Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 6

Project Titles and key words

- Gene function and autoimmunity

  Autoimmunity; Diabetes

- Glial neurotransmitter receptors in Alzheimer's disease

  Astrocytes, amyloid, calcium

- Exotic virus detection

- Skeletal homeostasis, remodelling and repair

  Arthritis, stem cells, cartilage, bone, regeneration

- Vascular cytoprotection during inflammation

  Inflammation, blood vessel injury, blood vessel growth, endothelium, atherosclerosis

- Factors affecting fish populations

  Fish, management, conservation, environment

- Senescence and its effectors in tumour suppression

  Senescence, autophagy

- Signalling Processes in Chronic Diseases

  Cancer, inflammation, viral disease, therapeutics

- The immunobiology of regenerative medicine

  Stem cells, Pluripotency, Degenerative disease, Immunological rejection, dendritic cells

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Gene function and autoimmunity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Autoimmunity; Diabetes,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Autoimmune disease occurs when a person’s immune system attacks the body’s own healthy tissues and destroys them, e.g. type 1 diabetes (T1D) and multiple sclerosis (MS). We will investigate the role of a newly identified gene of unknown function, which is important in risk of T1D and MS, in the normal function of the immune system of the mouse. We know that a person who is genetically protected from disease has a larger amount of the protein encoded by this gene in their white blood cells, but critically, we do not know how this protects them from autoimmunity.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work will help us to confirm and understand the role of this newly-discovered gene in autoimmunity. Specifically it will focus future efforts to prevent and treat autoimmune disease - we believe this gene may be a novel target for drug treatment.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Over 5 years we will specifically study the immune system of up to 100 mice in which the expression of this gene has been reduced.

Over the same period we will breed and study up to around 600 mice per year, in which the potential autoimmunity gene has been deleted.</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

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?	Our work with cells and blood samples suggest that that mice will be only mildly affected but any mice who develop more severe problems will be euthanased immediately. Theoretical possibilities include problems with breeding / survival of litters of young mice or failure of the immune system to develop properly. We will examine all animals frequently to minimise the possibility of any animal suffering undue distress. We will minimise the number of animals used by careful experimental design and statistical analysis. At the end animals will be euthanased unless they are to be studied in a different context with appropriate permissions.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All the work undertaken on animals will run alongside and be informed by our studies using tissue culture and human blood samples. Whilst such experiments can provide some insight into the role of the gene in individual cells, work in a whole organism is needed to explore the impact of changes in the gene on the whole animal. We have looked carefully to make sure that no one has already made the knockout mouse for this gene, to avoid unnecessary replication.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that statistical methods are appropriately applied to minimise the number of mice in each experiment and will keep variability to a minimum to ensure that experiments are neither underpowered nor use excessive numbers of animals. We will make samples available to other investigators to avoid the work being repeated elsewhere.

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.	It is not possible to use nematode worms or fruit flies since they do not have the gene of interest, nor are they suitable for studying the immune system. We considered zebrafish as a model but this is more technically challenging, the immune system is less well understood and there are no opportunities for studying the gene in the context of autoimmunity. Mouse models of autoimmunity are well defined, there is opportunity for looking at gene function only within certain body systems and techniques for 'knocking out' genes in mice are more well developed. We will ensure that for each procedure, we will use the most refined protocol possible, e.g. - local anaesthesia and analgesia where indicated - blood sampling only being carried out by skilled personnel - providing any drug treatment by a palatable oral route rather than injection where possible

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Glial neurotransmitter receptors in Alzheimer's disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Astrocytes, amyloid, calcium.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3 months</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Alzheimer’s disease is characterised by a decline in the ability to learn new things and recall memories. The brains of people who have Alzheimer’s have a high level of deposits of a protein called amyloid, which is thought to interfere with learning. One of the main ways we learn involves the release of a chemical called acetylcholine from neurons. Released acetylcholine binds to specific proteins (acetylcholine receptors) on other neurons, and so acts as a signal. It is also known that these proteins are present on other cells in the brain called glial cells. These support neurons and also have some immune roles. It is now known that amyloid can actually interact with the acetylcholine receptor. The research that we will perform will be to study the effect of amyloid on acetylcholine receptors on glial cells in mice which actually have a form of Alzheimer’s disease.</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>Identifying a new action of amyloid in the brain would be a major step forward in the understanding of Alzheimer’s disease. Knowing if amyloid acts at receptors on glial cells may lead to the discovery of a possible target for new drug therapies.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice 150</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td>Transgenic animals will be bred and maintained. No adverse effects are expected since mutation does</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	not cause painful or distressing effects. Severity is mild. For experimental use animals will undergo terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are currently no computer simulations available which model the astrocyte-neurone signalling that we investigate. Available cultured cell lines also have differences to native cells, which are likely due to the loss of anatomical interactions that glial cells have with neurons in the brain. Therefore the most appropriate system for our study is mouse brain slices.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have discussed with statisticians and performed calculations to determine the minimum number of experiments required for our studies. In addition, many brain slices will be taken from the same animal and different methods combined in the same experiments to increase the quantity and validity of the data. The appropriate number of animals will therefore be bred.
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 Alzheimer's mouse model proposed is a model that overexpresses beta amyloid and is of comparable severity to other Alzheimer's Disease models. A beta amyloid overexpressing model is the least severe type of model that is appropriate since a main aim of this proposal is to investigate the role of amyloid interaction with astrocyte acetylcholine receptors and the long term effects of such an association in the brain ie in transgenic animals.

Exotic virus detection

- Summarise your project (1-2 sentences)

To maintain and further develop the capability of isolating and detecting newly emergent and re-emergent viruses using laboratory rodents and/or reagents produced *in vivo* in support of diagnostic and surveillance services.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

This project supports the analysis of biological samples that are received from clinicians. This programme directly influences strategy and policy for pathogenic outbreak control and patient management.
Increasing numbers of *in-vitro* techniques are employed in virus detection services and whilst these are rapid and quite sensitive they are unable to detect novel or unexpected pathogenic agents nor antigenic or virulence changes in known agents. Therefore *In vivo* techniques remain the proven optimal method of isolating and detecting novel agents as they increase the probability of amplifying and isolating viruses from material derived from human and animal infections.

- Outline the general project plan.

This project has two parts, the first (Virus isolation) involves the introduction of suspensions of clinically derived material into suckling mice; with end of study samples being sent for microbiological and histological analysis.
The second part of the project (Antigen production) is to generate material and antigens to be used for further in-vitro diagnostic assays. This part of the project involves the use of litters of suckling mice, adult mice and adult guinea pigs and involves the dosing of stock antigen preparations via various routes.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

Both protocols involve the intracerebral inoculation of suckling mice which carries a small risk of physical neurological damage during injection. The animals will be very closely monitored immediately following inoculation to ensure full recovery from the injection. Following this initial period, both protocols ensure that the animals are closely observed for signs of illness or distress resulting from the infection. Any animal suffering from signs of illness or distress will be killed by a Schedule 1 method.

Protocol 2 also involves the use of adult mice and guinea pigs for antigen production (for subsequent use in in-vitro assays / assay development. The routes of administration will depend on the study design, but will be limited to intraperitoneal, subcutaneous, intradermal or intravenous routes all of which involve transient discomfort.
Due to the potential novel nature of the virus there is a chance that the animals could show clinical signs of infection. Due to the regular monitoring of the animals health, should this be the case – the animals will be humanely euthanized and tissue taken and analysed / used.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

The potential beneficial outcomes of the work conducted under this licence are numerous and include:

- Rapid and accurate identification of newly imported viral infections in patients entering the UK.
- Early identification of exotic viruses to alert government agencies and provide information and advice for strategies and policies that can be employed regarding travel advice, patient care/clinical measures, public health and outbreak control or clinical measures and appropriate vaccine development programmes.
- Reduction of use of animals for identification and detection of newly emergent and re-emergent pathogens.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

Protocol 1 - This work will involve the use of up to 150 litters of mice. Suckling (<3 day old) mice are considered the gold standard as most viruses will grow in these tissues and generally yield high titres. Animals will only be used to amplify and identify the virus in question. Any further work (other than that identified in Protocol 2) will be authorised under a separate PPL.

Protocol 2 – in a similar manner to the above, the suckling mouse is likely to be the most appropriate model for the production of viral antigens for subsequent in-vitro assay development. Up to 50 litters of mice are included in this protocol.
As new born mice may not always be the most appropriate model, small numbers of adult mice (x50 animals) and adult guinea pigs (x100 animals) are included in Protocol 2, as these may be a more relevant model for some viruses.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

The number of animals used within this programme of work are significantly reduced (by 90%) from similar projects historically. This reduction is due to the increase in suitable alternative methods of virus isolation, with this remaining portion only being required for novel and / or unexpected virus isolation.

The programme of work supports other projects intending to develop a range of reliable well established *in vitro* laboratory diagnostic systems for virus isolation and identification in order to reduce the dependence on the use of animals in routine detection assays. Work will evaluate and compare *in vitro* and *in vivo* methods of virus isolation from material derived from human and animal infections caused by newly emergent and re-emergent agents.

- Explain why the protocols and the way they are carried out should involve the least suffering.

The protocols are designed to ensure that suffering is minimised by close monitoring throughout the study. Should an animal become ill, then it will be humanely killed and the tissues collected for analysis and further use. At this point the investigation will have achieved its objective and the study can be ended. By definition, if an animal becomes ill the tissues will be positive for the virus under investigation and the tissues will be scientifically useful for use in further *in-vitro* tests (including the development of in-vitro tests to replace the in-vivo studies).

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Skeletal homeostasis, remodelling and repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Arthritis, stem cells, cartilage, bone, regeneration</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Arthritis is a leading cause of disability. Joint destruction is the result of an imbalance between tissue breakdown, often caused by inflammation or trauma, and repair. Postnatally, stem cells maintain and repair tissues and organs of our body. Our knowledge of the location and functional regulation of the stem cells in the adult joint is poor. We propose to characterize resident stem cells naturally present in the normal and diseased joint and to investigate their role in the maintenance, remodelling and repair of joint tissues in the adult life. For such studies, no in vitro system would be able to reproduce the complex in vivo environment with continuous interactions among tissue and organ systems across the whole body.

In clinical conditions characterized by large defects (e.g., non-union fractures or advanced osteoarthritis), endogenous stem cells are not sufficient to ensure repair. In these circumstances, the transplantation of exogenous stem cells (either in suspension or as engineered body parts) would be necessary for replacement of missing tissue components. Proof-of-concept studies in humans support the clinical utility of stem cells for bone and cartilage repair. A major problem, however, is the large variability in clinical outcome, partly due to inconsistency of the stem cell preparations. There is, therefore, an unmet pressing clinical need for assays that allow quantitative estimation of the bone and/or cartilage-forming potency of human stem cell preparations. Such potency assays would allow development of quality controls for efficacy of</td></tr>
</table>

[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

	stem cell preparations, a prerequisite for routine use in clinical practice.
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 ultimate goal of this research is to develop cell-based treatments for patients with skeletal disorders such as non-union fractures and arthritis, including osteoarthritis and rheumatoid arthritis. These conditions are characterised by extensive damage of skeletal tissues such as cartilage and bone. Current treatments are often unsatisfactory. For patients with arthritis, medications can halt inflammation but are unable to achieve regeneration/repair of the damaged tissues. Our research could lead to novel cell-based therapeutic strategies for replacement of damaged tissue via transplantation of stem cells or via the administration of drugs that target the stem cells that are naturally present in our body.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice. This species is most used for preclinical studies and allow assessment of function via genetic modification. The number of animals is kept to a minimum as we extensively use in vitro experimental systems in the first instance and only for validation we require in vivo experimentation. Considering our past experience and on the basis of power calculations for adequate group sizes, we have estimated to use up to 3,000 mice over 5 years. The availability of state-of-the-art in vivo imaging equipment including x-ray, densitometry, MRI and PET allows longitudinal studies, thus minimising the number 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?	Immunodeficient mice (unable of immune rejection) will be used for transplantation of human stem cells obtained from adult individuals to assess the potency of these stem cells. These protocols are well tolerated and the risk of adverse effects in our experience is very low. A possible side effect is local inflammation at the site of injection. This is rare and does not cause any signs of distress to the animals. Mouse models of traumatic joint injury, osteoarthritis and inflammatory arthritis will also be used to study the native stem cells in the skeleton, with a focus onto the joint. These models are also well tolerated. Like human patients, mice may develop joint pain and swelling. Possible side effects are related to the anaesthesia and local or systemic infections. Appropriate anaesthetic regimes suitable for the species and the duration of procedures will be administered and maintained by trained personnel. Any possible infections will be

	treated appropriately. After irradiation prior to bone marrow transplantation, a temporary weight loss of ~20% can occur. Any animal losing >20% will be reviewed by NVS or NACWO. Animals will be humanely killed at the end of the proposed experiments or in case of excessive suffering, distress or lasting harm. The possible benefits to patients, NHS and society largely exceed the harms to the experimental animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We routinely employ several in vitro assays to determine the capacity of stem cells obtained from tissues such as bone marrow to form cartilage and bone. All our assays are optimised to mimic the in vivo environment. However, evidence indicates that often they are an overestimation of the true in vivo capacity of stem cell populations. Hence, normally after extensive screening in vitro we proceed to confirm the in vitro findings with appropriate in vivo experimentation, as this is normally a required step for any clinical translation. In vivo studies are necessary to identify and characterise the native stem cells naturally present in the joint and define their role in joint tissue maintenance, remodelling and repair. None of these experiments could be performed in vitro. Cell or tissue culture cannot mimic this, as the interactions between the different tissue and cell types within the joint and in the entire body are lost. To study the role of stem cells in arthritis there is a requirement to look at the cells in the full picture consisting of a continuous interplay across multiple cell types, tissues and organs in a living body with circulation through bloodstream of a myriad of molecules.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The in vivo procedures involved in this study are well-established and can therefore be performed in a way that provides maximum information with the minimum number of animals determined using statistical analysis. Our state-of-the-art in vivo imaging facility allows longitudinal studies in mice, thus reducing considerably the total numbers of animals used when time-point analysis is 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	We have carefully selected the species and models to use in our study. The mouse is the most convenient mammalian species and also now considerably more valued in pre-clinical models. The in vivo procedures in this study are internationally well-established and routinely used

measures you will take to minimise welfare costs (harms) to the animals.	by academic and industrial institutions for preclinical studies and assessment of novel treatments. They are therefore performed in a way that provides maximum information but minimal distress to animals. Several similar experiments have been carried out previously and have provided sufficient information to enable us to set early end-points (based on appropriate and relevant scoring systems) for experimental protocols and thereby keep animal suffering to a minimum. When needed, anaesthesia and analgesia are ensured. Mice are monitored regularly and, if needed, extra bedding is provided. When necessary, mice are provided with a “mash-type” diet for ease of eating. Veterinary staff is always accessible for advice and assistance in matters pertaining to the welfare of the animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Vascular cytoprotection during inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Inflammation, blood vessel injury, blood vessel growth, endothelium, atherosclerosis.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td>Yes</td><td><s>No</s></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The principle focus is the study of arterial injury and angiogenesis (the development of new blood vessels) during inflammation. It has become apparent in recent years that these processes are intimately associated with human disease. Thus, arterial injury predisposes to atherosclerosis, the cause of heart attacks and strokes, and the biggest killer in the Western world. Angiogenesis is involved in diseases including diabetic eye disease, rheumatoid arthritis and cancer.

The work will study the endothelium, the layer of cells that lines all our blood vessels including arteries. Principle objectives are: identification of mechanisms important for maintaining a healthy endothelium, and those working to repair damage. We believe that by understanding these processes we will discover new targets for the treatment of against diseases including heart attack and stroke.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The work proposed is original and expected to further understanding of the mechanisms involved in vascular injury, the regulation of protective repair pathways and the development of new blood vessels. The potential benefits include discovery of the means by which the vascular endothelium may be conditioned by drug treatment to prevent vascular injury and accelerated atherosclerosis in patients with systemic inflammatory diseases, and to modulate angiogenesis, either by inhibiting or inducing angiogenic signalling pathways.
.</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice, up to 1000-1500 per year 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 majority of mouse strains we use are healthy and should not suffer adverse effects. By interbreeding, we will obtain novel strains. Some of these may have increased susceptibility to infection and will be carefully monitored (see below). During breeding, tissue is required for genetic analysis. This will be obtained by collection of 1-2 mm tail snips under local or short-acting general anaesthesia. There is a small risk of bleeding and mice will be carefully monitored until the bleeding has stopped. Mice undergoing surgical procedures may suffer pain and they will receive effective analgesia to prevent this as advised by the Veterinarian. Less than 1% of animals suffer complications of surgery and they will be monitored carefully post-operatively. Any animal showing signs of adverse effects such as of localised skin lesions, haemorrhage, malaise, ruffling of fur, loss of appetite, reluctance to move or weight loss (greater than 20%), will be carefully observed and where necessary we will seek the advice of the Vet and/or kill the animal humanely.
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 in cultured human endothelial cells. However, in spite of the large amount of information on underlying molecular mechanisms accrued by this work, inflammation and angiogenesis are complex multicellular responses that cannot be modelled completely in the laboratory with isolated cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Over the last 10 years we have developed protocols to minimise animal numbers required. These are the subject of constant review and we strive to improve them and to develop alternative approaches that do not require animals. For example, extensive use of post-mortem tissues in different projects optimises output and reduces the total number of animals required.
3. Refinement Explain the choice of species and why the animal model(s)	Mice have proved invaluable for our studies, largely due to the availability of genetically-engineered strains to model human disease. For example LdlR

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.	knockout mice are a good model for human atherosclerosis with minimal animal suffering. Our protocols have been devised and refined so that they are all mild or moderate. We have taken advice from the vet to achieve this. For example, when anaesthesia and/or surgery is required, animals are recovered in a warmed cabinet and analgesia administered pre- and post-procedure following expert.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Factors affecting fish populations.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, management, conservation, environment,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall objective of the project is to generate reliable and robust scientific evidence on the impact of selected environmental factors affecting fish populations in support of specialist advice provided to stakeholders, national and international governments and other international organisations on the conservation and management of fish stocks in England and Wales.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The project will examine how man made activities within the aquatic environment and modifications to the freshwater and marine environment may affect fish at the individual and population level so that steps can be undertaken to manage these impacts in a sustainable manner and protect a range of fish species within a changing environment.
Specifically, the project will focus on diffuse and point source pollution, renewable hydropower schemes, angling baits and construction activity within rivers, estuaries and coastal waters in order to better manage and conserve important fish stocks.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The project will focus on migratory fish species that move between the freshwater and marine environments. These will include Atlantic salmon, European eels, shads, lampreys, sea trout. In addition, species that are popular in sports fishing will be also studied. These include, carp, roach and barbel. It is expected that approximately 25,000 fish including eggs will be studied over a 5 year period.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>There are a range of potential adverse effects on the fish, including a small risk of wound infection, and modifications to swimming and migratory behaviour. The overall expected level of severity is</td></tr>
</table>

[9] Delete Yes or No as appropriate.
[10] At least one additional purpose must be selected with this option.

level of severity? What will happen to the animals at the end?	considered to be moderate. Depending upon the protocol, the fish will be either released to the wild or humanely 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	**There is no alternative to the use of living animals, as the principal aim of the work is to describe the behaviour of fish in relation to changes in their natural aquatic environment in order to conserve and manage populations.**
2. Reduction Explain how you will assure the use of minimum numbers of animals	In developing the project, advice has been obtained from a statistician experienced in animal research, regarding animal numbers and design in order to use the minimum numbers required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The species chosen represent those where there is a requirement to manage and conserve populations, such as the Atlantic salmon and European eel. Fish models will not be used as these generally do not represent natural populations. To minimise welfare costs to the fish, an appropriate level of anaesthesia will be ensured by careful monitoring of the animals throughout any procedure. Any fish released to the wild will be assessed and monitored by a suitably qualified individual to ensure that it is fit to be release back into the environment

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Senescence and its effectors in tumour suppression</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Senescence, autophagy,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>Yes</td><td>~~No~~</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Why are benign tumours benign? One promising avenue of investigation into how some tumours remain benign is through the cells ‘going to sleep’ through senescence. That is overcoming their predisposing genetic mutations and stably ceasing to multiply.

We aim to understand how senescence is regulated and how it contributes to tumour suppression. In this programme of work, effector mechanisms of senescence identified in our cultured cell model will be progressed to validation in vivo.

This programme of work has three main objectives:
1) Establishing, and utilising, our laboratory’s primary mouse models of in vivo senescence
2) Characterisation of mice with genetic modifications to senescence effector genes.
3) Use of standard grafting tumour models, as necessary, to validate the tumour suppressor / oncogenic (cancer causing) activity of candidate genes identified through studying senescence in cell culture.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The short-term benefits of this project will be the advancement of our knowledge of the process of senescence in healthy, ageing and disease contexts. In the longer term we wish to translate our findings into treatment(s) for patients, through our search for a means of therapeutically inducing senescence in vivo. Senescence induction potentially offers a powerful means of combating cancer.</td></tr>
</table>

[11] Delete Yes or No as appropriate.
[12] At least one additional purpose must be selected with this option.

What species and approximate numbers of animals do you expect to use over what period of time?	Mice 5000 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 genetic modifications to some of the mice bred under this licence are expected to lead to the development of chronic disease and / or a susceptible to cancer. Any animal showing distress or pain reaching a moderate severity limit or clinical signs suggestive of tumour growth will be killed and processed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible our laboratory carries out experiments on cultured cells. However, the interactions of cells with other cells within a tissue are too complex to recapitulate in a culture dish; for this reason it is essential to carry out some experiments on animals. However, results from cell culture experiments will be used to design our animal work. Mice represent the ideal model organism available, because of the ability to manipulate gene expression and study the tumour within the context of a whole organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of mice, we will keep stocks of frozen sperm and embryos so that if a mouse line is not continuously required we can avoid unnecessary breeding. To maximise the data obtained from each animal we will collect samples from multiple body sites whenever possible and, if requested, provide samples to appropriate scientists to prevent duplication of their experiments. Experiments will be designed in consultation with our in-house statistician in order to use the minimum number of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Genetically modified mice permit specific genes to be knocked out, or removed, at specific time-points in the animal's life appropriate to the development of cancer. This removal can also be localised to a specific organ, e.g. liver, kidney, or skin. Mice are the only vertebrate species where gene knock-out experiments can be carried out. Mice also breed quickly allowing inter-crossing in a reasonable period of time. Mice have a short lifespan enabling the study of genetic manipulations from birth through to old age. Crucially, they recapitulate well human cancer behaviour. There is no other model

	system that is capable of providing the type of data necessary for our studies. In order to minimise animal suffering, animals will be closely monitored and any animal displaying any signs of suffering will be immediately killed. This will apply during normal husbandry and breeding but especially while the animals are undergoing regulated procedures. We have refined all animal procedures through training and advice, to reduce any pain and/or distress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Signalling Processes in Chronic Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, inflammation, viral disease, therapeutics</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To identify, evaluate and develop novel drugs for the treatment of chronic diseases such as cancer, inflammation and viral diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The aim of this project is to develop novel therapeutics from in vitro studies through animal studies and eventually via clinical evaluation in humans. Successful therapeutics will be used to treat chronic diseases to alleviate suffering and prolong life. The scientific concepts behind and the data supporting the development of these therapies will be published in the scientific literature.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice approximately 9000 animals per year.
Rats approximately 1000 animals per year.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The project will evaluate the novel therapeutics in a step-wise manner. At each step the novel drugs that do not fulfil the required criteria will not be to the subject of further studies in animals. At the end of the procedures the animals will be killed by an approved method.

The first in vivo evaluation of any drug will be to determine the pharmacokinetics (that is the adsorption, distribution, metabolism and excretion) of the drug by administering low doses of the drug and determining the amount of drug in blood or tissues. The low concentration of the drug is very unlikely to cause any adverse effects and therefore</td></tr>
</table>

[13] Delete Yes or No as appropriate.
[14] At least one additional purpose must be selected with this option.

	this procedure has a mild level of severity. Drugs that have the required pharmacokinetic characteristics will then be tested at higher doses to determine if the drug can achieve sufficient levels in blood or tissue to exert a beneficial effect in disease. As the concentration of drug increases the potential for adverse effects increases, and therefore these procedures will have a moderate level of severity. Potential adverse effects could be reduced feeding, body weight loss or short-term reduced movement of the animals within the cage. Prior to evaluation of selected drugs in animal models of disease, we may wish to determine the maximal tolerated dose of the drug. These studies are performed using small groups of animals administered increasing levels of drug. Animals will be closely monitored for behavioural changes and the body weights recorded daily. The aim of these studies is to determine the dose of the drug that will not cause adverse effects in the disease models which often use larger groups of animals. We will determine the maximal tolerated dose for a drug up to a dose that can be formulated and not higher than a dose we believe will have an efficacious effect. We aim to keep the level of severity to a maximum of moderate, but on a rare occasion the drug under evaluation can cause death and thus the level of severity is graded as substantial. Only those drugs which fulfil the required criteria will be evaluated for efficacy in larger groups of animals in disease models. In general, disease models require prior treatment of the animals (such as injection of cultured cells) before drug administration. Drugs may be administered for prolonged periods and animals will be monitored throughout this period for signs of adverse effects such as body weight loss, reduced feeding, reduced movement. The level of severity in these models will be moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Before any studies are performed in animals we will do extensive in vitro studies of the drugs against the target enzyme or target protein and in selected cultured cell systems. Only those drugs with the required degree of activity will be progressed to animal studies. Animals will be used for certain studies because we cannot determine all the required characteristics of the drug using in vitro techniques alone.
2. Reduction Explain how you will assure	We will use the minimum number of animals to evaluate the drug in each of the above procedures.

the use of minimum numbers of animals	The numbers of animals used will be sufficient to achieve statistical significance and based on good scientific practise. For example, to determine the low dose pharmacokinetics of the drug we will use three animals for each time-point and then analyse the data as a composite of all the time-points. We may have up to seven time-points to characterise the drug pharmacokinetics but we will minimise the number of animals by repeat bleeding of the same animals for a prescribed number of samples. The number of animals used will also be minimised by not testing drugs that do not fulfil the required criteria for next step/procedure.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use mice or rats for our studies as these the lowest vertebrate group that have been well characterised for both pharmacokinetics and efficacy studies. We will minimise animal suffering by using the appropriate models and by using gaseous anaesthesia (when appropriate) to reduce stress. Analgesics will be administered when appropriate after surgery.

The immunobiology of regenerative medicine
Stem cells, Pluripotency, Degenerative disease, Immunological rejection, Dendritic cells

- Summarise your project (1-2 sentences)

  The ageing nature of the population demands new approaches to the treatment of chronic and degenerative diseases, for which the use of stem cells may prove effective. This project will seek to define the extent of the immune response that implantation of stem cell-derived tissues will elicit and the prospects for mitigating its impact.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

  Although the substantial increase in life expectancy over the past century is to be welcomed, it has been responsible for a dramatic rise in incidence of chronic and degenerative diseases, which pose the greatest medical challenge of the 21st century. Pluripotent stem cells (PSC) have recently been generated capable of differentiating into any of the cell types that make up the mammalian body, providing a potential source of tissues for replacement of those lost through disease or the process of ageing. The greatest obstacle to realising their potential is, however, the immune response of the recipient which may cause rejection of the implanted tissues. The purpose of this project is, therefore, to systematically investigate the extent of the immunological barriers encountered and possible ways of overcoming them through the establishment of immunological tolerance.

- Outline the general project plan.

  The project will be conducted in two parts. Firstly, we shall generate the necessary PSC lines which will require occasional tissues derived from healthy mice that have been humanely killed using a Schedule 1 method. Secondly, the PSC will be coaxed to differentiate *in vitro* into various cell types that may be useful for the treatment of chronic diseases in the future and these will be transplanted into recipients to gauge the extent of the immune response targeted against them. In the event that such tissues are rejected, we shall investigate the feasibility of modulating the immune system through the administration of cell types or pharmacological agents that have been implicated previously in the induction of transplantation tolerance. In order to address the cellular and molecular basis of tolerance we may generate and breed strains of mice carrying genetic modification of scientific interest.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

  In order to investigate the extent of the immune response that is likely to be mounted to tissues differentiated from PSC, it will be necessary to implant them under the kidney capsule of recipient mice, a site of implantation that is easily accessible and well-vascularised. Such experiments cannot be conducted under sustained general anaesthesia since rejection may take up to 28 days. Mice will, therefore, be allowed to recover completely from surgery with appropriate pain relief and post-operative care, similar to humans who have undergone routine surgery. Since the immune response

will target only the transplanted tissue, on which the animal's health is not dependent, there should be little collateral damage capable of causing suffering. In the event that the immune response proves limiting, we shall investigate ways of preventing it, the most promising involving administration of monoclonal antibodies or cell types, such as dendritic cells, known to be involved in tolerance induction. Their administration will be either via the intraperitoneal or intravenous routes which is likely to cause only momentary discomfort.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

  The findings of this project will provide essential information regarding the viability of stem cell-based therapies in the future, on which the prospects of regenerative medicine are based. Should the immune system of the recipient prove to be the limiting factor in their success, the experiments outlined in this project will provide an evidence base capable of informing future treatment options.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

  Although we will require significant numbers of healthy, untreated mice to maintain the viability of colonies and for breeding purposes, the numbers used in experimental procedures is unlikely to exceed 500 per year. Numbers will be kept to a minimum by calculating the smallest group size required to achieve robust statistical data and to enable unequivocal conclusions to be drawn. Furthermore, by differentiating many of the cell types we require from PSC, we will substantially reduce the number of tissue donors required. To meet our requirements for genetically-modified mice, we shall breed up to 4000 mice per year but ensure numbers are kept to a minimum by rapidly screening progeny for expression of the transgene of interest and by carefully matching the colony size to the scientific requirements of the programme of research.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

  Since the immune response and immunological tolerance are phenomena that require complex interactions between multiple cell types within the architecture of the secondary lymphoid tissues, they are meaningful only in the context of living organisms and cannot be recapitulated *in vitro*. Indeed, attempts to recreate the immune response *ex vivo* have universally demonstrated that such metrics are not predictive of the outcome of transplantation. We therefore intend to use mice to study this process since they represent the lowest vertebrate species for which the necessary inbred and genetically-altered strains are available.

- Explain why the protocols and the way they are carried out should involve the least suffering.

  All the procedures outlined in this project have been performed routinely and successfully over many years and progressively refined to ensure that they cause the least possible suffering. Nevertheless, all procedures are subject to ongoing refinement as we trial improvements from other laboratories or alternatives we have identified from the scientific literature. All mice will be monitored during and after intervention using proven behavioural metrics routinely used to assess their well-

being. This will allow the earliest possible veterinary intervention to be sought, should signs of pain or distress be identified. Furthermore, humane end-points have been defined for each procedure in order to permit action to be taken in advance of the onset of suffering.