Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 2

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

Project Titles and keywords

1. **Cellular electrophysiology of cardiac arrhythmias**
    - Atrial fibrillation, atrioventricular node, ion channel, myocyte, ventricular tachyarrhythmia

2. **Cell mechanisms controlling small blood vessel diameter**
    - Arteries, vasodilatation, endothelium, smooth muscle

3. **Genetic and pharmacological analysis of thrombosis**
    - Bleeding, thrombosis, platelets

4. **Examining SLFN14 function in megakaryocytes and platelets**
    - Platelets, megakaryocytes, haemostasis, bleeding

5. **Genetic & environment effects on heart development**
    - mouse embryo, development, heart, diabetes, hypoxia

6. **Image Guided Therapy for Cardiovascular Disease**
    - Imaging, heart, stroke, ischaemia, therapy

7. **Gene cell therapies for ischaemic disease**
    - Myocardial infarction, Ischaemia, Diabetes, Stem cells

8. **Re-defining the roles of integrins in angiogenesis**
    - Angiogenesis, integrins, endothelium

9. **Platelet formation and function in thrombosis and repair**
    - Platelets, bleeding, thrombosis, regeneration, heart-attack

10. **Mechanisms of cardiovascular regeneration**
    - Regeneration, Epicardium, Neovascularisation, Vascular Protection

11. **The regulation of platelet function as a central mechanism for the control of cardiovascular function and development**
    - Platelets, thrombosis, heart attack, stroke, clotting

12. **Zebrafish: development, physiology and disease modelling**
    - Zebrafish, development, physiology, disease, transgenic

13. **Regulation of Leukocyte-Endothelial Interactions**
    - Inflamation, Atherosclerosis, Leukocytes

14. Cardiac remodelling and heart failure

- atrial fibrillation, heart failure, ion channel, myocyte, ventricular tachyarrhythmia

15. Morphogenesis during early mammalian development

- Embryo, congenital defect, cell movement

Project 1	**Cellular electrophysiology of cardiac arrhythmias**	
Key Words (max. 5 words)	atrial fibrillation, atrioventricular node, ion channel, myocyte, ventricular tachyarrhythmia	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The heartbeat is normally initiated by an electrical impulse that arises in the upper right-hand chamber of the heart where the natural pacemaker is located and the impulse is then conducted throughout the heart muscle. Disturbances in heart rhythm (termed arrhythmias) arise from problems with pacemaking, for example when rogue impulses arise in other regions of the heart, or abnormalities in impulse conduction. Arrhythmias can be dangerous and are often treated using drugs. Anti-arrhythmic drugs work by either reducing the generation of rogue impulses or by affecting conduction (or both). However, many of these drugs have potentially dangerous side-effects in themselves causing arrhythmias. In addition, many drugs used for treating other diseases also cause arrhythmias as an unwanted side-effect. The atrioventricular node plays an essential role in conducting electrical impulse from the upper to the lower chambers of the heart and is therefore key to co-ordinating the pumping action of the heart. Abnormal atrioventricular node function leads to arrhythmias and a condition known as ‘heart block’ in which the delivery of the electrical impulse to the ventricles is either impaired or prevented completely. It is not understood how heart block arises, although	

	it is likely to involve problems in regulation of AV node function. In comparison with other regions of the heart, very little is known about how the atrioventricular node is regulated. This project examines the mechanisms and targets of action of anti-arrhythmic drugs and addresses specific areas where there is a lack of information regarding the regulation of the atrioventricular node.
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 is likely to reveal new targets for anti-arrhythmic drugs and ways of minimising the risk of arrhythmia associated with the use of therapeutic drugs. Heart block is associated with poor patient outcomes and treatment often involves implanting of a pacemaker device. This project will generate new information about the mechanisms regulating the atrioventricular node that will advance our basic scientific understanding of this under-studied region of the heart. It is anticipated that a better understanding of the regulation of AV node function will lead to the development of novel therapies for the treatment of AV node dysfunction including heart block and arrhythmia.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will be conducted using cardiac preparations from rats (585 over 5 years), mice (690 over 5 years), rabbits (520 over 5 years) and guinea pigs (475 over 5 years). The project will also use 5,400 mice over 5 years for the maintenance of colonies of genetically altered mice in which the TASK-1 or caveolin-3 genes have been ablated or the caveolin-3 gene inserted.
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 procedures in this licence are for the harvesting of viable cardiac preparations or for the maintenance of mice with non-harmful genetic modifications. The animals will be given a single injection and then killed. The expected severity limit is mild and no adverse effects are anticipated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no alternative to the use of animals for these studies as the study requires viable cells that can not currently be grown or maintained in cell culture systems
2. Reduction Explain how you will assure	Sample size calculations will be performed to calculate the number of repetitions required in order for the study to be adequately statistically powered to

the use of minimum numbers of animals	avoid the unnecessary use 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.	The rat represents a species for which there is extensive information regarding physiology of the cardiovascular system. It is particularly useful for the study of the basis to atrial arrhythmias. The rabbit represents the best characterised model for studying atrioventricular node function; however, it will be necessary to carry out a limited number of experiments on the AV node of other species to examine the importance of species differences in mechanisms of cardiac conduction. The mouse represents a species that is accessible to genetic manipulation for the understanding of mechanisms of cardiac conduction and arrhythmia abnormalities. The procedures to be undertaken have been refined to minimise suffering.

Project 2	**Cell mechanisms controlling small blood vessel diameter**	
Key Words (max. 5 words)	Arteries, vasodilatation, endothelium, smooth muscle	
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 research outlined in this project will investigate the cells that form very small blood vessels, which signal between each other and so ensure integrated vasoconstriction/dilation. This is essential, as it allows coordinated changes in blood vessel diameter, ensuring blood pressure and flow are appropriately controlled. In particular, we are interested in the role played by the vascular endothelial cells. These cells are in contact with the flowing blood and by increasing or hyperpolarizing the potential across their cell membrane they cause vessel diameter to increase (vasodilatation). Apart from being essential in the normal day-to-day physiological control of blood pressure and flow, the ability of the endothelial cells to keep blood vessels open (by vasodilatation) is disrupted at an early stage in cardiovascular disease, such as hypertension. Knowing how this comes about will enable the identification of new targets for drug therapy to reduce high blood pressure and prevent strokes and heart attacks.	
What are the potential benefits likely to derive from this project (how science could be	This research will provide fundamental new knowledge about the way that the cardiovascular system is controlled physiologically, and reveal what	

advanced or humans or animals could benefit from the project)?	happens when this control is disrupted by cardiovascular disease. As such, it will advance our scientific understanding of a major body system and inform the rationale design of novel therapy for cardiovascular disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Total maximum numbers over the 5 year project will be: rat 1000, mouse 1000 for protocol 1, 3000 mice under protocol 2, 1000 for protocol 3.
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 procedures are unclassified as they represent minimal intervention under anaesthesia and are all non-recovery. Animals will be killed using a schedule 1 procedure at the end of each experiment. The use of genetically modified mice under protocol 2 is classified as mild as no adverse effects are expected. A limited number of GM mice, such as db/db and ApoE, will be studied under protocol 3 and these have the potential to be classified under moderate severity
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research is not possible without using animals, because vascular cells grown using cell culture approaches change rapidly, so they no longer behave in the same way as the cells found in intact living blood vessels. They are therefore not representative of functional cells within the cardiovascular system. Also, and most importantly, the different cell types that form the artery wall work together using cell signalling mechanisms, the mechanisms we are planning to investigate. So it is essential to study intact blood vessels where the main cells, the smooth muscle and endothelial cells, can interact with each other.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When blood vessels are removed for in vitro studies we ensure the maximum number of vessels are removed for multiple parallel experiments. In this way we minimize the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	The research will use tissue from the rat and mouse. Rats represent the most widely characterized group of lower vertebrates as far as the regulation of the cardiovascular system is concerned. The majority of our in vitro data has also been obtained in this species and with new data will inform in vivo

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	experiments. Vessels from the mouse will be studied to identify any notable differences and to form the basis for studies with genetically modified animals.

Project 3	**Genetic and pharmacological analysis of thrombosis**	
Key Words (max. 5 words)	Bleeding, thrombosis, platelets	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Platelets are small circulatory cells that are essential to prevent bleeding. However, platelets also form a clot (called a thrombus) in an artery when an atherosclerotic plaque ruptures, which can block the artery. If a coronary artery is blocked, blood flow to the heart is reduced and can lead to a heart attack. The aim of the project is to better understand how platelets are activated and how they contribute to thrombus formation.	
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)?	Coronary heart disease leading to heart attacks is one of the biggest causes of death in the UK. The cause of death is often a clot (thrombus) inside a blood vessel supplying the heart. Drugs that reduce platelet activation and their contribution to thrombus formation could help reduce the incidence and burden of coronary heart disease. To develop such drugs, we need to better understand the molecular mechanisms that control platelet activation.	
What species and approximate numbers of animals do you expect to use over what period of time?	This project proposes to use mice. Approximately 2650 mice will be used over 5 years.	
In the context of what you	The majority of the animals maintained under this	

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?	licence are not expected to show any detectable adverse effects. Some genetically altered mice that have platelet defects, however, may develop bleeding defects, in which case they will be kept under close observation in case of injury and .receive special care. All animals will either be killed at end of procedures whilst terminally anaesthetised or otherwise humanely killed, after which various tissues samples will be taken for 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 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 themselves do not have a nucleus. This means that they cannot be grown in culture and cannot be analysed through standard genetic techniques used in other cell types. Mouse gene knockouts provide a powerful approach to understand the role of a gene in platelet function. Mouse gene knockouts are mice that have been genetically altered so that they are missing a specific gene and the protein encoded by the gene. The increased specificity of this approach, compared to pharmacological tools, enables us to draw clear conclusions. Unfortunately, this cannot be applied to human platelets. Moreover, platelets do not act in isolation from other cells. Rather, cardiovascular disease is a product of the interaction between multiple cells including platelets, vascular smooth muscle cells, endothelial cells and other blood cells. Our analysis of this complex process combines experiments on platelets in isolation from other cells, with experiments in a whole animal setting. This is vital to allow us to analyse gene function in the setting and context of these other cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers bred for use on this Project will be minimised as far as possible by matching breeding to experimental requirements. Pilot studies and power calculations will be employed to refine the number of animals used. The methods chosen will generate the greatest amount of data for the fewest animals used. We routinely expect to derive multiple data sets from a single animal, by extensive use of modern approaches that allow us to analyse very small blood

	samples.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the species of choice 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. Genetically-altered mouse technologies are becoming increasing sophisticated, where genes can be deleted within specific cells whilst leaving the rest of the animal unaffected. Such genetically altered animals will be used wherever possible in this project and will greatly reduce the risk of adverse effect to the mouse. This is because only the platelets will be genetically altered, whilst all the other cells in the mouse will be normal.

Project 4	**Examining SLFN14 function in megakaryocytes and platelets**
Key Words	Platelets, megakaryocytes, haemostasis, bleeding
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

To determine how platelet function and megakaryocyte development is regulated by a novel gene/protein called SLFN14, and how this defective gene/protein leads to a reduced platelet count (thrombocytopenia) and excessive bleeding in humans. The fundamental role of platelets and megakaryocytes (their precursors) in blood clotting in humans is shown by the excess bleeding associated with thrombocytopenia (too few platelets) which can be potentially life-threatening. The ultimate goal of our work is to identify new targets for development of novel antiplatelet agents with increased efficacy and reduced bleeding tendency for use in prevention of thrombosis and other platelet disorders.

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)?

Reduced numbers of platelets are associated with excessive bleeding in humans. By understanding how platelets and megakaryocytes are produced we can identify new targets for drug development. A drug that can prevent excessive bleeding that occurs in these patients will save lives

What types and approximate numbers of animals do you expect to use and over what period of time?

Over 5 years, we would expect to use no more than 13,500 mice in total – 3,500 animals for the experiments and 10,000 to breed the genetically altered strains required

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

We expect the mouse model defective in SLFN14 to mimic the excessive bleeding observed in patients with SLFN14 mutations. This includes increased cutaneous bruising and prolonged bleeding after minor wounds. These symptoms are expected to be mostly mild to moderate in severity. The animals will be checked regularly for signs of bleeding, pain and distress, and will be culled if these symptoms or signs of excessive bleeding occur.

Application of the 3Rs

Replacement

The essential role of platelets and megakaryocytes (their precursors) in blood clotting in humans is illustrated by the profound bleeding associated with thrombocytopenia (too few platelets) which can be potentially life-threatening. The ultimate goal of our work is to identify new targets for development of novel antiplatelet agents with increased efficacy and reduced bleeding tendency for use in prevention of thrombosis and other platelet disorders.

The study of novel genes and use of mutant mouse models implicated in bleeding such as the novel protein SLFN14 is a key step in determining their roles in platelet and megakaryocyte formation.

Mammalian platelets do not have a nucleus and so cannot be genetically modified and megakaryocytes are extremely difficult to isolate in large numbers. We will use genetically-modified mice to study the role of the protein in platelet production. We cannot address this with limited patient material and the difficulty of obtaining megakaryocytes from patients.

The novel protein SLFN14 has little or known function and there is little clue from the in *vitro* studies that have been carried out by us to date.

Reduction

Reduction will be achieved by first performing experiments on primary megakaryocytes, iPSC cell lines and human patient platelets, using gene manipulation techniques such as CRISPR and shRNA to identify the mechanism through which SLFN14 regulates megakaryopoiesis and platelet function.

Reduction will be achieved by first performing experiments *in vitro* on primary megakaryocytes, iPSC cell lines and human patient platelets, using gene manipulation techniques such as CRISPR and shRNA to identify how SLFN14 regulates megakaryocyte and platelet function.

Statistical analysis to ensure that we use the minimum number of mice per group will be performed. Where appropriate we use power calculations to ensure that we are using the appropriate number of mice.

To maximise the information gained from a single animal we aim to take perform multiple *in vitro* analyses on each individual.

Refinement

We cannot use a non-mammalian species for this work, as mammals are the only animals to have platelets. In mice, there is established and reliable transgene technology, and established models of platelet function. There are a large number of genetically modified mutants that are available and there is an extensive amount of work that has already been performed and published using mouse models of thrombosis and haemostasis.

Choice of methods

Blood collection

The major route of blood collection is through terminal anaesthesia and humane method of euthanasia which causes minimal harm, and preserves the integrity of the platelets. In cases where serial samples are required, a safe volume of blood is removed from a peripheral surface vein.

Bone Marrow Collection

The major route of bone collection is through terminal anaesthesia and a humane method of euthanasia as this causes minimal harm, and preserves the integrity of platelets to be collected simultaneously. Femurs will be removed after euthanasia and marrow flushed from the bones in order to isolate megakaryocytes.

Further refinement of studies during the course of this project

We cannot use a non-mammalian species for this work, as mammals are the only animals to have platelets. In mice, there is established technology and good models of platelet function.

During the experiments we will monitor the health status of the animal on a daily basis. This includes mice which have received injections of substances to alter platelet function or undergone tail bleeding time measurement.

Animals will be humanely killed if they lose more than 20% body weight compared to littermate controls or show any physical signs of adverse effects or inactivity which will be monitored daily. If any of these signs are observed constantly for over 24hrs, the animal will be humanely euthanised.

For all procedures, we will continually monitor the literature for methods of refinement and consider whether the use of animals is necessary to address the experimental question under investigation. As a routine measure, during invasive protocols each animal will receive analgesia which in not anti-inflammatory in nature to reduce pain and distress.

<table>
<tr><td>Project 5</td><td colspan="2">Genetic & environment effects on heart development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">mouse embryo, development, heart, diabetes, hypoxia</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="9">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>x</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td></td><td></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">Scientific and clinical unknowns

Congenital heart disease (CHD) is the most common human birth defect, with an incidence of up to 1% of live births. It is defined as a structural abnormality in the heart that is present at birth, and it is a major cause of infant mortality and morbidity, requiring ongoing medical treatment throughout life. Currently only — 20% of CHD can be attributed to known causes such as genetic syndromes. This is partly because our understanding of the genetics of embryonic heart development is incomplete, and partly because non-gtic factors, such as maternal diabetes or embryonic hypoxia, can perturb this process.

Purpose of the project

This project uses wildtype and genetically-altered mouse lines to investigate the roles of particular genes in the processes of embryonic heart formation. When the function or expression of such genes is perturbed, then they are likely to predispose, or cause, heart defects. It also investigates how environmental stressors, such as maternal diabetes or embryonic hypoxia, can also cause heart defects. The work plan consists of: (i) identifying genes important in the</td></tr>
</table>

	shaping the developing heart, and in particular those involved in the formation of the outflow tract of the heart; (ii) investigating environmental factors that can perturb normal embryonic heart formation, arid lead to heart defects; and (iii) identifying new methods or pathways for preventing heart defects by 'correcting' heart development in the embryo or foetus.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	**Expected benefits** (I) increased understanding of embryonic heart development, both normal and abnormal leading to heart defects (ii) improved methods of genetic diagnosis and genetic counselling in humans, which should follow from discovery of genes that cause heart defects in mice (iii) design and testing of therapies to reduce the incidence of environmentally caused heart defects.
What species and approximate numbers of animals do you expect to use over what period of time?	**Numbers of animals to be used** In this project, the majority of mice will be used for purposes of breeding to maintain colonies of genetically altered strains. It is extremely difficult to estimate the numbers required as this depends on what proportion of mice will be affected with heart defects. We estimate that up to 2,000 mice per year will be involved in the breeding programme; and up to 1,000 mice per year will be mated to produce pregnancies containing embryos for the study. Mated mice will be killed to remove embryos for the studies, or recycled into the breeding colony. We will use up to 3,000 embryos per year that are more than two-thirds through gestation.
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 and maintenance of genetically altered lines in the vast majority of cases (90%, i.e. 10,800 of the total 12,000 animals within the project) will have no outward phenotype and lie within a mild severity category. In approximately 1-2 lines (total of 1,200 mice) there may be increased severity of adverse effects as associated with cardiac insufficiency (including increased heart rate, breathlessness, inactivity and failure to feed). These animals will be monitored and where distress exceeds a moderate severity limit they will be humanely culled. In the studies involving administration of substances to alter transgene expression and/or induce diabetes in pregnant mice, treated adult female mice will normally be humanely culled prior to birth of their litter (19 days

	after mating) for embryo collection. Prior to culling, they will have their blood glucose levels and behaviour monitored closely, and where distress exceeds a moderate severity limit, they will be humanely culled and their embryos collected early. Embryos will normally be studied at an early stage of development, before pain or other sensations have been acquired. These embryos are killed almost the moment they are taken, so there is minimal potential for suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are investigating how the specific shape and function of the heart forms in the embryo. Mice have a four-chambered heart that develops in a very similar manner to that of humans. In addition, the genes, proteins, signalling pathways and cells Involved are almost identical. Embryonic heart development is a four-dimensional process (i.e. varying in space and time), and therefore requires the analysis of whole developing embryos. Direct genetic studies of foetal (embryonic) humans are difficult practically, and only descriptive analysis is possible, with experiments ruled out on ethical grounds. Tissue culture systems, although they can provide useful information on certain molecular or cellular phenomena, cannot mimic the complexity of a functioning organ such as the heart, let alone the developing embryo. Computer simulations can be valuable in extending theoretical approaches to embryonic development, but cannot tell us about the real biological processes occurring in the embryo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Appropriate calculations will be performed in order to obtain statistically relevant results and to ensure that the maximum amount of scientific information is obtained from each individual animal. Where possible, when animals used in procedures are sacrificed, their tissues will be shared with other laboratories. This should help minimise animal use.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare	We have chosen to study a mammalian species, the mouse, so that the principles emerging from our research have the greatest chance of applying to the human situation. Mice have been used throughout scientific history and are today considered one of the best models of development. Thus it is the most refined choice for our studies. If we used simpler animal model, we would have to develop the appropriate genetic tools, ultimately resulting in more

costs (harms) to the animals.	animals being used. It would also not accurately reflect human heart development, and hence would reduce the clinical application of our data.

Project 6	**Image Guided Therapy for Cardiovascular Disease**	
Key Words (max. 5 words)	Imaging, heart, stroke, ischaemia, 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
		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)	Cardiovascular disease is the leading cause of death worldwide, and its prevalence is still increasing. Hence there is a requirement to find new methods to accurately diagnose pathology and develop and evaluate new therapies to treat heart disease and stroke. We will develop and apply a range of novel biomedical imaging techniques (MRI, ultrasound, nuclear) which will be able to give more accurate information on what causes cardiovascular disease and more accurate measurements of how effective novel treatments are. We will test a range of novel therapies aimed at reducing damage caused by disease and repairing the tissue that was lost. These approaches will include testing of new drugs to reduce damage from stroke, testing stem cells as means to regenerate the heart, and giving gene therapy that can prevent heart failure. Importantly, we will develop and utilise our novel imaging methods to directly visualise and measure the effects of these treatments, guiding the optimisation of novel therapies.	
What are the potential benefits likely to derive from this project (how science could be	Innovative imaging techniques that can directly inform of the processes of disease. This will allow us to visualise the processes that cause disease within living animals and eventually humans. This can then be applied to	

advanced or humans or animals could benefit from the project)?	diagnosis of disease in patients and in veterinary animals. By performing these experiments using medical imaging methods in live animals, the same animals can be images at different stages of the disease, reducing the number of animals needed in research. Novel imaging methods to follow the mechanisms through which novel treatments work. This will allow us to visualise how treatments prevent disease within living animals and eventually humans. This can then be applied to diagnosis of treatment of patients and veterinary animals. By performing these experiments using medical imaging methods in live animals, the same animals can be images at different stages before, during and after therapy, reducing the number of animals needed in research. New treatments that can prevent disease and repair damaged organs. By using imaging to guide the optimisation of treatments we hope to develop effective new therapies for some of the leading causes of mortality and morbidity, namely heart disease, peripheral artery disease and stroke.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use rats (approx. 1650) and mice (approx. 2150) 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?	Models of myocardial infarction pressure overload and genetic/cardio- toxic heart disease will result in moderate adverse effects, such as breathlessness and lethargy. Peripheral artery disease can cause pain, but analgesia will be administered to alleviate suffering. Stroke can cause temporary disorientation and moderate discomfort. After the indicated periods of experimentation, animals will be sacrificed using approved methods and their tissues will be extensively studied so that the maximum amount of information can be gained from each animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The objective of this study is to develop translational non-invasive imaging methods which can provide information essential for guiding and optimising novel therapies for cardiovascular disease. Although imaging of liquids in tubes will initially be used to test imaging techniques, this does not incorporate the complex physiology of an in-vivo study. Although drug and gene therapies will be tested in cells grown in a dish in the lab, it is also necessary to test therapeutics in animal models, owing to the complex

	nature of cardiovascular diseases. Lower species, are enough to humans to allow development of translational imaging. Hence to progress towards our objectives, there are no alternative to the use of experimental animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Monitoring animals by in vivo imaging makes a major contribution to the reduction in animal numbers as each animal can be used as its own control. Sequential experimental design in which the same animal is monitored longitudinally over a number of time points provides paired data acquired before and after treatment, normalising baseline variability and increasing statistical power, meaning that fewer animals are required to achieve statistical differences. Live imaging allows multiple imaging methods to be applied to the same animal and hence more scientific information to be acquired, reducing the number of animals that will need to be included in a 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.	Mice and rats are the most suitable model species for these investigations, as organ anatomy, physiology and injury response is similar to that of higher mammals. The anatomy and inherent regenerative capacity of lower species, such as zebrafish, make them unsuitable for this research, while at present our imaging capabilities are limited to small animal, making large animal research unfeasible. All the models described in this licence are routinely used in research and have been well refined to cause minimal suffering and to provide reliable data, We have extensive experience of the surgical models described and will take guidance from collaborators when needed. Our use of ultrasound guided injections offers a method for delivery of cells and substances to the animals without having to perform surgical procedures. Using imaging to track the retention of cells and biomaterials invivo refines the currently used technique of serial sacrifice of animals, with histology performed at each time point. When using imaging measurements of small morphological changes in animals, it is often possible to use milder disease than with other assessments. live imaging of animal disease models can provide earlier humane endpoints and rigorous inclusion/exclusion criteria.

Project 7	**Gene and cell therapies for ischaemic disease**	
Key Words (max. 5 words)	Myocardial infarction, Ischaemia, Diabetes, 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)	To investigate the potential of genes, proteins and cells therapies for the treatment of heart disease and limb disease caused by occlusion of arteries and capillaries, a condition particularly frequent in patients with diabetes. The ultimate goal of this research is to develop new therapies that are able to meet the clinical needs of cardiovascular patients with or without diabetes.	
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)?	Current treatments for heart attacks and poor circulation to lower extremities reduce symptoms but do not result in a complete repair. Diabetic patients represent the most challenging problem. This project aims to benefit cardiovascular and diabetic patients by identifying new treatments that are able to repair damaged tissues.	
What species and approximate numbers of animals do you expect to use over what period of time?	The study will be conducted in mice and rats for the duration of 5 years and include a few thousands of animals.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects may occur as a consequence of the — procedures employed to induce heart and limb muscle damage similar to that observed in patients with cardiovascular disease and diabetes. These procedures have a moderate severity level: they carry the risk of possible surgical bleeding and postoperative pain. The modelled disease carries symptoms caused by reduced pumping capacity of the heart, poor circulation to peripheral tissues and dehydration associated with uncontrolled diabetes. Specifically, animals may experience fatigue, shortness of breathing, irregular and accelerated heart beats, inappetence, and difficulty in deambulation, which are the typical symptoms experienced by cardiovascular/diabetic patients. The treatments we are investigating here are intended to correct the cause of these symptoms. In fact, from our previous experience using these interventions the vast majority of animals recover well and show no signs of suffering or distress. At the end of the experiments or early if they manifest adverse effects, 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	We will be using rats and mice for these studies because the complexity of cardiovascular disease/diabetes and the therapeutic efficacy of proposed treatments cannot be effectively tested in non-animal systems. However, preliminary studies of cell functions will be carried out before engaging with animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use statistical approaches which is based on our previous experience and will allow us to determine the minimum number of animals needed to achieve our aims
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	Mice and rats are the less neurological developed species that can be used to test the therapeutic interventions we hope to progress into the clinical setting. We will use medicinal and environmental measures to attenuate pain, discomfort, infections and stress. We will adopt refined microsurgical techniques to minimise the adverse effects of surgery. All animals

take to minimise welfare costs (harms) to the animals.	will be carefully monitored after surgery and recorded individually. Wherever possible animals will be group housed and provided with an enriched environment. _____

Project 8	**Re-defining the roles of integrins in angiogenesis**	
Key Words (max. 5 words)	Angiogenesis, integrins, endothelium	
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)	Angiogenic therapies directed against endothelial integrins offer great hope for treating a large number of human disorders, such as cancer or recovery from a heart attack. They have not yet reached their full potential, This project explores in detail how these proteins function and cooperate to regulate both normal and abnormal angiogenesis so we can understand how to develop better treatments.	
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 improve upon current angiogenic treatments, particularly those used to treat cancer patients, that are meeting with some success but whose effects are often short-lived (e.g. extending lives rather than curing disease).	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 17,400 genetically altered and wild-type mice over the 5 years of this project.	
In the context of what you	We expect no more than moderate discomfort from our	

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?	surgical procedures, tumour implantations or inductions, or substance administrations. At the end of each procedure, all animals will be killed by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	During development of disease, angiogenesis occurs in the context of whole tissues, involves a large number of different cell types, and happens under blood flow. Currently these attributes cannot be adequately replicated outside the organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We do use cells and explants where possible, meaning we can gain large amounts of information from a small number of animals. We immortalise cells where possible, which allows us to reduce numbers even further. Prior to experiments we perform statistical Power Calculations to determine the smallest number of animals possible to achieve meaningful data, and we routinely perform meta-analyses on pooled experiments to uncover subtle differences between groups that might have otherwise required repeated 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 mice because they are genetically altered in the expression of proteins we believe are- critical for angiogenesis; this gives us a strong experimental tool to explore their role(s) in both normal and abnormal angiogenesis. Our protocols are routinely examined to ensure they are up-to-date and incorporate current best practice. Where surgery is required we employ analgesia to ensure minimal discomfort. We use early humane endpoints for all studies.

Project 9	**Platelet formation and function in thrombosis and repair**	
Key Words (max. 5 words)	Platelets, bleeding, thrombosis, regeneration, heart-attack	
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 aim of this project is to broaden our mechanistic understanding of platelets, a critical 'gatekeeping' cell in the bloodstream that serves multiple roles in physiology and pathology. The specific objectives of this project address key clinical needs, relevant to patients with bleeding diathesis requiring platelet transfusions and to cardiovascular disease patients at risk of, or recovering from a heart attack. For instance, it is estimated that approximately 155,000 people in the UK will die annually as a result of cardiovascular disease. To address this we aim to: 1. Improve our understanding of how platelets are produced and released into the bloodstream; 2. Identify novel genes that regulate platelet-dependent thrombus formation; 3. Investigate novel roles for platelets and biomolecules released from platelets in regulating the regeneration of cardiac tissue following a heart attack.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This proposed work will enhance our understanding on the regulation of platelet production and function in the body. By identifying new molecules that regulate platelet numbers and function we aim to translate these findings into advances in clinical diagnosis and the development of new therapies that can be used in the primary prevention and secondary management of cardiovascular diseases, such as heart attack and stroke.
What species and approximate numbers of animals do you expect to use over what period of time?	This project only proposes to work with mice and it is expected that approximately 2,000 mice per annum (including breeding and maintenance) will be used over the 5 year lifetime of the project (totals 10,000 mice).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most protocols proposed are of a mild severity category, for which we do not foresee expected adverse effects associated with these procedures. For our intravital microscopy work, assessing platelet production and thrombosis, and our model of myocardial infarction involving non-recovery, all mice used in these procedures will be under general anaesthesia. Subsequently, mice will be humanely killed by a schedule 1 method and tissues removed for further study and analysis. For the mouse recovery protocol following heart attack and limb ischemia, where the severity level is severe, mice will be given painkillers and be closely monitored in the ensuing days or weeks. Any mouse that displays signs of discomfort or distress (increased heart rate, breathlessness, inactivity and decreased feeding) will be humanely killed.

Project 10	**Mechanisms of cardiovascular regeneration**	
Key Words (max. 5 words)	Regeneration, Epicardium, Neovascularisation, Vascular Protection	
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)	While cardiovascular diseases remain the primary cause of mortality and morbidity worldwide, there is an urgent need to tackle the clinical and economic burden of heart failure. Our project aims to understand how to protect blood vessels against disease, such as heart attack and stroke and, since this is not currently possible and heart attacks are prevalent, to encourage the heart's own repair processes to replace damaged muscle in heart failure patients. Our previous research led us to identify a powerful growth factor that functions in the body to repair wounds. We found that Thymosin β4 is required in the embryo for development of stable, muscle-coated blood vessels and for migration of cells from the outer layer of the heart (epicardium-derived cells; EPDCs) to build coronary vessels. Significantly, we found that, if we treat adult mice, in which we surgically induce a heart attack, with this factor, these "dormant" embryonic processes can be reactivated to replenish muscle and restore blood	

	supply, leading to an improvement in heart function. Although very promising, the proportion of new muscle that can be created from EPDCs is currently inadequate. We will build on these important findings and i) investigate the role of growth factors in vascular disease and potential to repair diseased vessels; ii) explore strategies to enhance EPDC-based heart regeneration.
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)?	Damage to blood vessels causes accumulation of cholesterol and leads to destruction of muscle and elastic layers, to weaken the vessel. Rupture of weakened vessels causes life-threatening events (heart attack, stroke or aneurysm – balloon-like swelling of the body's main artery). Our studies to date have given us some insight into the signalling that occurs on the surface of vascular smooth muscle cells to protect them from destruction. We hope to increase our understanding of these pathways and tests key growth factors that could be used therapeutically to protect against these major life-threatening diseases. After a heart attack, a significant portion of the heart's muscle is irreversibly damaged, leading to heart failure in an increasing number of patients. By showing that dormant cells in the outer layer of the heart (called EPDCs) can be re-activated to make new muscle and blood vessels, we have already identified a promising new target to treat heart failure. However, we believe the process can be made far more effective, if we understand more precisely the signals that control EPDCs. Encouraging the heart to rebuild itself by making new muscle may provide a powerful treatment for the 900,000 heart failure patients in the UK and millions worldwide.
What species and approximate numbers of animals do you expect to use over what period of time?	Our work will be performed in mice. We expect to use a total of 16,000 mice over the 5 year term of the project. 12,000 of these will be used for breeding and collection of tissue samples; only 4,000 will undergo more invasive procedures as part of our experiments.

Project 11	**The regulation of platelet function as a central mechanism for the control of cardiovascular function and development**	
Key Words (max. 5 words)	Platelets, thrombosis, heart attack, stroke, clotting	
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)	Through our research we aim to understand how the blood cells know as platelets that cause the blood to clot are controlled. This is important because they need to be able to sense when injury has occurred in order to prevent excessive bleeding. The function of platelets is also be triggered by diseases of the heart and blood system, which cause thrombosis, or blood clots in the circulation. Thrombosis is the trigger for heart attacks and strokes that are major causes of death and illness. By understanding the molecules and processes that platelets use when they encounter healthy, injured or diseased blood vessels will enable us to develop new medicines that interfere with these processes, make platelets less active (in a controlled way) and therefore reduce the risk of heart attack or stroke. So our questions include what are the proteins on the surface of platelets that function as probes to detect injury or disease, what do they detect, and how to they then control the proteins	

	present within platelets that turn blood clotting on or off.
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 goal of this research is to determine how we could design new drugs to prevent thrombosis. Current drugs that target platelets and suppress their activity to prevent thrombosis are effective in many patients, but a large number of patients gain no benefit and go on to have a heart attack or stroke. Worryingly, most current 'anti-platelet' medicines also cause lots of side effects. Of particular importance are bleeding side effects, which can be life threatening and these occur frequently. Understanding the precise molecules in platelets that control each aspect of their function will enable us to identify mechanisms that may be more effective and safer (i.e. fewer side effects) targets for new anti-thrombotic medicines. Platelets are also involved in other diseases such as cancer, and any drugs that control their function are likely to be beneficial in the treatment of these additional conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	We will breed mice in this project and use these to ask specific scientific question. We expect to breed up to 2000 mice per year, or 10000 over 5 years. Most mice that will be used in these studies will be genetically modified, which means that we need to breed these ourselves.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In most cases the breeding of genetically modified mice will result in mice that may have mildly defective blood clotting, but are essentially healthy. On occasions it is possible that a genetically modified mouse may suffer from unhealthy bleeding or thrombosis, although in our experience this is very rare. Should this occur and exceed acceptable limits of severity (mild or moderate), mice will be killed humanely as soon as possible. The experiments that we do with the genetically modified mice to test bleeding and thrombosis will be performed under general anaesthesia without recovery, so the mice to not feel anything and do not wake up after the experiment. These protocols therefore have a non recovery severity limited. Some mice will receive

	drugs that affect platelet function or platelet number prior to experiments, or on rare occasions will be allowed to recover following analysis of bleeding. In some cases mice will be exposed to radiation and bone marrow transplantation. In these cases the health of mice will be studied very closely, and should they fail to thrive, show evidence of isolation, distress , bleeding or are generally unwell, they will be killed humanely as soon as possible.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Platelets do a specialised job that occurs specifically in blood flowing in blood vessels. For this reason if we need to study their ability to trigger blood clotting this needs to be performed in a living animal. All of our early fundamental research is performed using human platelets in the laboratory including the study of platelet clot formation in flowing blood, but in order to study the implications of specific processes in these cells on thrombosis and bleeding, the use of animals, and particularly mice, is essential because these processes are only triggered in conditions that are present in the body. In addition, platelets lack a nucleus so they are unable to divide in culture. This also prevents us from using molecular biology to alter genes in human platelets in the laboratory. Transgenic mice are therefore used for this purpose, where the mouse itself produces genetically modified platelets for our experiments. The mouse is considered a good model for studying thrombosis in a way that is similar to thrombosis in humans. Well-established standardised tests allow our data to be compared with a large scientific literature.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use statistical power calculations to ensure that we use the minimum number of mice in order to answer each of our scientific questions. This will be based on our experience of the tests that we perform and how variable the readouts from these are, the size of change that is physiologically meaningful, and statistically how reliable the data are (i.e. to rule out things that might happen by chance).

	Mouse usage will also be minimised in the following ways: • Use of efficient statistical designs to increase precision, e.g. use of litter mates as controls • Use of efficient breeding protocols that minimise the production of unwanted animals (numbers or genotype) • Utilisation of surplus mice that do not have the correct genetic makeup (for experimental use) for breeding. This avoids the need for additional mice for this purpose • Use of pilot studies where new types of mice are used • Use of modern efficient laboratory tests that allow fewer animals to be used When necessary, or appropriate, a statistician will be consulted to ensure an experimental design is optimal and minimises the number of animals required
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are established models for human haemostasis and thrombosis. While there are subtle differences between mouse and human platelets, in most cases mice model normal and diseased processes in humans well. Mice also allow us to overcome the limitations that are caused by an inability perform molecular biology on cultured platelets, due to the resource of available genetically modified mouse strains, to produce platelets that are genetically altered (removing or adding genes). Combined together the advantages of using mice rather than other species have allowed mice to become the preferred pre-clinical model for studies of platelet function. In recent years we have been involved in studies that have resulted in the development of more refined measurements of thrombosis in mice that allow more precise and sensitive analysis in which variability is minimised. The ability to analyse multiple parameters simultaneously in a single mouse, in a single thrombus, represents an important development since this improves the depth and quality of information than is obtained while also reducing the

	numbers of animals used. The breeding of new genetically modified mice may result in unexpected levels of suffering. All mice will therefore be very carefully studied for signs of ill health and killed humanely if necessary. Based on our experience these occurrences are rare due to having first performed relevant experiments using human platelets. Measures are therefore taken to avoid making transgenic mice in which substantial health issues are likely to be encountered.

<table>
<tr><td>Project 12</td><td>Zebrafish: development, physiology and disease modelling</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Zebrafish, development, physiology, disease, transgenic</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td></tr>
<tr><td>Regulatory use and routine production</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To learn more about vital processes that occur during development of vertebrate animals and how these lead to disease in humans when they are defective.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>1.) To provide an improved understanding of how the kidney functions in healthy animals, and how defects in kidney function lead to disease in humans. This information will inform treatment of patients with kidney disease and can be exploited to develop new drugs for treatment of such disease.

2.) To provide an improved understanding of how the skull and facial skeleton and the heart are formed. The research will also reveal how defects in the development of these tissues lead to disease in humans.</td></tr>
</table>

	3.) To determine the mechanisms and pathways that are required for the formation of new blood vessels. New blood vessel formation is a vital process during development and also for the maintenance of health. A better understanding of how blood vessels are formed will inform better therapy for a number of human diseases including cardiovascular disease and cancer. 4.) To reveal the mechanisms that occur in brain haemorrhage. The work will also lead to design of better screens for testing drugs that may be used to treat or prevent brain haemorrhage.
What species and approximate numbers of animals do you expect to use over what period of time?	168,540 zebrafish 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?	Zebrafish will be genetically modified and this may lead to developmental abnormality or genetic disease that could incur suffering (although zebrafish have much lower neurological complexity than mammals) in a limited number of animals. This will be mitigated by frequent inspection and early intervention. Zebrafish will also be injected with molecules that are unlikely to cause any suffering. All animals will be sacrificed at the end of the experiments using humane methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The processes described in this proposal occur in the context of an entire tissue, and cannot be effectively recapitulated using non-animal models. Invertebrates also cannot be used as the processes under investigation are poorly conserved in such species compared to humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Efficient experimental design and statistical techniques such as power analysis will keep the number of protected animals used to a minimum.

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 is the vertebrate of lowest neurological complexity that can be genetically modified to study the processes of interest. Any genetic manipulation indicated to interfere with feeding, locomotion, respiration or cardiovascular function, or inducing significant behavioural or other physiological abnormality will result in immediate termination of the organism concerned and other animals sharing the genotype.

Project 13	Regulation of Leukocyte-Endothelial Interactions
Key Words	Inflammation, Atherosclerosis, Leukocytes
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The objective of this Project Licence is to identify new targets for the development of medicines that will be beneficial to people with cardiovascular disease. Although there have been excellent advances in the treatment of heart disease in recent years, atherosclerosis, the hardening of arteries supplying the heart with blood, is still a major cause of morbidity and mortality in the developed world. Inflammation plays an important part in many cardiovascular diseases including atherosclerosis (hardening of the blood vessels supplying the heart) yet there are no effective anti-inflammatory treatments currently in use to treat this common disease. The inflammatory response is mediated by white blood cells that interact with the cells lining blood vessels. This process is controlled by changes in the adhesiveness (or stickiness) of both types of cell to one another. Learning about how these processes are regulated will provide useful information for the development of new medicines for the treatment of the underlying causes of cardiovascular disease.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

This project will give us a great deal of information about how cells and molecules that are involved in normal processes in the body may also contribute to inflammation and, ultimately, cardiovascular disease.

What types and approximate numbers of animals do you expect to use and over what period of time?

All of the animals used in this licence are mice as they are the lowest animals on the evolutionary scale where suitable models of inflammation and atherosclerosis have been developed. Over the 5-year duration of this licence we will use a total of 6800 mice.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Approximately 40% of the mice will be used to supply blood for the isolation of cells to be used in in vitro experiments that will give us information that we can use in designing subsequent experiments in animals. This procedure involves an injection of an anaesthetic overdose followed by collection of blood from the heart. Approximately 20% of the animals will be used to look directly at the interaction of white blood cells with the cells that line small blood vessels. This method is called intravital microscopy and provides important information about the number of cells that roll along and stick to the vessel wall as well as the speed at which they do this. These measurements will be recorded under anaesthesia so the only time they may feel any discomfort is at the time they are anaesthetised. Another 20 % of the animals will be used to look at the next stage, which is the movement of cells out of the vessel and into an area of inflammation, in this case, the peritoneum. In these experiments, the animals are given an injection into their peritoneum that causes inflammation and the movement of cells into this cavity. The animals are then humanely killed followed by removal of samples such as blood and the cells washed out and collected from the peritoneum for analysis. The remaining 20% of animals will be given a high fat diet in order for them to develop atherosclerosis. This will be done for up to 12 weeks, is very mild and produces no outward signs of distress or discomfort to the animals. The animals will then be humanely killed and samples such as blood and blood vessels taken for analysis. Most of our experiments are carried out under terminal anaesthesia.

Application of the 3Rs

Replacement

The use of animals is required due to the complex nature of the adhesion cascade; this requires multiple factors, including blood flow, to be present simultaneously to evaluate the complete effect. Although some experiments will be carried out using an *in vitro* flow based system to investigate microvesicle/leukocyte-endothelial interactions, this does not recapitulate the complexities of an *in vivo* system. We also require genetically modified experiments to be carried out and this can only be done in a mouse model. Although use of morpholino injection in zebrafish is used in some studies as an alternative to mouse experiments, we have found that the roles of the selectins are altered in this model and the phenotype of leukocytes in this model do not reflect those in humans. There are only a limited number of rat transgenic and

knockout strains and none of the molecules we wish to investigate are available.

Reduction

As part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, details of the experimental material, and the size of the experiment (number of groups, numbers of animals/group); and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and with the treatment differences that are to be estimated).

Refinement

As mentioned above, mouse models are the most appropriate to use for these studies due to the availability of genetically modified strains and active inhibitors. We have extensive experience in all of the models and methods to be used in this project and are confident that they are the most appropriate to address our research questions.

We will use the least invasive procedure and carry out our experiments under terminal anaesthesia in order to minimise animal suffering. We will continuously monitor the outcome of our procedures in order to effectively minimise this suffering.

Project 14	**Cardiac remodelling and heart failure**	
Key Words (max. 5 words)	atrial fibrillation, heart failure, ion channel, myocyte, ventricular tachyarrhythmia	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	High blood pressure and cardiac injury caused by blockage of a coronary artery are major causes of heart failure and of disturbances to heart rhythm – known as arrhythmias. Arrhythmias in the lower chambers of the heart (the ventricles) are a major cause of cardiac arrest while arrhythmia of the upper chambers of the heart (known as 'atrial fibrillation' – the most common arrhythmia) itself causes heart failure and is a major cause of stroke. Although it is known that high blood pressure and coronary artery disease cause changes in the structure and working of heart muscle cells that contribute to heart failure, cardiac arrest and atrial fibrillation, the precise mechanisms remain unclear. The overarching aim of the work covered by this licence is to understand the mechanisms underlying the changes to heart muscle cells that cause heart failure and arrhythmias.	

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 can be expected to advance understanding of the changes in the structure and function of atrial and ventricular heart muscle cells caused by high blood pressure and blockage of coronary arteries and how these changes contribute to the development of heart failure and arrhythmias. Work on this project may lead to the development of safer and more effective therapies for the treatment of heart failure and atrial fibrillation.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will be conducted using cardiac preparations from rats (550 over 5 years) and mice (380 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 procedures in this licence involve the induction of heart failure using surgery, either to constrict the major blood vessel receiving blood from the heart or by injuring the heart through tying off a blood vessel supplying the heart muscle itself (both approaches carried out in comparison with appropriate control groups). Once the animals have developed heart failure, the animals will be killed humanely and experiments conducted on preparations of heart muscle or single heart muscle cells. The majority of animals will experience moderate levels of suffering. However, it is possible that a minority of animals with heart failure could experience more severe symptoms and this will be minimized by regular checking of the animals and the use of early humane killing.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no alternative to the use of animals for these studies as the study requires viable heart muscle cells subject to the pathological changes associated with heart failure that cannot currently be grown or reproduced in cell culture systems
2. Reduction Explain how you will assure the use of minimum numbers of animals	Sample size calculations have been performed to calculate the number of repetitions required in order for the study to be adequately statistically powered to avoid the unnecessary use 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.	The rat and mouse represent species for which there is extensive background information about the heart and well-established surgical models so that heart failure can be achieved reproducibly. Suffering in the animals following surgery will be minimized by • Regular and frequent inspection of the animals to assess the condition of the animal and wound healing • Use of pain killers • Administration of fluids to prevent dehydration • Offering of high calorie food (e.g. broken chocolate biscuits) in the day following surgery to encourage appetite • Close monitoring during post-surgical recovery from general anaesthesia • Regular monitoring of body weight – progressive weight loss may indicate worsening condition and risk of death.. • Use of echocardiography to identify the onset of heart failure and thereby minimize the period for which animals experience heart failure.

Project 15	**Morphogenesis during early mammalian development**	
Key Words (max. 5 words)	Embryo, congenital defect, cell movement	
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)	Large-scale cell and tissue movements play a major role in shaping the mammalian embryo. For instace, the embryonic region that gives rise to the heart actually starts out in front of the region that forms the brain. Little is currently known about how the movement of cells and tissues is coordinated to give the intricately shaped structure of the body and how our genes control these complicated movements. The aim of this project is to understand at the cell and genetic level the movements that shape the body. We seek to clarify which cells give rise to various organs in the embryo and how cell movements generate the shapes of these organs.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	These questions are intrinsically interesting as they address the issue of how we (with a distinct front and back, organs of various shapes etc.) develop from a fertilized egg that is roughly spherical and has no features distinguishing any one region from another. They are also medically important as congenital	

project)?	abnormalities such as various heart defects, cleft palate, spina bifida etc. are the result of improper cell or tissue movements during development. Understanding which populations of cells give rise to different portions of these organs may help in preventing or treating such disorders. This knowledge may also identify stem-cell populations in the embryo or adult that could be used to treat various conditions in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rodents (mice and rats) as a source of embryos for the experiments we need to perform the address the questions outlines above. We expect to use 24,000 animals 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?	Since we study embryonic development, the vast majority of out animals (~90%) are used primarily as a source of embryos and therefore are simply maintained, mated and then killed so as to collect their embryos for experiments. We will breed and maintain wild type and genetically modified animals. The vast majority of mutants will have nil or minor/transient adverse effect. Genetically modified animals of greater severity (eg. mild limb defects such as short or entirely absent tail, tooth defects such as loss of specific teeth) will be bred on a separate moderate protocol. The only procedure the majority of our animals will be subject to is an ear biopsy or an injection, which is of mild severity. The majority of animals will be killed humanely at the end. Those that are not killed will be transferred to other authorized scientists who might be interested in studying them.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Embryonic events cannot be recapitulated using tissue-culture cells. Computer simulations are limited to an extent by what we have experimentally determined using biological samples, so are limited in their ability to tell us completely new things about

	embryogenesis. Therefore, to understand this process we need to perform experiments on actual embryos.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to reduce animal use, we are developing mathematical models that will help us to rule put certain experiments using computer simulations
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 genetic basis for the development of mouse embryos is sufficiently similar to that of humans for it to be a viable model of human development. Moreover, mice are the lowest mammalian species in which it is relatively east to make the genetic modifications required for this project. Non-mammalian species such as the zebra-fish are not suitable for this work as they do not show several features of this development seen in mammals (such as implantation in the womb, or a four chambered heart). Since the project focuses on embryonic development the majority of mice will be used only as a source of embryos and therefore will not suffer any experimental procedures. The mice will be killed humanely in order to recover embryos from them.