Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 49

Project Titles and key words

- Fish behaviour & physiology relating to fin damage

  Fish; fin damage; behaviour; welfare

- Neuronal processing of complex sounds
- Molecular mechanisms of CFTR channel gating
- Gene therapy in the cardiovascular system

  Angiogenesis, heart, VEGF, cancer

- Pharmacological Mechanisms
- Regulation of iron metabolism

  Iron, haemochromatosis, obesity, pregnancy, hepcidin

- Mechanisms of graft rejection

  Transplantation; immune response; rejection; tolerance; stem cells

- Testing products and methods for aquaculture
- The measurement of baseline ecological parameters in badgers

  Badger, Ecology, Movement, Social Organisation

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Fish behaviour & physiology relating to fin damage</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>fish; fin damage; behaviour; welfare</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>4 years</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>~~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[2]</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 aim is to advance understanding of fin damage in salmonids. This will provide a foundation for improvements in production systems and fish welfare. To do this the project will develop and apply simple measures of fish aggression, determine those conditions which contribute to aggression and fin damage in salmonids (including both environmental factors and episodic events), and study the time delays between injury and the appearance of observable fin damage and recovery from damage.</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>Aquaculture is an important part of the UK’s farming industry. It is a major employer, makes a large contribution to the economy and it is the main provider of oily fish to UK consumers. It is a growing sector. Production of salmon, which makes up 90% of the UK industry, has increased 5 fold over the last 20 years. Despite progress in addressing many welfare concerns, fin damage is an issue that has persisted in both the salmon and trout industry and fin damage is the dominant injury seen in farmed salmonids. This welfare issue affects a very large number of animals with UK hatcheries incubating around 135 million salmon and trout eggs each year. This project aims to further understanding of aggression and fin damage in salmonids with the final objective of proposing practical strategies that will reduce aggression and fin damage during commercial production of salmon and trout.</td></tr>
<tr><td>What species and approximate numbers of</td><td>Atlantic salmon and rainbow trout (the most important commercial species reared in the UK).</td></tr>
</table>

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

animals do you expect to use over what period of time?	The maximum number of animals will be 5120 over 4 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?	Other than collecting blood samples, the studies will involve protocols that are used in routine management in aquaculture and restocking (e.g. anaesthesia, marking, weighing and measuring). Changes in environment and aggression tests will also be designed so that situations are akin to those fish may experience on farms. All the procedures within the project are categorised as 'mild'. Few adverse effects are expected aside from change in fin condition and this will be monitored very closely throughout the studies. Induced change in fin condition will be kept within levels that are seen in commercial environments. The procedures that will involve handling of fish are performed routinely within the licenced establishment and optimal techniques will be used to minimise risk of secondary infection and any transient pain caused by labelling or blood sampling. Should any infections occur, these will be treated appropriately. Animals will be euthanised at the end of the studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This work will be studying the interplay between environment, fish behaviour and physiology and as such there are no suitable alternatives to the use of live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be designed according to the results of: previous work on fin damage in trout, a survey that will review aggression and fin damage in fish and the results of a study carried out at commercial sites that will collect relevant information on husbandry factors. All experiments will be designed to use the minimum number of fish necessary to produce statistically valid data that will be of value to the aquaculture sector. In addition, as part of the project, existing technologies will be developed to produce methods for remote monitoring of fish behaviour and fin damage (e.g. by video monitoring) and these methods will be utilised wherever possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Work will be carried out on Atlantic salmon and trout. These are important species in commercial aquaculture in the UK. The project involves procedures which are all categorised as 'mild' and good handling techniques and appropriate anaesthesia will be used throughout. Few adverse effects are expected and fish will be closely monitored. The staff that will be involved in the studies are experienced in fish care,

	and have all undertaken suitable training.

Neuronal processing of complex sounds

The project outlined in this application has two specific goals: 1) to model how complex sounds are represented in neuronal activity throughout the auditory pathway, from the auditory nerve to the cortex, and 2) to model how the neural representation of complex sounds is altered by auditory processing deficits such as age-related hearing loss, noise-induced hearing loss, and tinnitus, and to determine the extent to which therapies such as hearing aids and cochlear implants can restore the representation to its normal state.

Our current understanding of auditory processing is insufficient; if we hope to understand the auditory system well enough to restore its natural functionality in the hearing impaired we must make major advances in our understanding of how complex sounds are represented in the brain. Our experiments must be performed in animals, because the auditory system cannot currently be replicated in cell culture, and because the functioning of the auditory brain is not well enough understood to be simulated accurately on a computer.

However, we make heavy use of computer modelling in our work to reduce and refine the use of animals whenever possible. We also minimise the number of animals needed by choosing our methods and designing our experiments to ensure that each animal can provide tens, hundreds, or even thousands of data points for analysis, not just one.

We minimise suffering by anaesthetising animals for any procedures causing more than mild discomfort, and by ensuring that animals receive analgesic and appropriate post-operative care after any surgeries. We perform our experiments primarily in mice and gerbils, which are arguably among the least sentient species with a mammalian auditory brain; we also use rats and guinea pigs where experimental methods would be more successful in a species with a larger brain. Procedures include breeding and maintaining genetically modified mice; inducing hearing loss during development or adulthood; raising or housing animals in controlled sound environments; training animals on sound discrimination tasks; recording brain activity in anaesthetised animals; and recording brain activity in awake animals implanted with recording devices.

Many animals experience minimal or no adverse effects, because they undergo only procedures causing no more than mild discomfort, or because they are subjected to procedures only when under terminal general anaesthesia. Other animals may undergo surgeries under general anaesthesia with recovery; post-operative pain and discomfort in these cases are minimised by appropriate use of analgesics and good post-operative care.

Overall, we aim to achieve insight into how complex sounds are represented in neuronal activity throughout the auditory pathway, understand how this representation is perturbed by hearing deficits, and probe the extent to which therapies can restore this representation, all while minimising adverse effects on our experimental animals.

Molecular mechanisms of CFTR channel gating

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

Cystic fibrosis (CF) is the most common fatal inherited disease in the UK. It is caused by low or absent activity of a channel protein called CFTR. On the other extreme, excessive CFTR activity is responsible for both dehydration during secretory diarrhoeas (e.g.cholera) and polycystic kidney disease. The CFTR channel is a gated, specific hole: it provides a pathway for anions to cross membranes, but only when it assumes an “open” position.

Here we propose to study in depth how CFTR works. We will use two distinct, complementary, approaches. In Aim 1 we will focus on opening of the channel. We will use an established physico-chemical analysis, REFER, in a novel way. Our REFER study will investigate the temporal sequence in which different parts of the protein move during opening and, in particular, when a particular “joint” of the protein (the NBD/TMD interface) moves. This analysis will give information on the structure of an unstable, transient state called transition state which determines how frequently the channel will open.

In Aim 2 we will also focus on the NBD/TMD interface but we will extend our studies to steps following opening in the CFTR functional cycle. We have preliminary evidence suggesting that a movement at the NBD/TMD interface might occur while the channel is open, during a step which is followed very rapidly by channel closing (and therefore controls how fast channels close). Our investigations will allow us to determine how strongly two positions on opposite sides of the NBD/TMD interface interact, and on how this interaction changes during the various steps of CFTR’s functional cycle. The results of experiments in Aim 2 will therefore allow us to determine the direction, as well as the timing, of movements occurring at the NBD/TMD interface during the entire gating cycle.

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)?

Over the past 10 years academic efforts (including ours) have led to a major breakthrough in understanding how CFTR works. We now also have some information on the 3D shape of CFTR and in particular on two positions it is likely to assume. But these are like snapshots, we do not know yet at what point of the functional cycle CFTR takes these positions, nor how the protein moves to go from one position to the other.

Modulating CFTR function would be beneficial for treatment of several diseases (cystic fibrosis, secretory diarrhoeas, polycystic kidney disease). At present there is a large gap between industrial efforts to obtain compounds that affect CFTR activity (based on high-throughput screening of compound libraries), and academic research aimed at understanding how the CFTR protein works. While random screening has identified several promising compounds, unfortunately, the wealth of information that has emerged from basic research has yet to have an impact on drug discovery. Here we will address that gap: we will concentrate on one area of the protein, the NBD/TMD interface, which could be a good binding site for a CFTR-specific drug, and on two strategic steps in the functional cycle which are best suited as intervention points for altering CFTR activity (the opening step and the step triggering closure). Together with

structural data, the results of our investigations could lay the foundations for the rational design of CFTR potentiators and inhibitors.

n addition, many other transport proteins are closely related to CFTR (ABC transporters). We will use the ion channel CFTR as a model, to probe the conserved mechanism by which these proteins work. ABC transporters play fundamental roles in diverse physiological processes. They determine tissue distribution (preventing penetration in brain, fetus, lymphocytes and tumours) and oral bioavailability of most therapeutic drugs. Thus our studies might also suggest ways of beneficially altering the activity of other ABC proteins (e.g. inhibiting ABC transporters involved in keeping therapeutic HIV/AIDS drugs out of lymphocytes and brain).

Thus, while the importance of the studies we propose lies mainly in a deepening of our biophysical understanding of a molecular mechanism (how CFTR and ABC proteins work) our proposed studies also have the potential to improve the health of different patient cohorts (CF, secretory diarrhoea, central nervous system disorders, HIV/AIDS, cancer) in the longer term.

Gene therapy in the cardiovascular system
Angiogenesis, heart, VEGF, cancer

- Summarise your project (1-2 sentences)

  This project aims to identify molecules and mechanisms that play important roles in blood vessel formation and the development of cardiovascular diseases, and to develop approaches such as gene or cell therapy that can effectively target these molecules to achieve a therapeutic effect in human cancer and cardiovascular 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.

  Cardiovascular disease and cancer are the major causes of death in the developed world and rapidly increasing in developing countries. However, the mechanisms that cause, or protect against, these diseases are poorly defined, and there is a continued need for new therapeutic approaches. Both diseases involve blood vessels. We will identify molecules and mechanisms with important roles in heart disease, and disease-related angiogenesis and thereby identify new therapies or therapeutic targets.

- Outline the general project plan.

We will examine the effect of genes and other agents of therapeutic interest (such as proteins, drugs or stem cells) on formation of blood vessels and vascular regeneration in rodent, pig and zebrafish models which mimick human heart and vascular disease. This will identify targets for the development of novel therapies, and approaches that have effective therapeutic effects in these models that can be translated in future work to treatment of human disease.

- 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.

Our protocols are based on well-established procedures that have already gone through a considerable amount of refinement. Most animals will not undergo procedures that will inflict harm. Instead these animals will be used for phenotyping the effects of mutations using non-invasive imaging or analysis. Some animals will undergo procedures that include minor damage to the lining of a small region of a single artery in a mouse or rat or pig, or ligation of an artery in the mouse or rat that will restrict blood flow to the hindlimb, or cause minor heart attacks, or will involve implantation of tumour cells under the mouse skin, or in the zebrafish, injury to a small region of the heart, or injection of tumour cells in to the body cavity. Based on out experience, adverse effects are anticipated to be very limited in all our protocols and where they do occur to be very brief in duration. Adverse effects that may occur include (in rodents and pigs), lethargy, hunched posture, loss of appetite, weight loss, dehydration, diarrhoea, inflammation and infection, ulceration, difficulty of breathing, sudden death, and in fish, difficulty breathing, abnormal colouration, abnormal

swimming, feeding or schooling behaviour. All our protocols, except one, have a severity level of mild or moderate. Only one protocol has a severity limit of severe. All animals will be humanely killed at the end, and/or when signs of discomfort or pain are manifested.

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

This work will improve understanding of mechanisms and the key molecules involved that maintain cardiovascular health, or that stimulate angiogenesis associated with cancer. VEGF-linked signalling pathways, which are the focus of this application, are already known to be important for human cardiovascular health and in human diseases such as cancer. Since many of these mechanisms and molecules are conserved between vertebrate species, the work proposed here will have direct relevance for analogous process and disease states in humans. This work will therefore will advance knowledge and understanding of important processes underlying human health and disease. Furthermore, by identifying key novel molecules in these processes we will be able to identify novel targets for the development of therapeutic drugs, which may lead to the development of novel therapies for heart disease and cancer.

- 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.
-

Pigs (100)
Rats (2,000)
Mice (10,000)
Zebrafish (25,000)

Animal species were chosen mainly because protocols were established in those species, avoiding unnecessary pilot work. Small rodents (rats and mice) were chosen, as these are the simplest appropriate mammalian organisms. The choice of mouse and fish is determined by the unique ability to genetically alter these species. Use of zebrafish allows us to perform studies wherever possible in simpler vetebrate organisms. Limited use of pigs will be made where this is justified to provide stronger proof-of-concept data required for supporting development of a therapeutic approach for use in human disease, given the closer similarities between the pig and human cardiovascular systems.

Where necessary, pilot studies involving small numbers of animals will be performed to establish the proof-of-concept, and only if these small studies are encouraging, will we proceed to larger studies. Since protocols are already well-established in the chosen species, the minimum numbers of animals needed can be determined more accurately, and unnecessary pilot work can be avoided. Studies will be performed only using animal numbers sufficient to produce statistically robust results.

- 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.
-

While cell culture models have been helpful and we continue to use them extensively, there are no computer, tissue or cell culture models that successfully mimic human cardiovascular disease, angiogenesis or cancer. Two major reasons for this are: these

diseases develop in complex multi-tissue environments in living animals, which cannot be mimicked by non-animal models; they occur over long time periods which make it difficult to perform similar studies in non-animal models.

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

Protocols will be performed by experimenters who have experience in the models chosen. Measures will be taken at all appropriate stages of each protocol in order to prevent pain, discomfort or other adverse effects, and to promptly treat such signs. Experiments will be of sufficient duration to achieve our objectives, and persistence of adverse effects and any suffering in animals will be avoided by immediate humane killing.

Pharmacological Mechanisms

- 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.

The effective control of chronic pain remains suboptimal with only about 1 in 4 chronic pain patients gaining adequate pain control with present medicines. Chronic pain conditions are highly prevalent and represent a huge economic burden on society and in addition to causing unimaginable suffering, it has been reported that chronic pain also contributes to mortality. The successful development of novel analgesics, better use of present medicines and improvement of the diagnosis and recognition of pain as a major medical issue is contingent on a detailed molecular, cellular and functional understanding of the mechanisms of pain. This knowledge will be communicated to Health Care Professionals, ranging from pain experts through to nurses and physiotherapists and published in journals and also through interactions with Pain Societies and patient groups, with whom we have extensive contacts.

An overarching aim is to translate laboratory findings to the clinic in order to improve pain therapy. This involves identification and location of targets for new painkillers and also understanding where and how more established analgesics work. Where possible, we would attempt to show positive effects of novel compounds by studying the mechanisms and pathways behind pain, from where it starts and into the brain and the effects of drugs on these processes.

- Outline the general project plan.

We will investigate the how different pain messages are sent from the body to the brainin a select range of pain models that represent the most important clinically observed pain syndromes with a large unmet clinical need and healthcare cost to society. Using mostly in vivo electrophysiology where we can measure the activity of nerve cells to different pain stimuli in fully anaesthetized animals and some other end point measures we will study the consequences of how chemicals, nerve networks, drugs and genes play a role in the pain transmission. We wish to understand the mechanisms mediating the pain associated with tissue damage, cancer, peripheral nerve injury and osteoarthritis. These are the main types of pain in patients and many people have insufficient pain control with the existing painkillers.

- 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.

The procedures applied to the animals are as follows:

Inflammation – this includes injection of chemicals into the hind paw as a model of very short inflammatory pain, a UV irradiation procedure whereby the animal is covered only exposing a hindpaw (for UV exposure). This is a model of sunburn pain.
Osteoarthritis, a model with an early inflammatory component and a longer chronic nociceptive component, is induced by injecting monosodium

iodoacetate (MIA) into the knee joint. MIA is a drug which causes the loss of cartilage leading to the eventual erosion of bone in the knee joint, instability of the joint and subsequent pain, very similar to what occurs in patients.
Bone cancer induced pain – Secondary cancers in bone are common in many cancer patients. Here an injection of cancerous cells are implanted into the leg bone of the animal under deep anaesthesia. The animals are not be expected to show any adverse effects elsewhere in the body as a result of the operation or cell infusion. Eventually, as the tumour grows it will start to damage the bone and cause fractures but our studies will be over well before this stage.

Neuropathic pain – many people have this type of pain after Shingles or as a result of trauma, diabetes and HIV infections. Here the animals will be subjected to restricted damage of the peripheral sciatic nerve caused by tieing of the nerve or the nerve roots prior to entering the spinal cord under deep anaesthesia. Some post-operative pain is inevitable in this protocol. Previous experience suggests that animals recover rapidly after surgery in these models and are mobile and active soon after the cessation of anaesthetic. Infections due to the procedure will be rare due to the strict use of aseptic surgical technique. Here skin clips and non-absorbable sutures are used and to reduce local pain and/or necrosis we will remove them at approximately 7-10 days post surgery.

Electrophysiology - Measurements activity in nerve cells will be made in the spinal cord and brain nuclei of fully anaesthetised animals. The procedure involves inserting a microelectrode into CNS tissue and recording the output of neuronal responses following stimulation of the peripheral receptive field with a variety of non-painful and painful stimuli. Since the animals are unconcious at the time of the procedure they do not experience any suffering other than mild discomfort upon induction of anaesthesia.

CNS tract tracing – here anatomical tracing agents will be injected into specific areas of the brain or spinal cord to work out connections between areas. These agents do not in themselves produce any adverse effects and the procedure is performed under deep general anaesthesia.
The subsequent perfusion of the animals is performed under non-recoverable general anaesthesia, so no adverse effects are anticipated.

Genetically altered animals – here animals will be mated and subject to such other non-painful procedures as required for conventional breeding of animals with specific genetic defects or transgenes. This breeding will include cross-breeding with other genetically modified lines and conventional lines. Animals produced under this protocol are not expected to exhibit any harmful phenotype and as the genes targeted are those predicted to promote the transmission of pain, the behavioural consequence would most likely be reduced pain. However, it is not possible to fully predict the nature or severity of any potential defect and for all types of mice there will be careful monitoring for possible side effects.

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

Patients with pain should benefit from a better understanding of pain mechanisms and analgesic actions, both in terms of their own knowledge

and that of those treating them. Veterinary pain control will likewise benefit. Identification of pain targets could lead to more effective or better tolerated pain killers.

- 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 would use rats since they are resilient, and there is extensive knowledge regarding anatomy, physiology, psychology and toxicology. Their larger structure permits more ready access to peripheral nerves to enable surgical manipulation and easier access to spinal cord neurones during *in vivo* electrophysiology. They show a remarkable ability to rapidly recover from anaesthesia and surgery. The widespread use of this species (and the fact we use models that are used world-wide) allows comparison with published studies.

Mice represent an ideal species for certain experimentation as they are well adapted to laboratory conditions, small, economical and their genome has been fully mapped allowing phenotypic uniformity. This allows for genetic modification of identified targets, studies that are not possible in other mammals. All transgenic studies therefore have to be in the mouse.

A number of our published electrophysiological studies in the rodent have been shown to be excellent predictors of analgesic effectiveness in man and have also guided us towards the understanding of human hereditary pain disorders. The number of animals to be used in the studies is based on our extensive experience of *in vivo* physiological and pharmacological studies related to pain and ensures that the experiments are powered sufficiently to achieve the purposes of the experiment .The ability, in this species to produce extensive dose-response relationships within a single animal minimises the overall number of animals. Where study design allows obtain behavioural, neurophysiological and ex vivo immunohistochemical data from the same animal, thus minimising use of animals, and some cases the opposite non-injured side of the animal can be used as an internal control, thus reducing the need for separate control animals.

- 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.

Our work involves studying the role of pain pathways in an integrated system and as such cannot be replicated in a dish or modelled by computers.

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

The protocols selected are of mild to moderate severity, in most cases the procedure is carried out under full general anaesthesia, or the procedure involves minimal discomfort at best and so does not require analgesia. The animals are closely monitored following any surgical or genetic intervention. The large bulk of our work involves collating neuronal data as the end point measure, in fully anaesthetised non-sentient animals, and so there would be

little or no suffering experienced by these animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Regulation of iron metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Iron, haemochromatosis, obesity, pregnancy, hepcidin</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)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Preservation of species</td><td></td><td>No</td></tr>
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<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>
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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>Iron deficiency anaemia is the most frequent nutritional deficiency disease in the world affecting almost 2 billion people. In addition, anaemia due to chronic disease is the most common cause anaemia in hospitalised patients. Large section of population suffers from obesity which also leads to lower iron levels and physical performance. Furthermore groups of population on a diet rich in polyphenols (recommended as beneficial functional food for health) might at an even greater risk of iron deficiency. Conversely approximately 1:2000 people of Northern European descent posses a gene mutation that predisposes them to iron overload. Thalassaemics with increased capacity to make red blood cells also suffer from iron overload due to increase iron absorption. Disturbed iron metabolism also linked to many other common diseases such as heart disease and diseases of the brain such as Alzheimer’s disease. Hepcidin is a small hormone which plays a major role in maintaining iron levels at physiological levels. Levels of this hormone change in response to inflammation, infection, iron levels, low oxygen, and increase in rate of red blood cell production. It is of upmost importance to understand how the production of hepcidin is regulated and how it interacts with iron transporters to control iron homeostasis. The main goal of the proposed research is to understand the mechanism of systemic and cellular iron homeostasis and how it is perturbed in obesity, inflammation, low oxygen and</td></tr>
</table>

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

	dietary components, in particular, polyphenols.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project will provide new insights into mechanism of iron homeostasis and how this process is altered by diet and disease. The aim is to identify novel targets for controlling iron deficiency and iron overload. These results are expected to benefit the development of new strategies for preventing the detrimental effect of iron deficiency and iron overload on heart disease, vascular disease and neurodegeneration.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 2000 mice and 850 rats will be used over the period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Genetically altered models that will be used in this proposal have been characterised in detail by a number of different research groups and no adverse effects have been notes. Application has experience in all the other techniques to be used in this proposal. Administration of substances (including Intravenous, subcutaneous, intraperitonel and intramucular) will cause momentary needle stick pain. Administration will be according to current best practice in relation to carrier liquids and volumes recommended by The Royal Society for the prevention of cruelty. To avoid hypovolaemia and anaemia, no more than 10% of the tiotal blood volume will be withdrawn in any one occasion or no more that 15% in any 28day periods. Changes in the diet and administration of substances that modify body iron levels and inflammatory response will be dosed as such as not to cause lasting harm, pain, suffering to animals but might cause mild discomfort and distress. The animals likely to suffer dehydration as a result of a procedure will be fed with hydration jelly with glucose to prevent this.. The techniques described are of mild or moderate severity. All animals undergoing procedures will be weighed at regulator intervals and any showing loss of weight greater than 20% , associated with other clinical signs of distress , will be killed by schedule 1 method. In the event any animal shows signs beyond those describes in the licence, the NVS will be contacted for advice and treatment. If such treatment fails to alleviate symptoms, the animal will be killed by a schedule 1 method (cervical dislocation or anaesthetic overdose.)
Application of the 3Rs	
1. Replacement	Important aspects of this proposal are to establish

State why you need to use animals and why you cannot use non-animal alternatives	how circulating molecules which regulate hepcidin (hormone produced by the liver in response to external stimuli) levels, are recognised by the liver and how then messages are sent from this complex to modulate hepcidin production. This is then likely to involve liver releasing hepcidin into blood which then circulates around the body to act on multiple organs to have its effect of decreasing iron release, in particular intestine, macrophages, kidney, and endothelial cells. Obesity, pregnancy and serum iron levels and polyphenols are likely to affect these interactions. These complex interactions cannot be reproduced under cell culture conditions making these methods inappropriate for use in this project. In addition cell lines often do not behave like native cells in relation to their uptake and transport machinery.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Whenever possible changes in transport in different tissues will be established in one animal. In all our experiments blood and other main tissues (intestine, Liver, Kidney, spleen, adipose tissue, brain and heart and sometimes muscle) are harvested from each animal in order to correlate changes in iron levels with expression of iron transporters and iron binding proteins. This approach not only reduces animal usage but also provides the changes in different tissues when the animal genetics and diet are the same. This allows for more detailed and precise interpretation of data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In most cases choice of species is dependent on available disease models. In this project for most of the procedures mice will be used as KO and transgenic for iron genes are mostly in mice, which have been characterised and are available. In the case of obesity and pregnancy study we have chosen rat as leptin receptor deficient rat is available. Rat has advantage over mice that more tissue is available per animal so this allows us to do many more measurements on a single rat. The protocols used in this proposal have been used before in our studies, they have been designed to be most refined possible, using the minimum number of animals, to provide statistically satisfactory results. These protocols have been planned to cause least pain, suffering or distress whilst addressing the scientific question they have been designed to answer.

Mechanisms of graft rejection

Project summary

The purpose of our research is to gain a better understanding of the immune response to transplantation, which will help in the design of new strategies for treatment to prevent transplant rejection.

Background

Organ transplantation is the best treatment for many patients suffering from organ failure but patients need to take anti-rejection medicines for life to suppress the immune system otherwise the transplant will be rejected. These drugs are relatively non-specific and suppress the whole immune system, not just the response to the transplant; consequently, certain cancers are much more common in transplant patients while infection causes a third of the deaths in the first year in heart transplant patients.

The pathways of transplant rejection are complex. Cells and tissues express a set of molecules that are virtually unique to a particular individual. Following transplantation, these MHC molecules are recognised as “foreign” and the patient’s immune system attempts to destroy the cells by rejecting the transplant. The best solution for transplant patients would be if their immune system could be manipulated to recognise the MHC antigens on the transplant as “self” instead of as “foreign”. The immune system would then be tolerant of the transplant MHC antigens and it would not reject the transplant, so patients would not need anti-rejection therapy. In animal experiments, transplant tolerance has been achieved with several different types of treatment; also, some patients have been identified who no longer need to take their immunosuppressive medicines because they have spontaneously developed tolerance. In spite of this, scientists and clinicians have not yet managed to design a strategy for inducing immunological tolerance that works for all transplanted animals or patients, and this is because the mechanisms of rejection and tolerance are not fully understood. Clearly, we cannot, for ethical reasons, withdraw immunosuppressive treatment in patients, or try to alter their treatment to induce tolerance unless we have a better understanding of precisely how the immune system is responding to the transplant. For this, we must resort to working with animal models of transplantation.

Project outline

This project continues our existing program of transplantation research and builds on our previous findings. It involves performing skin, organ (hearts, kidneys, blood vessels) and cell transplants in inbred rats and mice. Strains of animals, including genetically modified mice, are selected to best answer specific immunological questions. Interventions such as treatment with immunological reagents or drugs are used to test well-defined hypotheses. Outcomes are measured by determining survival of the transplanted tissue and by sequential blood tests and imaging studies which provide essential information. Transplanted tissues and lymphoid tissues are further examined at the end of each study; parallel in vitro tests yield additional valuable information.

Possible adverse effects

All animals receiving transplants will undergo surgery from which they are expected to make a rapid recovery. Heart transplants are not life-sustaining models: the transplanted secondary heart, beating in the abdomen, is easily monitored by palpation. Rejection of some forms of tissue transplant, and some interventions may result in clinical signs including moderate weight loss, hunched posture, stary coat and poor appetite. Injection of stem cells in the Teratoma Assay may sometimes result in formation of a tumour (up to 12mm diameter) at the site of injection.

Predicted benefits of this project

Graft rejection and complications of non-specific immunosuppression continue to cause significant clinical problems following transplantation. Information provided by this research program will improve our understanding of mechanisms of rejection and induction of tolerance, and will provide new insights into the potential for stem cells to be used as replacement tissues for transplantation. A better understanding of the mechanisms of immunological rejection will inform future strategies for avoiding rejection and inducing tolerance.

Minimising animal use

The immune system involves the entire lymphoid system of the body and not just peripheral blood; it is therefore necessary to undertake “whole animal” studies. The physiological similarities between rodent and human immune systems have long been recognised and consequently, there is an excellent range of immunological reagents for rodents (especially mice) as well as an extensive research literature. The use of inbred, genetically identical strains of animals means that when transplants are performed between two different strains the rejection responses will be similar in each transplant recipient and rejection times will be predictable within a particular strain combination. Therefore small numbers of animals (5-6 per group) can give reliable experimental data that can be verified by statistical analysis.

Minimising suffering

Transplants are performed under general anaesthesia, together with pain relief; they are well tolerated and wounds heal rapidly. Animals are closely monitored for signs of rejection; rejection of skin or heart grafts or stem cells is unlikely to cause clinical signs. Rejection of kidney or aortic grafts may cause clinical signs; animals are killed before such signs exceed the moderate severity limit. Similarly, any interventions are unlikely to cause distress, other than transient, or clinical signs; animals are killed before such signs exceed the moderate severity limit. In all studies, we use the minimum number of animals, and only when animal use is unavoidable; methods are continuously refined to minimize their impact on animal well-being. Transplantation studies are supported by in vitro work using explanted tissues, and by less invasive Immunisation work to avoid the need for surgery.

Testing products and methods for aquaculture

We use fish to test potential new medicines and treatments that may be used to combat diseases and parasites. These may be drugs, vaccines or wrasse used as "cleaner fish".

In aquaculture there are diseases for which no cure exists, parasites that are developing tolerance of available medicines, and new problems arising with the expansion of the industry. We develop new treatments such as medicines, vaccines or biological controls as a service that also allows sensible combinations for drugs for new farm sites and integrated management. We study fish health on a small scale under controlled conditions that allow us to measure the effects of new husbandry methods or disease treatments. The work is needed in order to keep farmed fish healthy so that they don't suffer and to ensure that the methods employed are efficient" and ecologically sound.

80% of our research is for commercial clients in the feed, aquaculture and pharmaceutical sectors. Projects are usually a sequence of steps in identification, testing, safety assessment and approval of new medicines. Towards the end of the development process for a new product work to Good Laboratory Practice (GLP) guidelines. Other academic work is for the Institute of Aquaculture at University of Stirling, which can include making fish feeds more environmentally sustainable, developing vaccines, or growing cleaner fish.

Whenever we are testing efficacy, or effectiveness, of a medicine we have to give a disease or parasite which may cause harm. When we test a potential medicine for safety to the target animal we give several times the expected therapeutic dose, which can cause toxic effects that depend on the type of drug, or even death. If present in large numbers external parasites can cause irritation to the skin or gills that may lead to the formation of lesions or mortality. External bacterial or fungal infections can also lead to external lesions or mortality. Systemic bacterial or fungal infections can lead to internal lesions and mortality.

The aquaculture industry is an increasingly important source of protein for the diet of the world's population, and as more aquatic species are domesticated for intensive farming a limiting factor will be keeping up with the range of infections. In salmon aquaculture for example sea lice are controlled but are beginning to show reduced sensitivity to current medicines, while amoebic gill disease can only be controlled with fresh water or hydrogen peroxide in large volumes. The service we provide allows timely development of new treatments for such infections that will preserve the welfare of many millions of fish which will then provide food for millions of people.

Most of the animals we use are Atlantic salmon to developing treatments to support the salmon aquaculture industry. Wrasse are an ecologically-friendly cleaner fish in salmon farms, and since we share location with a commercial farm we help them improve rearing, feed, husbandry and use. We use small-scale efficacy tests to determine effect size and inform power calculations to estimate the numbers required for a significant result. We then plan experimental protocols to include the minimum number of animals that will guarantee a valid result taking into account a likely level of withdrawals and mortalities from coincident disease, handling injuries or other sources.

We are testing treatments for pathogens and parasites that can only reproduce and grow on their live animal host, such as sea lice that grow on salmon. Therefore we have to use live marine fish at least as the hosts to establish a supply of the pathogen

or parasite, even where it is possible to test the new treatments in vitro away from the fish. Our work is aimed at identifying and demonstrating the safety of methods for treating fish in large cages or ponds, either in freshwater or at sea. In these contexts the delivery of a treatment is usually either by incorporation into feed, exposure in a bath, or injection: and to obtain approval we have to test treatments using whichever of these is first choice for use in farms. Where it is possible to culture a pathogen or parasite in vitro for even a part of its life cycle or to test in vitro we do so, such as when hatching sea lice eggs or quantifying drug sensitivity of adult sea lice.

Many of the treatments that we test for pharmaceutical clients are already known to be highly selective for the target disease and safe in fish because they are licensed for use in agriculture, which minimises the risk of adverse reactions. We often carry out range-finding tests in vitro to establish the effective dose for a new drug before using it in ·vivo. We carry out small scale toxicity safety tests as the first live phase in order to avoid exposing large numbers of animals to harmful doses. When designing a live phase of a project we can usually choose a relatively low number of animals. This is possible because we are assessing whether a treatment has a strong therapeutic effect that is easily demonstrated statistically. Looking for large effects also means that we can set the level of parasite challenge low and demonstrate removal rather using more to induce pathology, such that even many untreated animals will never suffer.

We monitor the health of all the animals we use every day to ensure that we cause the minimum of suffering. This is written into every contract and protocol in order to ensure that we always have the resources available. If an animal is found to be suffering it will always be withdrawn from the study and humanely killed.

Project Title (max. 50 characters)	The measurement of baseline ecological parameters in badgers		
Key Words (max. 5 words)	Badger, Ecology, Movement, Social Organisation		
Expected duration of the project (yrs)	two		
Purpose of the project (as in Article 5)1	Basic research	Yes	
	Translational and applied research	Yes	
	Regulatory use and routine		No
	Protection of the natural environment in the interests of the health or welfare of humans or		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals2		No
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The principal objective of the current project is to provide data on baseline badger movements through the use of GPS devices. Spatial organisation of badgers will also be examined by the use of bait marking and genetic sampling. The study will provide unique information on the ranging behaviour and social organisation of badgers.		
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project is designed to increase the evidence base in order to assist in any future TB eradication strategy. Badgers are implicated in the spread of bovine TB and gathering ecological measurements for badger contact, badger movements and badger social organisation will provide data that could be used for to shape bio security measures to minimise cattle badger contact.		
What species and approximate numbers of animals do you expect to use over what period of time? 1..__._	Badgers (Metes metes) - 40		
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 procedures applied to animals in this project all have a mild severity limit and include animal capture, anaesthesia, attaching a collar to the animal, obtaining a blood sample, micro-chipping and collection of small hair samples. In terms of adverse effects, animals may get stressed, react to anaesthetics, have minor temporary bruising /		

end?	any adverse effects will be temporary and not cause any long lasting pain or distress. Animals will be released at the point of capture immediately after recovery from anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project aims to investigate the ecology of free-ranging badgers, to obtain baseline measurements. It would not be possible to meet the scientific objectives of the project without the use of live badgers in their natural environment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For the analyses that are critical to meeting the scientific objectives of the study the minimum sample size deemed appropriate is to sample and collar 2 badgers trapped at 20 setts (10 in each of the study areas).This will allow a maximum of 40 individual badgers to be fitted with GPS collars. These represent the minimum sample size to allow adequate statistical inference from the analyses that are required to be undertaken (spatial ecology
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 choice of species has been determined by the research requirement to address important knowledge gaps that exist in terms of obtaining baseline badger movement and social organisation. The majority of the methods used (i.e. anaesthesia, blood sampling) are standard that have been used to study badger populations for decades. Specific observation / training opportunities with colleagues in the techniques to be used in the current work programme will be available. Additionally, during initial badger live-trapping and handling a qualified veterinary surgeon will be on site to carry out or supervise badger anaesthesia