Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 48

Project Titles and key words

- In vivo imaging and image guided therapy

  Imaging Rodent

- Methods for genetically modified mouse production

- Interventions to reduce Campylobacter in poultry

  Chicken Campylobacter Intervention

- Evaluating therapeutic agents in human leukaemias

  Acute leukaemia, chronic leukaemia, therapy

- Development and maturation of respiratory rhythm generation

  Breathing, brainstem, maturation, development, rhythm

- Neural development and axon guidance

- Rabbit irritancy and toxicology studies

  Rabbit, Toxicology, Safety assessment

- Manipulating the nutrition and environment of pigs to **optimise** and understand their productive performance and welfare

  Nutrition, welfare, pig

- Posterior capsule opacification (PCO)

  Lens, cataract surgery, posterior capsule opacification, PCO, regeneration

- Improving the fertility, health and performance of dairy cattle through management, nutrition and genetic strategies

  Cattle, fertility, health, nutrition, management

- Strategies for brain repair

  Neurotransplantation, Brain repair, Parkinson's, Huntington's, Animal models

- Centrifugal modulation of olfactory bulb function

In vivo imaging and image guided therapy

- Summarise your project (1-2 sentences)

We will develop improved in vivo imaging techniques and image-guided therapy devices.

- 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.

Imaging is widely recognized as a leading tool in the diagnosis, treatment and monitoring of a wide variety of diseases.
Our work is problem-led by biologists and clinicians who require better imaging methods and we produce new and better methods to improve the study of in vivo biology.

- Outline the general project plan.

We will operate several parallel programs of research covering developments in the physical and engineering sciences and, once developed, these will be demonstrated in vivo.
Methods will be usually be developed using 'phantoms' (test samples made of plastic, metal and water). Translation to the live animal only happens when the new methods are expected to deliver quality data in vivo, and where imaging of the live animal is required. Methods are developed, refined and the resulting data is assessed to quantify the benefit of the new technique. The methods are then made available to the user community.

- 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.

Anaesthesia is usually required to minimise distress caused by the nature of the imaging techniques and the need to immobilise the animals for the duration of scanning.
For this work we will only perform terminal experiments so that the animals cannot experience any adverse effects.

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

We will deliver imaging methods that are faster, more sensitive, more specific, more tolerable or some combination of these, and which are immediately translatable to clinical practice. These methods will then be available for the biological research community at large.

- 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.

Most of our work will involve the mouse though some works will be performed in the rat, especially where the physical size of the subject to be scanned or treated affects the imaging or image-guided treatment process. We use these small rodents as these are the species most widely used in preclinical research, and our work is specifically aimed at improving measurements made in this arena.
We anticipate using approximately 500 mice and 100 rats over the course of this project, and expect that these works will lead to the development of at least 5 new and/or significantly improved imaging techniques. We can statistically analyse data as studies progress and we can cease development as soon as we achieve success. At this point data can be reported and the methods made available to others.

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

The live animal is required because many of the phenomena we need to image only exist in the living body. Some of these, such as tissue volume or constitution are the

target of the measurement whilst some of them, such as involuntary muscle jerks, the cardiac and respiratory cycles and peristalsis are confounds which corrupt the imaging process. We aim to develop imaging techniques that are insensitive to the confounds whilst maximally sensitive to the measurements required. The methods developed will be appropriate for use in repeated measurements in the same subjects so increasing the statistical power of the method and reducing the number of animals required for achieving success.

- Explain why the protocols and the way they are carried out should involve the least suffering.
- Terminal scans are used specifically so that animals experience the minimum level of distress.

Methods for genetically modified mouse production

- Summarise your project (1-2 sentences)

The aim of the project is to improve the way genetically modified mouse models are made in the research laboratory.

- 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.

Genetically modified mouse models are frequently used within the research community to explore gene function in a living organism and to allow animal models of human disease to be investigated. These models provide insights into disease mechanisms and provide information about therapeutic strategies.
Existing methodologies for the generation of genetically modified mice are not without their short comings and overall the outcomes can be unpredictable and highly variable. Consequently, larger numbers of mice are used in the laboratory to allow for this variability. Improving the reliability of the mouse models and thus reducing the variability of outcome has the potential to reduce the number of mice that are used in biomedical research and this project is focussed on developing new technologies that will contribute to these improvements.

- Outline the general project plan.

This project aims at combating the disadvantageous and unpredictable aspects of current methods for generating genetically modified mouse models by investigating:

a) chromosomal sites in the mouse where the insertion of genetic material can occur safely and at high efficiency
b) the use of DNA enzymes to allow genetic manipulations to occur
c) the use of enzymes which subtly modulate gene expression in a controlled and precise manner
d) manipulation of recombination processes which describes the shuffling of genetic information which occurs in every generation

- 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.

To establish and optimise new methods, it is necessary to test and validate them by making genetically modified mice which will require animal procedures. This involves procedures for the collection of embryos from sacrificed female mice, their manipulation in vitro and their surgical re-implantation into foster mothers. *Mice are expected to make a rapid recovery from the surgical procedure and no adverse effects are expected. For the proof-of-concept models, genetic elements will be used which are not associated within any harmful or detrimental phenotype.*

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

The likely benefits would impact the absolute numbers of animals used in transgenic biomedical research and it is hoped that the new methodologies would dramatically reduce the numbers of animals used in such studies. Furthermore, the increased predictability of the newly developed methodologies should lead to a situation where unexpected and non-specific adverse effects of the genetic manipulation are minimized.

- 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.

Approximately 2000 mice per year will be used in this project. Mice are the animal of choice for this project as more is known about their genetic makeup than any other animal and despite appearances they are genetically very similar to humans. Also the technology used to produce these models has been established and developed in the mouse so it therefore provides the best model for this work.

- 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.

To understand the complexity of biological processes and to model human disease, it is not possible to rely on cell-culture or computer simulations alone. The effects of genetic alteration on a living organism can be essential to understand the complex interactions that occur. Cell culture systems will be used where appropriate and many of the experiments will be attempted first in cell culture before proceeding to the mouse.

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

Animal use and suffering during the project will be minimized by establishing the key parameters for genetic manipulation in cell culture systems before moving to the animal model. Furthermore, when mice are used, only genetic elements and chromosomal insertion sites which are not associated with any detrimental or adverse effects will be selected. Furthermore, the genetic elements to be used in this study or the endogenous genes to be manipulated have been chosen as ones which are not associated with a detrimental consequence. Easy-to-monitor, non-invasive visible characteristics of the resulting mice, such as their weight or coat colour will be used.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Interventions to reduce Campylobacter in poultry</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Chicken Campylobacter Intervention</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)[1]</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></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>Campylobacter and Salmonella are common causes of food poising. Contaminated chicken meat or eggs, respectively, are the major sources. The Food Standards Agency has set a target to reduce the number of infected chickens and thus the incidence of human infections. This project aims to investigate the effect of various dietary interventions at reducing Campylobacter and Salmonella in the chicken.</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>Campylobacter accounts for a third of the cost of the burden of foodborne illness in England and Wales, estimated at more than £583m in 2008. An intervention which can be given to the birds at the farm that will reduce or stop Campylobacter and Salmonella getting into the chicken food chain would save money and benefit society.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Poultry will be used for this work as their gut environment cannot be replicated sufficiently in vitro A maximum of 6400 birds are expect to be required for the five year project.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The treatments that will be given to the birds are classed as mild and no adverse effects are expected. The only adverse effect that might occur in a very small number of birds would be diarrhoea. If this occurs, the animals will be immediately killed.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement
State why you need to use animals and why you cannot use non-animal alternatives</td><td>Both Campylobacter and Salmonella replicate and survive in the chicken gut from as young as 5 days of age or as an egg respectively, until death. It is very well adapted to the chicken gut which</td></tr>
</table>

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

	cannot be replicated in any other models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical calculations have been made to ensure the most statistically powerful observations are made using the least number of animals. If anything changes during the course of the project that allows the team to use less animals, then this will be actioned immediately.
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 interactions between the chicken gut and the organism that live there are very complex and cannot be replicated *in vitro*. The chickens will not be dosed with higher levels of *Campylobacter* or *Salmonella* than that found naturally occurring on a commercial farm. These bacteria do not cause any clinical signs of illness. Procedures will be kept to the minimum with only two mild procedures to be carried out at a maximum of 4 times each and never more than once a day.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Evaluating therapeutic agents in human leukaemias</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Acute leukaemia, chronic leukaemia, therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Leukaemia is a form of cancer, one that affects white blood cells. This is a condition that can affect people of all ages and leads to a number of clinical manifestations, inlcuding anaemia, increased susceptibiltiy to infection, tiredness. Although advances have been made in both the understanding of this condition and its treatment, there is a need to devleop better therapies. For example, in the treatment of childhood leukaemia, a significant proportion, (20%), relapse due to failure to eradicate the disease. The situation is worse for adult patients with relapse rates of around 50%. The mechanisms that underlie these failures are poorly understood and investigations into the mechanisms of resistance to medicines are essential if we are to improve the results of treating these conditions. The relapses in leukaemia are thought to arise from a population of cells that are resistant to current medicines. These resistant cells have the capacity to replicate themselves and hence subsequent disease relapses may arise from these cells. Characterisation of these leukaemia ‘stem cells’ is essential for the development of effective new treatments. This study proposes to characterise leukaemia stem cells and then assess the effectiveness of both established and new medicines against these stem cells in animal models of leukaemia.</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</td><td colspan="3">This project will enable us to increase our understanding of how leukaemia develops and grows and provide a system for the evaluation of new medicines that may be of value in the treatment of these conditions.</td></tr>
</table>

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

project)?	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use immune deficient mouse species that are the most suitable for evaluation of human haemopoietic cells. Over the 5 years of the project the proposed work will use no more than 4,600 mice, including those animals used for the purposes of breeding.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will inject leukaemia cells into mice to determine which cells can establish and cause leukaemia in the animals, so called leukaemia stem cells. We will monitor progress by examining animals and by removing blood samples in order to identify the presence of leukaemia cells. Treatments will be given, in the form of medicines which we believe are active against the leukaemia cells, these will be given by injection, in a similar way that they will be given to patients. At the end of the study, animals will be killed and we will undertake a post mortem examination in order to determine whether the treatments have eradicated the disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although studying leukaemia cells in cell culture can provide a lot of useful information, we need to study how they behave in the complex environment of a living animal, where they may become established in a number of different tissues e.g. bone marrow, spleen and lymph nodes. As a result it is necessary to take studies into living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Much of our work is undertaken in the laboratory using cells taken from patients who are suffering from the disease. The ability of leukaemia cells to divide and expand will be investigated in cell culture. We will also assess the sensitivity of cells to both current and new medicines. Consequently, only leukaemia cells that can grow in cell culture systems will be used in the animal models. Likewise, only those medicines that have shown potential to kill leukaemia cells in culture will be examined in the animal model. The laboratory-based experiments will provide essential information as to the suitability of the patients' cells and the medicines of interest for studies in mice and allow us to significantly reduce the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The species chosen are the most compatible for evaluation of human cells and their growth. Typically, we use mice that have an underactive immune system, so that when we inject leukaemia cells into them, the cells become established and grow. We make use of good experimental techniques with minimal intervention to avoid distressing the animals, expert preparation of samples for investigation, strict adherence to protocols and keeping the time for which an animal is under experimentation as short as possible.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Development and maturation of respiratory rhythm generation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Breathing, brainstem, maturation, development, rhythm.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><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[6]</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>Understanding how the brain controls breathing breathing is of considerable interest to human physiology and pathophysiology, since failure of the respiratory system can lead to serious health problems, a diminished quality of life and death. The aim of this proposal is to investigate the mechanisms underlying the development and maturation of the respiratory system.</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>An increased understanding of how the respiratory system matures and the vulnerabilities of the system that result in respiratory failure will eventually lead to improved diagnoses and treatment of respiratory disorders, particularly in the elderly and in newborns when the respiratory system appears to be most fragile.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats & Mice
Adults & neonates - Mice (600) and rats (250)</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>Minimal transient discomfort may occur during induction of anaesthesia or during drug administration. Steps will be taken to limit damage to muscle and tissues when implanting electrodes to measure muscle movements and brain activity during sleep and wakefulness. Surgery will be carried out using aseptic technique and analgesia will be given for as long as required in consultation with the named veterinary surgeon. Most animals will undergo a procdure at the end of the protocol which involves terminal anaesthesia followed by removal of the brain for further study. Those animals not undergoing this procedure will undergo a schedule 1 procedure.</td></tr>
</table>

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

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The research proposes to study pathologies of breathing in mammals during sleep and wakefulness. There are currently no substitutes available for this type of research in vitro. Control of respiratory rhythm is complex and influenced by state e.g. wake and sleep. Breathing is a complex behaviour that can be studied from a cellular to a systems level. The molecular mechanisms underlying rhythm generation can be studied using in vitro brainstem slice preparations. Combining in vitro and in vivo data will provide a full understanding of development and maturation of the respiratory system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot experiments on a small sample of animals will be conducted to select appropriate drug doses where these are not known from previous work or from the literature. Collaborators in France will also carry out certain pharmacological procedures in vitro, which will inform the proposed in vivo work, allowing better hypotheses to be formed and thus reduce the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats and mice are the lowest vertebrate group in which well characterised models of respiratory rhythm have been developed. The majority of work carried out in vitro in this field is performed on rodents, which is what the in-vivo work is built on. Many of the respiratory data we will collect comes from the use of whole-body, open-flow plethysmography, which is non-invasive and non-painful, and does not require physical restrain. The vast majority of drugs used for manipulating respiration are known to be safe and only result in minor changes.

Neural development and axon guidance

Our project sets out to unravel the mechanisms that are involved in brain development, including how nerve cells wire up with muscles and allow the precise control of movement. We currently have little knowledge of the cellular processes and molecules that govern these processes. Our research will also have direct impact on the understanding of human diseases such as neuro-developmental and neurodegenerative disorders.

The main aims of the project are:
1) To describe the organisation and axon projections of key groups of cranial motor neurons
2) To identify the molecules which orchestrate the organisation of these neurons and their axon projections
3) To manipulate expression of these molecules to give information about their normal function
4) To generate model systems for human movement disorders such as squint

We will use an integrated set of approaches, using animal models (fish and chick), tissue culture, and occasionally mouse models. Animals have to be used for these experiments, as in order to understand the development of nerve pathways we need to analyse them in an intact system. In order to create accurate models of diseases in humans, such as eye movement disorders, we will use zebrafish models. We will mainly utilise the zebrafish, and will combine use of wild-type and transgenic lines (protocol 1) to observe and make movies of normal nerve projections, along with labelling and testing the function of various genes by microinjection of fluorescent molecules and genetic constructs (protocol 2). More durable models of neural disorders will be made using transgenic lines (protocol 3 and 4). We will trace nerve pathways (protocol 5) and analyse behaviour e.g. filming eye movements (protocol 6). In a few selected cases we may also use transgenic mice (protocol 7) to validate results found in the fish embryo.

The numbers of zebrafish used will be 30,000, and 500 mice. The protocols used are designed to minimise any adverse effects and no animal will ever be subjected to pain or suffering. In the main, we will perform non-injurious analysis of living embryos, or fixed specimens. The only invasive procedures will involve microinjection into one cell embryos, which are not yet sentient, and fin-clipping under anaesthesia for genotype analysis. The transgenic lines we intend to make will not suffer adverse effects apart from ‘mild’ perturbations of eye movements. We have chosen the zebrafish due to the fact that cranial motor nerves are almost identical in layout between fish and humans. Also, the zebrafish has many advantages, including genetic and imaging studies, which we will exploit.

The benefits which will accrue from our studies will be a thorough understanding of cranial motor neuron development, in particular nerve projections to the eye muscles, and how these develop normally and abnormally with relevance to humans.

Through our interactions with the scientific community and with clinicians, we will shed light on neuro-developmental disorders and help eventually in the development of therapies.

Rabbit irritancy and toxicology studies
Rabbit, Toxicology, Safety assessment

- Summarise your project (1-2 sentences)

This project's objective is provision of non-rodent data for regulatory submission and/or for safety assessment purposes with the rabbit being the regulatory accepted test species. The data will be used to review substances under development and, where appropriate, satisfy governmental regulatory requirements necessary to gain clinical trial approval and/or marketing authorisation.

- 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.

Governments require and the public expects that substances we are exposed to are safe or their hazards are well understood. Regulatory approval is required to allow drugs to be tested in human or veterinary trials, or for chemicals, agrochemicals, food additives/substances or medical devices/articles to be marketed.

- Outline the general project plan.

Studies conducted in this project form part of a framework of studies designed to investigate potential effects of pharmaceuticals, chemicals, agrochemicals, food additives/substances or medical devices/articles to facilitate a review of substances under development and, where appropriate, satisfy governmental regulatory requirements necessary to gain clinical trial approval and/or marketing authorisation.

Regulatory testing requirements generally follow a tiered approach, with the extent dependent on the intended use of the chemical and its stage of development. Studies are designed to determine specific toxicity or regulatory endpoints, and/or for safety assessment, ranging from single-dose toxicity, irritancy or local tolerance studies to repeat-dose studies.

Project Title (max. 50 characters)	Manipulating the nutrition and environment of pigs to optimise and understand their productive performance and welfare		
Key Words (max. 5 words)	Nutrition welfare pig		
Expected duration of the project (yrs)	5 yrs		
Purpose of the project (as in Article 5)[1]	Basic research	Yes	
	Translational and applied research	Yes	
	Regulator/ use and routine production	Yes	
	Protection of the natural environment in the interests of the health or welfare of humans or animals	Yes	
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals[2]		No
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim is to provide government, industry stakeholders and the general public with informed and accurate knowledge and information on how to maintain a sustainable pig industry through investigation of five key areas which are sow nutrition, pig performance indicators, nutritive quality of feeds, ecological impact of pigs and pig welfare.		
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 main benefit is the advancement of science in the areas of sow nutrition, pig performance, nutritive quality of feed ingredients, environmental pollutants and pig welfare. Through the experimentation conducted in this project, animal productivity will be optimised whilst also optimising animal welfare. The excretion of nutrients and emissions of green house gases and other hazardous gases to the environment will be quantified from pig production and minimised therefore there are major benefits for the environment. The knowledge gained in this project will provide producers with knowledge to maintain sustainability and government with information to make informed decisions.		
What species and approximate numbers of animals do you expect to use over what period of time?	Pigs will be the species used and it is estimated that approximately 2530 will be used across all experiments over the 5 year period.		
In the context of what you	All procedures are mild and the vast majority of animals		

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?	will be returned to the pig herd. Where animals have suffered stress or are ill as a result of any of the protocols they will be treated appropriately under the instructions of an experienced Veterinary Surgeon. A few animals will have cannula inserted and at the end of the protocol these animals will be euthanised using a schedule 1 method.
	Application of the 3Rs
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For most objectives (sow nutrition, pig performance indicators, environmental impact, pig welfare), the studies must be conducted on the live animal since the main effects of treatment will be on the performance or behaviour of the animal. Currently there is no alternative means to test for animal responses such as performance and behaviour using a non-animal model. For nutritive quality of feed and environmental impact, the use of *in vitro* techniques and rapid non-destructive methods have been considered but the results so far have indicated that they are not reliable indicators of the in vivo situation. As such no non-animal model currently exists to provide reliable accurate data with regard to the nutritional quality of feed ingredients for pigs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all areas, a statistician will be consulted to determine the minimum replication required to account for natural variation and attain a statically robust result. The number of animals required will be further reduced through the use of appropriate experimental design, for example a changeover design will be used when determining ileal digestibility.
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 funding that this project relates to concerns pig experimentation. As such pigs will be the main species used. Furthermore, the research must be conducted on commercially applicable animals within commercially applicable housing and management practices so that results are directly transferrable to the local commercial industry. As such the level of animal suffering will be no more than that experienced under normal commercial conditions for the vast majority of the experimentation. Where animals are segregated for a specific experimental protocol, for example determination of digestibility, they will be monitored regularly and will always have sight of others. They will also be handled with extra care. Any adverse effects will be monitored and appropriate action will be taken. There are no severe protocols proposed.

Posterior capsule opacification (PCO)

KEYWORDS Lens, cataract surgery, posterior capsule opacification, PCO, regeneration

- **Summarise your project (1-2 sentences)**

  This project aims at furthering the knowledge of the mechanisms leading to complications of cataract surgery including posterior capsule opacification, the most common complication of cataract surgery and to lens regeneration, a potential novel approach to prevent PCO.

- **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.**

  Posterior capsule opacification (PCO) remains the most common complication of cataract surgery and causes reduced vision. PCO can be treated with laser but there are complications that can occur as a result of this treatment in addition to the inherent costs. Furthermore, current cataract surgery does not recover the ability of the lens to accommodate, which allows focusing at close and, thus, reading glasses are needed following cataract surgery. Research has been conducted in an attempt to reduce rates of PCO. So far, only modifications in the intraocular lens design (an intraocular lens is placed inside the capsular bag of the lens when the lens is removed at the time of the surgery; as the cataract represents the lens of the eye, if removed, it needs to be replaced by an artificial intraocular lens so that patients can focus following surgery) have been successful at reducing rates of PCO. Nonetheless, PCO is still a clinical problem and, in certain groups such as children and young adults rates of PCO are extremely high (>90%) if conventional cataract surgery is undertaken.

  The purpose of this research is to search for new treatments to reduce the occurrence of PCO.

  The current work aims at investigating the signals that control lens regeneration and the potential of using lens regeneration as a possible option to conventional cataract surgery. Thus, if "modified" cataract surgery is done, which entails removing the lens but leaving the bag where the cataract is inside the eye, the lens regenerates. This has been observed in animals but there are many reasons to believe that it could also occur in humans. The current research aims at understanding better the mechanisms that control lens regeneration and evaluate the possibility of using lens regeneration as a possible new treatment for cataracts. If a new lens were to regenerate, there would be no need to putting an intraocular lens during the surgery. The newly regenerated

lens would, potentially, have the same properties as the individual's own lens before it became a cataract and, thus, would be superior to an artificial intraocular lens.

- **Outline the general project plan.**

Lens extraction will be undertaken with conventional techniques (removing the anterior part of the capsular bag as it is routinely done in cataract surgery, with or without implantation of an intraocular lens) and different treatments will be used in an attempt to reduce rates of PCO. In addition, "modified" cataract surgery will be also undertaken, leaving the capsular bag and allowing the lens to regenerate. In both scenarios, the mechanisms that lead to PCO and to lens regeneration will be investigated.

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.

No harms are expected. Animals will undergo cataract surgery. We know from our experience in humans that this is not a painful procedure.

Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.
This study may provide new potential treatment strategies for PCO, even to prevent it by using lens regeneration as a possible treatment for cataracts.

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.

200 rats, 100 mice and 50 rabbits maximum. We have an excellent anaesthetic and surgical technique which we have been using in animals for the past 12 years. Importantly, we will be working together with our Named Veterinary Surgeon to assure the well-being of the animals at all times.

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.

Lens regeneration does not occur in vitro. Furthermore, although there is an in vitro model of PCO using donor human capsular bags, it is expensive and not all components of PCO occur (for instance, Soemmering's ring does not develop in this in vitro model). We will use in vitro studies to support our in vivo work.

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

As much as possible, we will anaesthetised the animals only twice during the study, at the time of the surgery and at the time of the sacrifice. We will use general anaesthesia combined with topical anaesthesia to minimise any potential discomfort the animals may experience at the time of the surgery and will be very vigilant following surgery so if any complications occur (which we know from our 12 year experience doing cataract surgery in rodents and over 20 year experience doing cataract surgery in humans is unlikely) they will be promptly treated. Experience doing cataract surgery in rodents and over 20 year experience doing cataract surgery in humans is unlikely) they will be promptly treated.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Improving the fertility, health and performance of dairy cattle through management, nutrition and genetic strategies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cattle, fertility, health, nutrition, management</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)[7]</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>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</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>Development of concentrate supplementation strategies for higher yielding dairy cows which will make more effective use of the concentrate and forage parts of the diet.

Identification of the role of concentrate supplementation strategies and management on dairy cow immunity, and on early lactation uterine health, and fertility.

Identification of the role of transition and early lactation supplementation strategies on dairy cow fertility and health, and subsequent performance.

To provide ‘genomic’ samples which can feed into a much larger ‘European’ data base, thus allowing difficult to measure (but potentially economically/environmentally important) parameters to be predicted with improved accuracy.</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 development of improved concentrate feeding strategies would allow concentrates to be allocated with a higher degree of precision, thus saving on feed costs, and potentially reducing phosphorus losses from dairy systems.

An improved understanding of links between nutrition, management and genetics and cow</td></tr>
</table>

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

	fertility, health and immune function, would ultimately lead to the improved health and fertility of the UK dairy herd. By adopting a genomic approach, genetic progress can be made across a range of difficult to measure traits, and at a much greater rate than achieved historically through conventional breeding programmes.
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle and sheep (1000)
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 procedures are minor, and any possible adverse effects will also be minor. These include stress as a consequence of handling, minor infections around blood sampling sites, possible increased risk of mastitis, stress due to confinement, skin rubbing when harnesses etc are fitted. In the case of the formation of a stoma, there is an increased risk of post surgical infection. Animals will return to the herd at the end of each procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The overall aim of this project is to evaluate the effect of nutrition, management and genotype on *cow performance, health, fertility, nutrient utilisation and metabolism.* By definition, this work needs to be conducted using cattle. No other animal model is suitable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise the number of animals subject to experimental regimes, careful consideration will be given when planning any experiment. In any experiment, the likely variability that will be encountered in terms of the variable being examined will be considered, and taken into account when planning the numbers of animals to be used. This will vary between experiments. The Biometrics team is always consulted when designing animal experiments to ensure that replication and design is adequate to achieve the stated objectives.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	In order to examine the effect of nutrition, management and genotype on animal performance, health, fertility and nutrient losses, cattle must be used. No other species is appropriate. Minimising

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	suffering is always a key objective in any research being undertaken. Most of the measurements proposed within the programme are non-severe. Ensuring that all staff are fully trained in the implementation of the techniques being adopted, and are alert to signs of suffering/distress within the animal, are key issues. Efforts are always made to improve techniques so as to minimise any distress/suffering caused. Staff are aware that any animal can be removed from an experiment at any time if its welfare is compromised.

Non technical summary following on from a retrospective assessment.

The original summary can be found in Volume 39

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Strategies for brain repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neurotransplantation, Brain repair, Parkinson’s, Huntington’s, Animal models</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></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></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[10]</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>This project seeks to develop novel strategies for treatment of brain damage, whether caused by injury or disease, with a particular focus on the development of novel cell and gene therapies for Parkinson’s disease (PD), Huntington’s disease (HD) and stroke.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work underpins clinical trials of fetal tissue transplantation in HD and PD taking place now, and provides the biological foundations for the next generation of major new applications using more efficient sources of cells, including pluripotent stem cells.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats and mice.
The project will use approx. 4000 rats and 5000 mice over 5 years.</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</td><td>The project involves surgical, anatomical, physiological and behavioural procedures of mild, or at most, moderate severity, including breeding genetically modified animals, that express modest impairments of motor and cognitive disability, that are the targets for structural repair and functional amelioration. The experimental procedures are</td></tr>
</table>

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

end?	reliable, and serious adverse effects are rare and not expected, but procedures are in place for rapid alleviation of distress in the case of unexpected adverse events being detected. All animals are killed at the end of each experiment by the most humane methods appropriate to the species.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Motor and cognitive behaviours are complex features of the living sentient animal, dependent upon the intact functioning of a complex living nervous system, and impaired in human neurodegenerative diseases. The survival, growth and connectivity of cells in this complex environment cannot be adequately modelled in vitro or in simulation. Thus, in order to develop effective new cell-based therapies for devastating human conditions, the experimental use of live animals is the only way to model the disease processes, to determine the survival integration growth and connectivity of cell repair processes, to test the effectiveness of alternative cell therapy procedures, to develop the transplantation technology and to test protocols for safety and efficacy prior to human application.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All protocols are designed for maximum sensitivity, and experiments are designed to maximise power to detect significant results with the smallest numbers of animals achievable. Non-animal alternatives e.g., tissue culture are used to optimise all cell preparation protocols prior to assessment in animals, but ultimately the in vivo situation cannot be avoided if the goals for human health are to be achieved.
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 organisation of motor and cognitive functions and of the brain systems that underpin them are relatively consistent among mammalian species but differ progressively from non mammalian brains. Rats and mice are used as the least sentient mammals to model the relevant systems and functions disturbed in human neurodegenerative disease. These species tolerate well living in the laboratory environment, and provide the most extensively validated models for addressing the physiological, anatomical and behavioural functions under investigation. All animals are housed in licenced facilities and cared for by professionally trained staff following procedures designed to optimise health and welfare, operating under a rigid inspection system to ensure compliance with full and continuous attention to

	welfare regulation and best practice.

Centrifugal modulation of olfactory bulb function

Sensory systems provide a neural representation of the external world that depends not only on “bottom up” sensory input, but also on “top down” input from central brain areas. This project seeks to increase scientific understanding of these inputs from central brain areas influence the processing and learning of information in the olfactory bulb, at the first stage of processing of the sense of smell. We will build on our previous work, which has characterised learning-dependent changes in the olfactory bulb during the learning of social odours and food-associated odours. We will record electrical activity from brain cells in the olfactory bulb to determine how noradrenaline, which is released by some of these central inputs, modulates responses to social odours and how it induces learning that allows a female mouse to recognise the identity of her mate. This memory is vital for the reproductive success of mice and so this work may provide fundamental information that will be useful in developing new, humane and species-specific forms of rodent control.
We will similar recordings , along with mathematical simulations, to analyse how oscillations in activity of cells in the olfactory bulb depends on feedback from central brain areas and how this influences sensory processing and learning. This type of reciprocal connection between brain areas is widespread throughout the brain but very little is known about its function. We can address this question by using injections of a genetically modified virus to selectively turn off these feedback connections and assess how this changes the way that sensory information is processed and learned.
We are particularly interested in an area of the brain called the NLOT, about which very little is currently known. It’s input to the olfactory bulb and its connections with the rest of the brain suggest that it might be involved in the brain system controlling how the sensitivity to food odours is increased when hungry and decreased when satiated. This idea is reinforced by some of the genes expressed in this brain region, which are associated with over-eating and obesity in mice. We aim to test: whether the activity in the NLOT is associated with appetitive state; whether input from the NLOT to the olfactory bulb is involved in the modulation of sensitivity to food odours; and whether the input from the NLOT to the olfactory bulb is required for the learning of appetitive or aversive behavioural responses to odours paired with food or bitter tastes respectively. If we find that the NLOT is involved in the attractiveness of food odours then it will open up an important new area of research into how the brain regulates food intake.
The aim of this work is to determine the role of long-range interactions between brain areas that can only be studied in the living animal. The project will use a total of approximately 900 mice. Wherever possible, experiments will be performed on terminally anaesthetised animals to minimise the potential for suffering. Around 500 of the mice will undergo surgery, but post-operative pain will be controlled using analgesics, and the experimental procedures are not expected to result in significant or lasting pain, although their sense of smell may be altered. Around 200 of the mice used will be genetically modified, which may cause obesity. These mice will be closely monitored to ensure they do not suffer discomfort or illness due to their obesity. The number of animals used will be kept to a minimum by careful experimental design incorporating power analysis wherever possible to optimise the number of animals used to detect a biologically-meaningful effect. Mice have been chosen for this project because almost all of our understanding of the sense of smell

has come from mice and mice provide unparalleled ability to study the effects of individual genes in a mammal.
The benefit of this project to society is primarily the advancement of science in terms of a greater understanding of processes affecting brain function, especially the influence of central inputs on sensory function and learning. This fundamental knowledge has the potential to be exploited in the development of more humane and selective forms of rodent control and therapeutic strategies to regulate food intake in an increasingly obese population of humans and companion animals.