients at risk for aortic dissection as a non-invasive, safe and more accurate tool for the monitoring and treatment of their condition.
What species and approximate numbers of animals do you expect to use over what period of time?	We would expect to use 6,400 mice and 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?	Mice or rats will be used to the model of aortic aneurysm and dissection, which mimic the main causes of human aortic aneurysm and dissection. Aortic aneurysm and dissection will be induced chemically, e.g. by infusion of agents that increase blood pressure, atherosclerosis, vascular inflammation. The expected level of severity for animals that develop aortic aneurysm/dissection is moderate as some aortic aneurysm/dissection may rupture this would change to severe. Aortic Aneurysm is known to be painful in humans, there is a 5-10% risk of a rupture occurring, if it did occur death would be in a matter of seconds. It is expected that in case aortic rupture occurs, death will be immediate due to extreme blood loss and therefore the animals will not suffer. However, this will be properly assessed. At the end of the experiment the animals will be humanely killed for anatomical, histological, biochemical and molecular analyses. Surgery will be controlled by the use of analgesia which will not be repeated.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because aortic aneurysm and dissection is complex condition and involves the interactions of the whole body system. It cannot be reproduced in-vitro cell studies. In-vivo animal studies cannot be replaced.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will perform procedures using minimum number of animals to achieve statistical significance. Wherever possible, in-vitro cell studies will refine the in-vivo animal 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.	Mice and rats will be used in this study because most relevant methods and techniques are successfully established in mice and rats. Animals will be closely monitored for the sign of suffering during all procedures.