Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 33

Project Titles and key words

- Efficacy Testing of Pharmaceutical Materials

  Efficacy, Toxicity, Protected animals, regulated procedures

- Investigations of poultry respiratory infections

  Poultry, Respiratory, infection, IBV, aMPV

- Improving joint repair by reducing OA-like changes in an ovine knee model.

  Cartilage, bone, ovine, osteoarthritis, repair

- Tissue engineering of the kidney

  Organ transplantation, whole organ decellularisation

- The role of the immune system in tissue damage

  Inflammation, tissue injury, immune system, autoimmunity

- Development, growth and repair of striated muscle

  Muscle, heart, zebrafish, mouse, growth

- Cardiac Muscle Cell Death and Regeneration

- Sand Fly colony maintenance

  Chemical Ecology, Host odour, Leishmaniasis, Pheromones, Sand flies:

- Autonomic Control of Cardiac Physiology

  Cardiac, Arrhythmia, Autonomic

- Neuronal dysfunction in models of CNS disease

  Dementia; Neurophysiology; biomarkers; cognition

- Birds as indicators of environmental change
-

  Seabirds – environmental change – fisheries – foraging

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Efficacy Testing of Pharmaceutical Materials</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Efficacy, Toxicity, Protected animals, regulated procedures</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td><s>No</s></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><s>Yes</s></td><td>No</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[2]</td><td><s>Yes</s></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">
Studies performed under this licence will generate data that will allow us to assess the potential effectiveness of a material in disease treatments.
The data generated may also be used to determine if a material is a more effective treatment against a particular disease than comparative medicines, or used to identify any superiority in efficacy between different species and/or strain of animal.
Where studies include toxicity assessments the data generated will provide support to drug discovery by early characterisation of toxicology liabilities and allow selection of optimal drug development candidates, refine discovery strategies and provide appropriate animal models to enable effective candidate selection.
Studies performed to provide toxicity, efficacy data and, biomarkers etc. will assist in the decision making process regarding whether to progress the clinical evaluation of substances and the design of protocols for clinical trials and will also be included in regulatory submissions. Data generated will help to minimise the
</td></tr>
</table>

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

	risk to volunteers and patients and will also increase the chance of regulatory approval of applications for clinical trials and marketing. Data from these studies may also allow later animal studies to be tailored to include specific endpoints to maximise efficacy and increase our ability to detect/characterise toxicity.
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 overall benefit of this project is that it supports the development of safe and effective new medicines to improve the health and quality of life of patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making. Achievement of the objectives of this licence will enable safe and effective development candidates to progress and will also help to remove unsuitable candidates from the development pipeline at an early stage, thus saving animals and resources.
What species and approximate numbers of animals do you expect to use over what period of time?	2000 rats and 2000 mice over a five year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The procedures performed will cause no more than transient discomfort. Treatment related effects are likely and may include reduced weight gain, lack of appetite, but will be no greater than Moderate severity. All animals will be routinely observed and if there is any animal that is unnecessarily suffering then it will be humanely killed. At the end of each study animals will be euthanized and subjected to a post mortem in order to determine toxicological effects on organs and tissues.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The efficacy of some treatments can be successfully assessed by using established *in-vitro* techniques and these will be used wherever practicable. However, it is generally accepted that the way in which a material is metabolised within a living body has a significant effect on how it works. Consequently, for the majority of materials it is imperative they are tested on living animals in order to replicate clinical use or to generate more robust data on the efficacy of a material. In terms of assessing the toxicity of a material, the use of alternative methods, including the use of dead animals cannot, at this moment in time generate relevant data which supports the

	submission of safety data to international regulators. Alternative methods such as *in-vitro* techniques will however be used as much as practicable to supplement the work involving protected animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Regulatory guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of a test substance. Core study designs are based on international guidelines where these exist. Otherwise reference is made to internal guidance on study designs to provide the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. Project specific variations are used as required. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of the studies to be performed.
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.	Generally the rat or mouse is the species of choice. There is a wide knowledge of the response of rats and mice to various chemical entities and a wealth of background data in the pharmaceutical industry. Rats are big enough to provide repeated blood samples for toxicokinetics, thus requiring significantly fewer rats than mice to achieve the same objectives. Where Genetically Altered animals are required these will usually be mice because most of the relevant genetically engineered disease models are only available in this species. Regular monitoring informed by knowledge or the expected effects of the engineered phenotype will be carried out and appropriate action taken to alleviate suffering if this becomes necessary. All animals will undergo regular health assessments and appropriate levels of care given. The procedures performed e.g. dosing and blood sampling will be performed the minimum frequency necessary to achieve the objectives of the study.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Investigations of poultry respiratory infections</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Poultry, Respiratory, infection, IBV, aMPV</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)[3]</td><td>
<table>
<tr><td>Basic research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</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>The objectives of this programme of work are:
to determine the virulence of newly emerging strains of Infectious Bronchitis Virus (IBV) in chickens and Avian metapneumoviruses (aMPV) in chickens and turkeys.
to determine the extent to which commercially available vaccines (given singly) protect chickens against the new strains of IBV in chickens, and aMPV in chickens and turkeys.
to determine the extent to which commercially available vaccines given in combinations protect chickens against a range of conventional and new strains of IBV.
to understand the immune mechanisms of enhancement and broad protection, including humoral and local antibodies and cell mediated immunity against IBV and aMPV
to determine and test possible reversion of avian metapneumovirus vaccine in chickens and turkeys.</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 will lead to the improved use of existing commercial poultry IBV and aMPV vaccines for better poultry welfare, health and production worldwide.

The IBV survey will highlight novel types of IBVs and vaccine trials will indicate if and where new vaccines are required. The results will be of interest to poultry virologists and corona-virologists in other animal species.</td></tr>
</table>

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

	The basis for the broad protection offered by two different genotypes of IBV vaccines will be better understood and will provide the rational for improved vaccine programmes. The new AMPV vaccine candidates should lead to improved vaccines. The virulence and molecular studies with AMPV will enhance our knowledge of the underlying mechanisms of how these viruses cause disease and prevention of it.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 1000 chicks and 300 turkey poults over five years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The reasons for doing the work are to optimise the use of poultry vaccines in the following ways: (i) by knowing what new types of IBV a prevalent in a country/region and adjusting vaccine protocols accordingly, (ii) by understanding the broad protection offered when two different IBV vaccines are given (iii) by developing a novel AMPV vaccine based on a recent isolate and (iv) by studying the interaction between different live vaccines given simultaneously in the short life of the chicken, so that temporal adjustments can be made to the programme. These diseases are <u>specific</u> to domestic poultry and in order to test the vaccines it is essential to use the host birds. Molecular or antigenic interrelationships between vaccine and field viruses are not in themselves helpful in predicting the outcome of vaccination challenge trials. The respiratory viruses to be used cause relatively mild infections, which normally resolve in about 7-10 days. The commercial vaccines cause no distress. All animals will be monitored 1-4 times daily pending protocol used. Numbers in experimental groups will be kept to a minimum consistent with producing meaningful results and allowing for individual variation. At the end of the experiments, all birds are humanely killed using Home Office Schedule 1 Methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	These diseases are <u>specific</u> to domestic poultry and in order to test the vaccines it is essential to use the host birds. Molecular or antigenic interrelationships

use non-animal alternatives	between vaccine and field viruses are not in themselves helpful in predicting the outcome of vaccination challenge trials. Protection studies in the respective host provide undisputable results and accepted by scientist, veterinarians and producers worldwide.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of birds used will be kept minimal and not affecting the scientific output of the studies. For this, based on past publications, protocols set by national and international regulatory bodies (e.g. European Pharmacopoeia).
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.	Chickens and turkeys will be used because they are the host animal to the diseases under study. The respiratory viruses to be used cause relatively mild infections, which normally resolve in about 7-10 days. The commercial vaccines cause no distress. All animals will be monitored daily. Numbers in experimental groups will be kept to a minimum consistent with producing meaningful results and allowing for individual variation. At all times, the birds are kept in the best environment, where floor-space, ventilation, light and lightings, feed and feeding, water and watering, behavioural needs (e.g. perching) and others requirements are provided to optimal standards. Any birds of welfare concerns, either due to health or not, would be put-to-sleep humanely.

Project Title (max. 50 characters)	Improving joint repair by reducing OA-like changes in an ovine knee model.
Key Words (max. 5 words)	Cartilage, bone, ovine, osteoarthritis, repair
Expected duration of the project (yrs)	5 years
Purpose of the project (as in Article 5)[5]	Basic research Yes Translational and applied research Yes Regulatory use and routine production Yes Protection of the natural environment in the interests of the health or welfare of humans or animals No Preservation of species No Higher education or training No Forensic enquiries No Maintenance of colonies of genetically altered animals[6] No
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to establish whether a tissue engineering approach can successfully improve cartilage and/or osteochondral repair techniques by reducing the initiation of Osteoarthritis - like changes in subchondral bone in an ovine knee model. Specifically the objectives are: 1. To identify subchondral bone changes, both molecular and structural, during cartilage and osteochondral repair. 2. To determine combinations of biomaterials, cells and biomolecules which enhance repair of cartilage and

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

	subchondral bone
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding the response of subchondral bone to cartilage damage and during the repair response following treatment is critical to developing improved cartilage repair strategies. This project will produce a detailed understanding of the changes in subchondral bone during the development of early osteoarthritis and the response to a number of treatments following meniscal cartilage or articular cartilage resection. Our use of novel repair material combinations with cells and growth factors using tissue engineering will provide pre-clinical evidence for the use of these approaches. A new programme of orthopaedic surgery in this area is being instigated to translate these findings into clinical practice.
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep, total of 240 animals over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All the animals will undergo surgery and therefore experience discomfort and pain immediately post surgery – in most cases this is of short duration. Some animals will experience transient mild lameness following surgery that is controlled by analgesics. A few (2%) may experience adverse effects such as wound breakdown. Regular analysis of the weight bearing of the operated leg will allow lameness to be monitored throughout the experiment. Animals are killed at the end of the experiment with an overdose of anaesthetic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project seeks to understand the interactions of cells in cartilage and bone in tissues over time and how this controls the repair of cartilage on the surface of joints. These interactions are complex and there are no adequate in vitro systems to model this process. We also intend to determine the most suitable strategies for repair prior to clinical investigation, which require successful modifying of these complex systems. The use of live animals remains the only method to carry out these investigations.

	The two alternatives to in vivo investigation are computer modelling and cell culture systems and whilst both continue to become more sophisticated there is still a huge divide between what they can tell us and the actual temporal events taking place following cartilage damage and repair. We do however use a number of cell culture systems to carry out preliminary studies of the interactions including detailed studies of cartilage cells and bone cells to determine what the cells are capable of, some aspects of the signalling pathways we are interested in and to ensure that the materials used for repair are not toxic and support the growth of the cells which they will contact following implantation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will always seek to use the minimum number of animals necessary to achieve the objectives of this project. We have considerable experience of the response of bone and cartilage in sheep using similar systems and we will conduct power calculations to determine appropriate numbers. We will also cross check these with studies published in the wider scientific literature. The design of each experiment will be based on the specific research question in order to determine the experimental groups for comparison and the appropriate time points at which to determine outcome. Again our previous experience and those of other research groups will be used to inform this process. All experiments will be hypothesis driven with the comparisons to be made and the statistics used determined before the study. Statistical advice is available where necessary.
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 sheep is considered to be one of the most suitable animal species for the evaluation of bone and joint surgery. This is because although it is a quadruped, it has bones of a size approaching in man and due to its size the loading produces mechanical forces through the tissues that are more relevant to humans than those in smaller animals. There is also a wealth of comparative information from other researchers including ourselves which can be used to improve understanding of the responses seen. The models we have

	chosen require access to the meniscal cartilage and joint surface without resecting the collateral ligament, as is done in other models. This reduces soft tissue trauma. Suffering will be minimised by careful monitoring of the animals following surgery. In particular the use of a force plate to monitor loading on the operated limb will allow earlier treatment of any problems with the joint that may arise.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Tissue engineering of the kidney</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Organ transplantation, whole organ decellularisation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>2</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>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[8]</td><td>Yes</td><td>No</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>End stage kidney failure (known as ESRF) is associated with major morbidity and mortality, and a person aged 60 with end stage renal failure requiring haemodialysis has a median life expectancy of 5 years. Renal transplantation is known to be the optimal form of renal replacement therapy in terms of survival, quality of life and cost-effectiveness and can restore function and life expectancy to virtually normal. However, this therapeutic option is severely restricted by the limited pool of organs available for transplantation from cadaveric donors.

The annual incidence of ESRF in the UK was 49,080 patients in 2009 but there is a huge disparity between this and the total number of kidney transplants performed in the same year i.e. 2,600. This disparity has been unchanging over the last decade even as the clinical developments in transplantation (both in surgical technique and immunosuppressive therapy) have made transplantation safer and more tolerable than ever, while ESRF is rising in prevalence worldwide in concurrence with the rise in conditions such as diabetes mellitus (which is the leading single cause of ESRF). Hence, an alternative source of organs for transplantation or implantation is both desirable and necessary. This solution may be potentially achieved within tissue engineering (including stem cell therapy), which is an interdisciplinary field that aims to generate biological tissue or organ replacement.</td></tr>
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[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

	This research project involves using a new technique to further this aim called 'whole organ decellularisation' which creates a naturally derived biological scaffold with all the structural and biological molecules native to kidney tissue as well as the complete 3-D organ architecture. This can be uniquely done by using the intrinsic blood vessel network to perfuse the tissue and completely remove all the cellular material to leave an intact 'whole kidney scaffold' which consists of the extracellular matrix (ECM). The ECM is the material that normally surrounds the cells in whole tissue, and has an essential role in cellular interactions and behaviour, as well as a mechanical 'scaffolding' purpose (as in the building construction sense). It is also crucial in tissue regeneration and healing. The kidney ECM can then be re-populated with various cell types as it has the ability to support cell growth and specialisation into potentially functional kidney tissue or even a whole kidney. The perfusion of the intact blood vessel network also allows 3-D *in vitro* culture and organ engineering on a complex level that was not previously possible. In this project we have produced whole kidney scaffolds using cadaveric rat kidneys and 'recellularised' these scaffolds with two different cell types, kidney cells and mesenchymal stem cells (a type of adult stem cell). The recellularised scaffolds or 'kidney constructs' will then be implanted inside live animals similar to a transplant procedure to see how well the cells survive and regenerate in vivo, and how they might develop into fully differentiated kidney tissue. There will be three types of kidney constructs implanted: those with kidney cells, those with mesenchymal stem cells, and those without cells. The three types will also be implanted for different time periods.
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 implantation of these kidney constructs could potentially lead to the development of living kidney tissue on these scaffolds, and show that they are viable and compatible within the body. This would be a further step in the process to developing fully functioning kidney tissue for transplantation.
What species and approximate numbers of animals do you expect to use over what period of time?	Species: rats Approximate numbers: 35-40 over 1-2 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	The typical animal will undergo one surgical procedure under general anaesthetic i.e. implantation of a kidney construct after removal of one of its kidneys. Surgically, this is essentially the

level of severity? What will happen to the animals at the end?	same as performing a transplant procedure which is known to be well tolerated in rats. The possible adverse effects are likely to be related to the surgery e.g. bleeding, infection, wound problems and the animals will be monitored and treated to minimise any discomfort. The removal of one kidney has no expected long-term adverse effects. The overall severity is moderate. All animals will be euthanized at the end of the project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to replicate the complex physiological *in vivo* environment and its biological effects on ECM scaffolds and their responses which allow 'remodelling' and tissue regeneration. It is also important to test the *in vivo* biocompatibility and viability of replacement tissues that have been tissue engineered. All stages of the work prior to implanting the recellularised kidney construct have been carried *in vitro*; this has also been extensively optimised and tested prior to *in vivo* implantation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As this is a preliminary study, the absolute minimum of animals are involved in each test group with the minimum allowance made for any mortality or morbidity (i.e. n=3+1 per group).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We are using a kidney transplantation model as this is exactly the purpose for which we are developing this tissue engineered kidney construct and the nature of the construct allows us to directly transplant it in vivo, using the standard transplant procedure and surgical technique, which is known to be well tolerated in rats. Animals will be subject to one procedure only and will be monitored closely post-operatively and treated to minimise any discomfort or complications. Any animals that suffer greater than minor complications or show signs of distress that cannot be ameliorated will be humanely euthanised.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The role of the immune system in tissue damage</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Inflammation, tissue injury, immune system, autoimmunity</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>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[10]</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>The immune system comprises a large network of cells and proteins that can detect and eliminate potentially noxious substances e.g. toxins or disease-causing microorganisms. Induction of an immune response in this setting is appropriate and a necessity for health. However, if the response is uncontrolled or indiscriminate it may lead to tissue damage and disease.

This project seeks to determine the molecular mechanisms causing the development of an abnormal response against self-components and the subsequent organ damage. The ultimate aim is the development of novel therapeutic targets for chronic inflammatory conditions and 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>The majority of chronic conditions develop as a consequence of an abnormal response of the immune system. This is either spontaneous or triggered by external factors and results in an imbalance between beneficial (anti-inflammatory) and harmful (pro-inflammatory) mechanisms leading to organ damage. Presently the treatment of inflammatory conditions relies on the use of immunosuppressant drugs that are non-specific and associated with a heavy burden of long term side effects. For the development of specific therapies a better understanding of the underlying mechanisms is mandatory.

In the current application we aim to continue our</td></tr>
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[9] Delete Yes or No as appropriate.
[10] At least one additional purpose must be selected with this option.

	studies on the underlying mechanisms resulting in an impaired tolerance towards our own body and to design new therapeutic strategies aimed at protecting against organ damage. Therefore the work we propose is of direct relevance to the understanding of human disease and will illuminate the extent to which the immune system modulates the development of tissue injury. We anticipate that, as proven by our successful track record, the experimental models generated under this licence will represent a unique and essential system to validate any therapeutic approach prior to its application in clinical trials.
What species and approximate numbers of animals do you expect to use over what period of time?	Only adult mice (wild type or genetically-modified) will be used for this project. We estimate that for all the procedures outlined in the application up to 4,000 mice per year will be bred and used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of animals will carry non-harmful genetic modifications and will be used for breeding and/or killed when appropriate. None of the experiments planned under this project involve procedures that are expected to cause the mice severe distress or discomfort. All procedures have been designed to be terminated if any of the animals appear to be suffering. A small proportion of animals (<10%) will undergo mild/moderate surgical procedure and will suffer no other adverse effect than those associated with post-operative recovery. To minimise associated suffering we have taken veterinary advice, will utilise anaesthesia and analgesia and terminate experiments early if required. Some animals (<15%) will be exposed to a tumour challenge For the mice undergoing this procedure additional humane end points have been listed to avoid unnecessary suffering. Some animals may develop renal impairment spontaneously or as a result of the experimental procedures. By measuring the presence of protein and blood in the urine (leaking through a damaged kidney) we will assess the development of renal impairment and mice will be culled at the onset of clinical signs of renal disease. In all experiments the mice will only develop minor or no symptoms before being humanely killed.
Application of the 3Rs	
1. Replacement	The majority of our work will be performed using *in*

State why you need to use animals and why you cannot use non-animal alternatives	*vitro* cell culture or other alternative approaches. However, inflammation and tissue damage are complex multicellular responses that cannot be fully modelled *in vitro.* Animal models are the only definitive way of testing hypotheses generated *in vitro* and studying novel therapies. In addition, for some of the work we have now reached the stage, after extensive and successful *in vitro* testing, where animal experimentation is required to demonstrate *in vivo* efficacy of the therapeutic strategies that are currently being pursued by us and other researchers.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Throughout this project we will pursue a vigorous policy of keeping the numbers of mice used in each experimental procedure as low as possible compatible with the achievement of the objectives of the experimental programme proposed. Animal numbers are minimised by: • examining multiple organs and tissues simultaneously and this approach will produce an abundance of data from each animal. • applying mild-moderate murine models, of the shortest duration, with clear end-points • using inbred mouse strains to reduce experimental variability • designing protocol with statistical advice to ensure significant results with minimum animals. • applying, whenever possible, longitudinal imaging studies to maximise the data output from live animals. • storing carefully tissue post-sacrifice to allow additional analyses at a later date without the need for further 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.	Mice are the lowest species to develop autoimmune and inflammatory disorders considered equivalent to those in humans and in which we can reliably measure clinical parameters analogous to the ones used in clinical practice. *In vivo* procedures will be based on findings made *in vitro*. Animals will be subjected to models of tissue injury, mainly kidney and skin, for which we have extensive experience. We also elected skin grafting as it is a very reliable and sensitive readout of

	immunity and tolerance. These models are all of moderate severity. In all cases these models have been chosen because they accurately mimic the human disease equivalents, do not result in systemic distress to the mice, and, to our knowledge, there is no alternative less severe *in vivo* protocol. Wherever possible non-invasive imaging techniques will be used to measure outcome. Animals will be inspected regularly to ensure general well-being and any animal showing signs of illness will be humanely killed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Development, growth and repair of striated muscle</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Muscle, heart, zebrafish, mouse, growth</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)[11]</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[12]</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>Our Objectives are to determine how striated muscle (skeletal and cardiac) is:
integrated into tissues and systems during growth, development and repair
regulated by physical force and/or exercise
defective in diseases and ageing
best treated to repair or prevent such defects.</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>Understanding of:
How force is perceived by cells
How muscle growth is regulated
How heart muscle growth is regulated
Methods to :
prevent muscle weakening in the aged
enhance regeneration of diseased or injured muscle
permit regeneration of heart muscle</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:
Zebrafish 27,000
Xenopus 50
Rats 50
Mice 1000
Chickens 100</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>For the vast majority of zebrafish (~25,000) and mice (~800) they will live a normal life within the animal facility with no adverse effects and be used for breeding until they are humanely killed around 18 months of age.
Procedures on Xenopus and chicken eggs will be mild, including nothing more than injections.
Rats (50), mice (200) and zebrafish (2000) will</td></tr>
</table>

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

	receive surgery rated as mild or moderate, such as induction of a muscle wounds. Some animals may be permitted to become old to assess muscle weakening in ageing. All will be humanely killed after the procedures. Small numbers of zebrafish (20 adults or 1000 surviving embryos) may be treated with mutagens of substantial severity. All will be humanely killed after breeding. In one procedure, limited numbers of adult fish will have wounds made in their heart muscle to mimic human heart attack. There is some immediate death from this substantial procedure, although survivors appear to swim normally and regenerate their heart. All treated fish will be humanely killed after a period of heart muscle regeneration.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are examining growth and repair of striated and heart muscle. These muscles do not mature and grow in tissue culture, or form proper connections to the skeleton, nerves and blood vessels. We also want to study the role of force or exercise in promoting of muscle, which requires the tissue to be within the body.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To prevent the need for severe procedures, we employ advanced genetic approaches with single cell resolution to determine outcomes. A suffering animal in less informative than one with a specific minor defect that can nevertheless be analysed in detail. For this reason we use zebrafish, whose optical clarity permits use to track the behaviour of single defective cells in an otherwise healthy animal. Where more severe experiments are essential, statistical methods, such as the resource equation, will be used to ensure that cohorts are the minimum number needed to give reliable results. Pilot experiments will assess the likely appropriate size of these cohorts.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Most of our experiments will be performed on zebrafish, both because they have experimental advantages and because they are regarded as less sentient than mammals. Results will be verified in rodents to ensure relevance to man, while minimizing the number of total rodents used. All moderate procedures involve the use of anaesthetics and pain relief as advised by veterinary staff.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Cardiac Muscle Cell Death and Regeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td></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)[13]</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[14]</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>Heart failure, the inability of the heart to meet the needs of the body, is a major cause of morbidity and mortality in the developed world, and is increasingly becoming prevalent in the developing world. It can be caused by heart attacks and genetic mutations. Current therapeutics, with the exception of heart transplant, do not address the underlying cause of heart failure, namely the death of up to 1 billion heart muscle cells. The greater the amount of cell death the worse the prognosis for the patient. Ordinarily there is little or no cardiac regeneration after injury, making the rescue of cardiac muscle cell number an urgent problem for patients with heart disease. The objective of this project is to employ 2 strategies to address the loss of healthy heart muscle. Firstly, reducing heart muscle cell death, by improving our understanding of the mechanisms by which it occurs in order to develop new drugs. Secondly, we aim to increase heart muscle cell number by improving our understanding of cardiac regeneration and strategies to enhance such regeneration (e.g., cell grafting using cardiac progenitor cells).</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>To date there are very few clinical trials using agents to reduce cell death and, therefore, is a current unmet need in the clinic. By investigating new targets for preventing cell death and the development of drugs aimed at these targets the number of patients developing heart failure could be decreased and the severity of the disease could be reduced.</td></tr>
</table>

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

	Injection of bone marrow cells into patients that have had heart attacks has shown that they can have a small benefit. This is likely because the cells are not truly regenerating the heart directly but by releasing favourable paracrine factors. Cardiac progenitor cells are more likely to directly regenerate the heart and as such are being investigated for such purpose. If they do have great regenerative potential then it is hoped that they could be used clinically to repair injured hearts.
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of the experiments will be conducted in mice due to the availability of transgenic mice. Over the period of the programme it is estimated that 9125 mice will be used in the breeding and breeding related protocols. For all other protocols it is estimated that 5000 animals will be used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The most likely adverse effects to occur during the protocols to be used in the current programme relate to development of heart failure. They may be a result of breeding the transgenic mice (particularly where bi-genic animals have resulted from a cross between 2 lines with mild phenotypes and can result in marked potentiation of heart failure or where the deleted gene has an essential function) or of the surgical procedures. The symptoms of heart failure include oedema, breathlessness, cyanosis, lethargy and loss of appetite. Animals will be monitored daily for these symptoms and is likely to be seen in 10-15% of animals. Symptoms would be classed as moderate/severe. Appearance of these signs will result in killing of the animal by a Schedule 1 method and a post mortem will be conducted in order to confirm cause of illness.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to understand the mechanisms behind processes involved in heart failure and regeneration the intact heart itself is required, not a reductionist model system that lacks fidelity to the mature adult myocardium and clinical syndrome. While all experiments have undergone thorough testing in cell culture, circulating factors and other organs are involved in both cardiac muscle cell death and regeneration and therefore an intact mammalian system is required for more conclusive and physiological evaluation.
2. Reduction Explain how you will assure	Power calculations have been conducted in order to ensure that the minimum numbers of animals are

the use of minimum numbers of animals	used while still yielding useful data. In vivo experiments will be planned along with ex vivo analyses carefully in order to obtain the most information from each experimental animal, thus reducing the number of animals used. Furthermore, analytical techniques are constantly being refined so that fewer animals are required to obtain usable data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	At this time, only mice are amenable to the complex genetic manipulations. In experiments that do not require gene deletion or transgenesis, mice are used simply for direct comparability to the animal models used in the many experiments that do. Whereas rats and rabbits would be suitable in the latter case, the change in animal model would be arbitrary and would introduce many confounding complications (differences in surgery, differences in imaging, differences in molecular reagents) that would surely render the pace of work less effective than using a single small-mammal platform. The surgical models have been chosen as they mimic human disease. Surgery technique is refined to be as non-invasive as possible and close monitoring is used to enable early identification of problems and subsequent early management, thus reducing animal suffering.

Sand Fly colony maintenance

Our project aims to understand and exploit the chemical ecology of blood feeding sandflies, in order to develop new tools to control populations of these important leishmaniasis vectors, and therefore prevent the spread of the disease.

- **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.

Leishmaniasis is a debilitating and potentially fatal disease which affects over two million people each year. There is no vaccine to protect people from the disease, and treatment can be both unpleasant and expensive, and is often ineffective.

Leishmaniasis is spread to people and animals by blood-feeding insects called sandflies. Our research programme aims to develop new ways of controlling leishmaniasis, and reduce the number of cases in humans and animals, by creating new tools for controlling sand flies.

In order to achieve this aim, we require colonies of live sand flies study. We used these insects to test whether we can use naturally-occurring chemicals, produced by sand flies and the animals on which they feed, to attract sand flies away from people and their animals, to stop them blood feeding, and therefore prevent the spread of the disease.

- **Outline the general project plan.**

The general plan of our project is to keep a colony of sand flies for us to study, in order to develop new methods of controlling leishmaniasis. Because sand flies require a blood meal in order to produce eggs, we must feed female sand flies on an anesthetized mouse to maintain the colony in the laboratory. We use the insects from the colony to extract potentially attractive chemicals and the proteins which make them, and to conduct behavioural tests to identify chemicals which could be used to control sand flies and leishmaniasis in the field.

- **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 maintain sand flies colonies, we must feed female sand flies on blood from a live animal. To achieve this, we anaesthetize a mouse, and place it into a cage containing female sand flies, and allow them to feed. The mouse remains anaesthetized throughout the procedure.

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

Our research will lead to the direct development of new methods of controlling sand flies, and reduce the number of cases of leishmaniasis worldwide. We have

already proven that the technologies developed through our research can be used to attract and kill sand flies in the laboratory and field.

- **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.

We estimate that over the course of 5 years we will use a maximum of 2650 mice for the purposes of maintaining the colony. We chose mice because they are well suited to the laboratory environment, and make excellent hosts for sand fly feeding, thereby minimizing the total number of animals required.

- **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.

In a previous project, we showed that it was impossible to feed sand flies without using a live host. Therefore we use anesthetized mice as a means of feeding sand flies, while minimizing animal suffering. In order to minimize the number of sand flies we use, and therefore the number of times we have to blood-feed, we use very sensitive equipment to detect and analyse the chemicals that sand flies produce, and design our experiments using live sand flies to be as efficient as possible.

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

Our work requires a single protocol for feeding sand flies. A mouse is placed under general anaesthetic, and placed in a cage of sand flies for a maximum of 1.5 hours. Because the mouse is unconscious, it is unaware of any pain or discomfort associated with the sand fly feeding. We constantly monitor the mice to ensure they do not react to sand fly feeding, and each mouse is used only once.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Autonomic Control of Cardiac Physiology</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cardiac, Arrhythmia, Autonomic</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 Yrs</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[15]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</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>Do all drowning victims drown? The answer to this seemingly odd question, is no. It is clear that falling into cold water activates two powerful reflexes which can cause severe and even lethal disturbances in the rhythm of the heart. We have evidence that this may be an important factor underlying death following immersion but we also believe that this phenomenon may be more widespread and occur in many every-day situations, contributing to sudden cardiac death. People with other risk factors, such as ischemic heart disease, or on some medications, may therefore be vulnerable to this form of sudden death. These studies will investigate this hypothesis by testing how modulating the nervous input to the heart, superimposed on a variety of other background situations, may induce disturbances in the rhythm of the heart.</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 aims to provide novel insights into how lethal disturbances in the rhythm of the heart occur. These arrhythmias underlie a very significant proportion of sudden death in people that may be triggered by a variety of environmental and pathophysiological stimuli. Our data will address fundamental questions regarding this complex phenomenon and may help inform future clinical practice through the development of novel treatment strategies.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rabbits – 600 over 5 Years (120 per year)</td></tr>
</table>

[15] Delete Yes or No as appropriate.
[16] 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?	No animal is expected to experience more than mild severity. This will be as a consequence of pre-treatment with chemical agents given to induce specific changes in the heart and the way it functions. After this treatment the heart will be removed from the rabbit under anaesthetic and used for experimentation outside the animal's body. The animals will be given an anaesthetic from which they do not recover and blood vessels connecting the heart will be dissected so that the heart can be removed with the parts needed for the subsequent experiment intact.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cardiac arrhythmias are complex phenomenon involving the interaction of multiple factors and cannot, at the present time, be studied using existing in vitro methods. Our understanding of the processes involved and their relative importance limits our ability to use computer modelling, though this is a clear goal we are working towards.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experimental data will be continuously analysed and assessed in order to achieve the aims of the project with the minimum number of animals. All protocols will be refined and conducted by trained individuals to reduce error and this experimental numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rabbits are the smallest laboratory species relevant for the study of cardiac arrhythmia, being most similar to humans when compared to guinea pigs, rats and mice. The rabbit will provide the most clinically relevant data and the data are easily translatable to humans. Our experimental model has been developed to limit harm to the animals, being short in nature and mainly conducted under general anaesthesia. We will continue to make efforts to refine protocols and further reduce the welfare costs.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Neuronal dysfunction in models of CNS disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Dementia; Neurophysiology; biomarkers; cognition</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)[17]</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[18]</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>In this project we aim to elucidate some of ways in which electrical signalling in the brain dysfunctions in dementias such as Alzheimer’s disease and fronto-temporal dementia. We will use state of the art recording techniques to examine electrical signals in individual and groups of connected neurones. We will use rodents which have been genetically altered or treated with specific compounds in such a way that they develop characteristic pathological features of dementia.

We specifically hope to discover how the electrical properties of individual neurones and groups of neurones are affected by genetic and or pharmacological treatments which produce these dementia-like symptoms. We will also examine how synaptic communication within and between brain areas is affected in these disease models. Finally, we will test established and experimental medicines in an attempt to reverse these changes in electrical activity.</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 specific benefits we hope to derive from this project fall into 2 broad categories:
1) Greater understanding of the changes to electrical signalling in the brain that occur in dementias, such as Alzheimer’s disease;
2) development of disease biomarkers: that is a signature change in the electrical signalling that can be observed in both animals and humans (using EEG). These types of functional, translational biomarkers are urgently needed to help develop new medicines to treat dementia.</td></tr>
<tr><td></td><td>Mice, approx. 1000 over 5 years</td></tr>
</table>

[17] Delete Yes or No as appropriate.
[18] 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?	Rats, approx. 250 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the genetically altered mice will not suffer any adverse effects, since most will be bred and then killed for tissue extraction without undergoing any procedures. The genetic alterations themselves mainly cause cognitive deficits and are not expected to cause any overt behavioural effects. A few mice and rats will undergo surgical procedures to implant recording devices and/or infuse specific substances in their brains. A small proportion of these animals may experience some adverse effects associated with the surgical procedures, including bleeding, infection and post-operative pain. Animals which have undergone surgical implantation of electrical recording devices will then be attached, via a cable, to additional equipment to amplify and record the electrical signals. The animals may experience some level of stress associated with the tethering procedure, although the vast majority (>90%) rapidly become accustomed to this. Some animals will undergo testing in various behavioural tasks designed to assess cognitive performance. Most of these tasks will be fairly non-aversive and are unlikely to result in any adverse events. One task, known as the Morris Water maze, involves animals swimming in a pool of warm water and as such may result in a certain level of stress. Other tasks may involve restricting access to food, in order to motivate them to perform the various tasks, in which food is provided as a reward for correct performance on the task. This will necessarily result in a certain level of hunger. Finally, some animals under this licence will be treated with clinically approved or experimental medicines in an attempt to improve the symptoms of dementia. Some of these medicines have known side-effects, such as diarrhoea, whilst for other we do not have any toxicological information. However, since all of these medicines are designed to affect the central nervous system, it is possible that some of these medicines may produce neurological adverse effects. At the end of each study the animals will be killed

	and usually their brains will be taken for physiological and/or pathological analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mammals such as mice and rats have comparable complex brain anatomy and physiology to humans, which cannot be accurately, modelled using other non-human alternatives such as flies, worms and computer models. Furthermore, the most accurate models of dementias such as Alzheimer's are genetically altered mice or rodents which have been surgically infused with proteins excessively present in dementia.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Wherever possible, genetically modified mice will be imported onto the authority of this licence from external breeders, rather than breeding a separate colony ourselves. This reduces the numbers of animals generated wasted from the breeding process. We will carefully control the conditions under which the animals are maintained in an attempt to reduce animal-to-animal variability. For example, all animals used in a particular study will be bred and housed under the same conditions, therefore reducing environmental variability and reducing the overall statistical variability in data sets. This inevitably leads to a reduction in the number of animals required for any given 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 can be easily genetically altered to produce specific pathological features of dementia. These genetically altered models represent the current gold-standard for examining the physiological effects of specific dementia-inducing pathologies. An additional, complementary approach to understanding dementia like pathology is to infuse dementia-related proteins such as β-amyloid or tau into the brains of rodents and allow them to spread in much the same way as they do in the disease. This is a refinement of an older version of this model, where animal are studied a short time after infusion, thus missing the clinically relevant spread of disease pathology. The in vivo recording techniques outlined here provide the best compromise between obtaining high resolution and high quality data and animal welfare. Future developments in wireless technology will be monitored and employed when it becomes sufficiently useful. The behavioural tasks used in this project are

	generally minimally or non-aversive and, where possible, animals will be motivated to perform these task using positive food rewards only, rather than aversive stimuli.

Birds as indicators of environmental change
Seabirds – environmental change – fisheries – foraging

- Summarise your project (1-2 sentences)

Understanding how birds respond to environmental change requires detailed individual-based research that can be scaled-up to population-, community- and ecosystem-levels. My research uses a combination of population genetics, diet reconstruction using elemental analysis, in tandem with tracking technology to address fundamental and applied questions about avian communities during a period of rapid change.

- 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.

Seabird populations are currently declining faster than any other comparable group of birds. Understanding factors influencing these declines requires a detailed knowledge about the demographic process and the proximate factors influencing changing population trajectories. My research makes use of elemental analysis of avian tissues (feathers and blood) to reconstruct foraging behaviour and relate this to changes in prey availability (such as that attributed to changes in fisheries management via the EU Common Fisheries Policy). Understanding individual-level responses is fundamental for understanding population-level processes. Moreover, analysis of tissues with different rates and timing of growth provide an insight to diet and foraging behaviour throughout the annual cycle. This is especially relevant for marine birds that spend long periods of the non-breeding cycle away from land and therefore away from conventional research techniques. This type of research has been complimented by the use of a range of bio-logging techniques (e.g. GPS loggers, geo-location loggers and time-depth recorders) to reconstruct fine-scale behaviour throughout the annual cycle.

Environmental change may not impact all members of the population equally. Sex-specific differences in foraging and migratory behaviour are well known but for species where males and females cannot be identified based on external characteristics, they can be sexed using genetic markers amplified from non-destructively sampled tissues.

- Outline the general project plan.

Sample tissues from seabirds breeding at multiple populations throughout the North Atlantic and using species specific genetic markers test for population structuring.

Analyse stable isotope ratios in the tissues (grown at different stages of the annual cycle) of seabirds and their prey to determine the relative contribution of different resources at different times of the year.

Collect tissues using non-destructive sampling methods and amplify sex-specific markers to determine gender of study birds.

- 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.

Severity of procedures is mild - withdrawal of feathers and blood. These approaches may cause some discomfort but volumes of blood are extremely unlikely to cause *anaemia or hypervolemia* (>8% by volume of body weight) and the number and type of feathers

unlikely to impact on flight performance or thermoregulation. Bio-logging techniques are unlikely to have harmful impacts, as indicated but current research in this area. On-going monitoring will be conducted to determine whether or not this is indeed the case.

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

The aim of the current research is to inform conservation for seabird populations currently under severe threat. These iconic animals provide an essential function in marine ecosystem dynamics but also have considerable intrinsic value for people. In the UK alone, we have approximately 8 million breeding seabirds of 25 species and for 8 of these species we have >30% of the global population. Moreover, at one small seabird colony in Scotland the local economy is boosted by ~£750,000 per annum because of visitors to this site. Without effective research-informed conservation, the future of seabird populations is bleak.

- 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.

30-40 Manx Shearwaters will be sampled from a number of different populations. In addition ~150 northern gannets will be sampled each year for five years to determine how diet and foraging vary over a period of expected change in climatic conditions and fishing activity in the NE Atlantic.

- 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.

Field-based applied research requires sampling of free-living animals – there are currently no viable alternative model systems available to address the questions at the core of my research. However, this research will be complemented by ecosystem modelling.

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

Wild birds will be caught, handled and released to minimise disturbance and handling time. Mild protocols will only be performed on those animals considered suitable (based upon reproductive state, condition and qualitatively assessed stress levels).