Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 39

Project Titles and key words

- Strategies for brain repair

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

- Blood products and antibodies for research

  Antibodies; blood; complement; immunology

- Mechanisms of Ischaemia Reperfusion Injury in the Kidney

  Ischaemia, Reperfusion, Injury, Kidney

- Creation, breeding and maintenance of genetically altered rodents

  Creation, breeding, genetically, altered, rodents

- Role of the innate immune system in an animal model of multiple sclerosis

  multiple sclerosis, central nervous system, inflammation, demyelination, immune system

- Production and maintenance of GM zebrafish

  Zebrafish, genetic modification, cryopreservation, IVF

- Function and regulation of the immune system

  Autoimmunity, cell signalling, tumours, lymphocyte biology

- Regulation of breathing by hypoxia and hypercapnia

  AMPK, LKB1, hypoxia, breathing, pulmonary

- Neuronal cell development and survival

  Disease, Motor neuron, Axonal transport, RNA metabolism

- Effects of phytase in farm production

  Efficiency, environment, enzyme, pig, nutrients

- Management of insulin resistance in ponies

  Insulin resistance, laminitis, equine metabolic syndrome Equidae, nutraceutical

<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)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>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[2]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g., the scientific unknowns or scientific/clinical needs being addressed)</td><td>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 end?</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 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.</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] 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	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.

Blood products and antibodies for research
Antibodies; blood; complement; immunology

- Purpose: The purpose of the project is to generate unique and high value proteins, antibodies and assays for use in analysis of the roles of the complement system, a key component of innate immunity, in health and disease.

- Objectives:
  The project is being undertaken in order to create the tools needed for in-depth analyses of the complement system, a critical part of the immune system essential for defence against bacterial infections. The proteins and antibodies made in the project will be used in studies in vitro, in vivo in animal models, and in translational studies in assays to measure complement parameters in man in health and disease. The importance of the complement system in a broad range of inflammatory and infectious diseases has become increasingly recognised over the last decade and the proposed project will play an important part in unravelling how complement contributes to disease and guiding novel therapeutic directions targeting complement. The scientific unknowns and uncertainties are many – knowledge of precise mechanisms of involvement of complement in many diseases remains at a low level and much more research, supported by excellent tools, is needed to bring understanding of the events. The clinical need for assays and interventions in the complement system is acute; the project will go some way to meet the need by building on work programmes already established in the Group to define roles of complement in chronic inflammatory conditions.

- Outline the general project plan.
  Blood and other tissues will be harvested from animals and used as raw material for the purification of Proteins of the complement system and related immune systems. Animals will be immunised with purified complement proteins and related immune proteins from animal and human sources in order to raise specific antibodies against these proteins. The antibodies will be used in tests of the functions of the proteins both in test-tube assays and in animal models of human diseases. Antibodies will also be used to develop tests that can be used to measure the complement proteins in human blood samples as a way of helping to diagnose and monitor disease.

- Predicted harms:
  Animals will be bled from superficial veins using methods that cause minimal trauma to the animal. Bruising and bleeding at the site is a possible but rare problem that is easily avoided by good technique and managed by application of pressure. In some circumstances, animals will be deeply anaesthetised and bled out by inserting a needle into the heart. Deep anaesthesia ensures that the animal does not suffer during this terminal event. For antibody production animals will be injected with small amounts of the protein of interest mixed with a stimulant of the immune system. In rare cases ulcers and erosions may develop at the injection site. With good technique and attention to cleanliness of the injection site these events are very rare. If ulcers are seen then they will be treated vigorously with topical agents.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.
  There will be a better understanding of how complement and related immune molecules contribute to inflammatory and infectious diseases. The project will aid the development of new and improved tests for changes in the complement system and guide future attempts to treat these diseases by interfering with the complement system.

- 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.

For protein and antibody production we estimate that we will use over the five years of the project a total of 1150 mice, 380 rats, 50 guinea pigs and 100 rabbits. Mice and rats are the most suitable currently for monoclonal antibody production, although increasingly, rabbit are being used for this purpose. Rabbits and guinea pigs are particularly useful to obtain polyclonal antisera in the large volumes needed for the development of clinical tests. Good technique and long experience in the Group will ensure that the number of animals used in each part of the project is kept to a minimum. For example, our high success rates in creating monoclonal antibodies means that we need only immunise groups of, on average, three mice or rats.

- 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.
  Antibody production is dependent on the use of animals; there are currently no viable alternatives. Cell culture alternatives for the bulk production of monoclonal antibodies have already been adopted, markedly reducing animal use. As other new developments emerge we will take advantage of them to further reduce animal use.

- Explain why the protocols and the way they are carried out should involve the least suffering.
  The protocols have been optimised in the Group over the last two decades with the aim of increasing efficiency of reagent production while reducing both number of animals used and suffering to each animal. We remain alert to new technical developments and eager to test those that might further reduce suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms of Ischaemia Reperfusion Injury in the Kidney</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ischaemia Reperfusion Injury
Kidney</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Ischaemia Reperfusion Injury (IRI) is a common and serious problem following kidney transplantation. There are multiple complex processed involved at the cellular and molecular level with lots of unknown interactions. Understanding these mechanisms with a view to identifying novel targets for manipulation would help to ameliorate IRI.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Identifying biomarkers of the extent of IRI and identifying novel molecular targets for manipulation will allow us to develop therapies that can reduce the deleterious effects of IRI. We hope that such research would be translated into clinical practice in humans.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Adult Lewis Rats.
88 number over the 3-year period.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The animals will undergo an operation to create the Ischaemia Reperfusion Injury to the kidneys, from which one of the adverse effects is postoperative pain. This may be of moderate severity and will be controlled with effective analgesia. The animals will be observed twice daily for signs of any distress.

After a period of up to 72 hours of observation, the rats will be terminally anaesthetised and relevant post mortem tissue samples will be taken.</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] 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	Ischaemia Reperfusion injury involves multiple complex biological processes and unknown interactions. To date there is not a suitable non-animal model and therefore the use of animals is the only current method available for the experimental assessment of these interactions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculation based on previous literature and results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The rat is a reliable subject for surgical work and its use as a model of renal IRI is well known and of widespread use. We are experienced with the surgery involved and the facilities are well established within the university to facilitate both the procedures and husbandry of the rats.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Role of the innate immune system in an animal model of multiple sclerosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>multiple sclerosis, central nervous system, inflammation, demyelination, immune system</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)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of this proposal is to identify new treatments for multiple sclerosis (MS) and clarify some of the unknown immunological mechanisms of this disease. We will use an animal model of MS, called experimental autoimmune encephalomyelitis (EAE) for our studies.
We want to do this work as there is substantial knowledge on the role of the adaptive (highly specific) immune system in MS, but little knowledge on the role of the innate immune system (less specific but rapid and potent). MS is an autoimmune disease and we also want to find new treatments based on new knowledge from this project. Existing immunotherapies are not yet satisfactory.
We plan to identify which chemical and biological compounds that act on the innate immune system could be used therapeutically. These substances could either ‘block’ harmful actions of the innate immune system or promote its beneficial effects. In our previous studies we have already identified some of these substances.
Unfortunately there is currently no alternative to the use of an animal model. This is because no cell culture system or computer simulation can yet approach the level of complexity of a real living organism in predicting what the effect of a substance will be in people with MS or other diseases. EAE is very valuable because it resembles MS in many ways. We carefully calculate the number of mice for each experiment to ensure that the minimum number is used that will</td></tr>
</table>

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

	yield robust scientific results.
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 advance our knowledge of the mechanisms of disease in MS. The programme of work is also expected to clarify how some of the currently used medicines used in MS work and to lead to the identification of novel compounds which will then be taken to clinical trials. Thus it is expected to make a difference in the quality of life of patients who suffer from MS by providing new treatment options.
What species and approximate numbers of animals do you expect to use over what period of time?	Only mice will be used. The maximum estimated number will be 1,750 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals with EAE develop a motor paralysis that is similar to that observed in people with MS. To induce EAE, anaesthetised mice receive an injection under the skin that includes proteins derived from the nervous system. Mice will be used as they represent the 'lowest' animal species that develop this type of MS-like disease. We will use different strains of mice in which EAE has a different clinical course (for example, one in which mice develop an acute disease attack followed by incomplete recovery and a chronic mild paralysis, and another type, in which the first attack is followed by recovery and then one or more 'relapses'). In all cases, to minimise suffering, humane endpoints will be predetermined to establish when mice should be humanely killed (for example, if they show any signs of respiratory distress). Regular veterinary examination of the animals will take place to ensure that appropriate welfare measures are in place. All animals will be humanely killed at the end of the experiments according to Home Office approved methods. Tissues including spleen, lymph nodes, spinal cord, brain and other organs will be collected for scientific analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use murine models of MS to characterise *in vivo* the integrated responses of the nervous and immune systems to pharmacological, biological and cellular stimuli. Such responses reflect the complexity of a living organism and cannot be reproduced *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers	We will use power calculations to ensure that we always use the minimum number of mice required to produce statistically significant data that have

of animals	scientific validity.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice will be used because a) they are the lowest susceptible vertebrate group, b) EAE in the mouse is sufficiently similar to MS clinically and pathologically, and c) more biological reagents and genetically altered strains are available in the mouse than in other species. We understand our duty to minimise the adverse effects in every case for every animal (causing the least damage and for the least possible time).

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Creation, breeding and maintenance of genetically altered rodents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Creation, breeding, genetically, altered, rodents</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></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</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 will create, breed and maintain rodents with genetic alterations (GAAs) and supply them for fundamental research within this establishment. GAAs are important for understanding fundamental biological processes and causes of disease. They may be used as disease models and to test treatments for disease.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>During the last 24 years, GAAs have made significant contributions to the research at this establishment. However, the function of many genes is still not known or not fully understood, either individually or in the ways they interact to produce their intended effects, or how they are dysfunctional in disease. The use of animal models is necessary to determine these processes.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The mouse provides an appropriate species as the genome has been sequenced and can be manipulated, the required reagents are available and its basic biology and pathological processes are similar or identical to those in other mammals, including man
Over a five year period the aim is to:
Produce a maximum of 35,000 GA mice. A maximum of 19,000 mice will be used to create strains; supply donor embryos, recipients, for receipt of implanted embryos for development of young to term, and vasectomy or maintenance of sterile males for production of pseudopregnant females.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>The protocols should not result in more than temporary pain, suffering of distress to the animals. Appropriate pain relief should minimise the effects of surgical procedures. The effects of the genetic</td></tr>
</table>

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

level of severity? What will happen to the animals at the end?	alteration for the majority of the animals will be negligible and of a mild severity at most. Some GAA lines may have abnormalities causing moderate severity suffering. These effects can be minimised by appropriate breeding, husbandry and/or veterinary measures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As there are no non animal alternatives, the use of the rodent (mouse) model is needed in order to breed strains which show genotypes and/or phenotypes typical of human disease in order to help develop new novel therapeutics for the treatment of human disease. Many of the research projects will involve the use of in-vitro systems such as cell culture, human tissue assays, computer modelling to complement the animal work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Unnecessary creation or production will be avoided by database searches to ensure the required strain is not already available. Consideration to use tissue provided from established strains will be given prior to any new animal creation. A centralised service is administratively effective, with breeding controlled to produce batches of animals as needed and any spare can be made available for use by several research groups. Written requests for animals will be made and approved by the Animal Welfare Ethical Review Board (AWERB). Subsequent production and use of animals will follow standard protocols. Each request will justify the use of animals and explain why they cannot be replaced by other non-animal alternatives.
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.	Scientists often choose to use mice rather than other species of animal, since it is possible to alter mouse genes manipulate the mouse genome to produce models for particular human disease or conditions. Due to these scientific developments allowing a greater specificity of the development of the transgenic models, the ability to study many human diseases is possible. Mice, due to their short reproductive cycle time are the lowest sentient vertebrate group on which breeding for production of transgenic animals can be performed with genotypes and/or phenotypes, which mimic models of human disease.

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<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
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<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
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<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td></td><td>No</td></tr>
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[10] At least one additional purpose must be selected with this option.

	yield robust scientific results.
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 advance our knowledge of the mechanisms of disease in MS. The programme of work is also expected to clarify how some of the currently used medicines used in MS work and to lead to the identification of novel compounds which will then be taken to clinical trials. Thus it is expected to make a difference in the quality of life of patients who suffer from MS by providing new treatment options.
What species and approximate numbers of animals do you expect to use over what period of time?	Only mice will be used. The maximum estimated number will be 1,750 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals with EAE develop a motor paralysis that is similar to that observed in people with MS. To induce EAE, anaesthetised mice receive an injection under the skin that includes proteins derived from the nervous system. Mice will be used as they represent the 'lowest' animal species that develop this type of MS-like disease. We will use different strains of mice in which EAE has a different clinical course (for example, one in which mice develop an acute disease attack followed by incomplete recovery and a chronic mild paralysis, and another type, in which the first attack is followed by recovery and then one or more 'relapses'). In all cases, to minimise suffering, humane endpoints will be predetermined to establish when mice should be humanely killed (for example, if they show any signs of respiratory distress). Regular veterinary examination of the animals will take place to ensure that appropriate welfare measures are in place. All animals will be humanely killed at the end of the experiments according to Home Office approved methods. Tissues including spleen, lymph nodes, spinal cord, brain and other organs will be collected for scientific analysis.
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2. Reduction Explain how you will assure the use of minimum numbers	We will use power calculations to ensure that we always use the minimum number of mice required to produce statistically significant data that have

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<table>
<tr><td>Project Title (max. 50 characters)</td><td>Production and maintenance of GM zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>zebrafish, genetic modification, cryopreservation, IVF</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><u>Yes</u></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>
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<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td><u>Yes</u></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 Service/Production licence is for the support of projects associated with the Zebrafish Facility, specifically the generation and/or the preservation of genetically modified (GM) zebrafish lines.</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 purpose of this project licence application is to establish a service license to create, breed and maintain genetically modified zebrafish in support of the Zebrafish Facility, a core university facility. This project licence is in support of existing licences and new users.
There is increased recognition of the benefits of zebrafish as a model organism, especially with the consideration of the 3Rs, therefore it is believed that there will be increased demand for the production of GM lines and their preservation.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Zebrafish (Danio rerio), over 5 yrs the expended number to be used is 9000 fish</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 generation and/or the preservation of genetically modified (GM) zebrafish lines (mild severity limit) are not expected to have abnormal clinical signs; however, in all cases of zebrafish exhibiting any unexpected harmful abnormal phenotypes will be killed by approved humane method before the free feeding stage. Natural-occurring matings of fish can, at times, produce offspring with significant but naturally-occurring mutation. These will also be killed humanely.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement</td><td>The study of genes and disease aims to model</td></tr>
</table>

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

State why you need to use animals and why you cannot use non-animal alternatives	human disease processes as accurately as possible. Due to the complex interactions of tissues and genetic systems, and because Humans cannot be manipulated genetically for research purposes, a whole animal model is required. Zebrafish are now a recognized suitable model to study human disease. Unfortunately, there are no alternative in-vitro model systems that can be used for the purpose of this licence. However, amongst all the currently used model organisms in biomedical scientific research, we have chosen a lower vertebrate model species compared with mammals; zebrafish are considered less sentient than mammalian model species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The production and maintenance of GM zebrafish lines under this license can be subdivided into two main categories. (1) Generation of new transgenic lines (2) Breeding and maintenance of fish carrying harmful mutations or genetically modified fish Most experiments are conducted on zebrafish embryos prior to free-feeding, thereby reducing the total number of animals used. However, justification for each experiment prior to the start provides an opportunity for the researcher to discuss the minimal number of animals that may be required. To further reduce the number of zebrafish lines maintained, cryopreservation will be offered and utilized to reduce long-term breeding programme.
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 zebrafish is a lower vertebrate species for biomedical research compared with mammals. Unlike other vertebrate model systems used in the laboratory, zebrafish embryos are externally fertilized, and are transparent during early embryogenesis, the optically clear zebrafish larvae provide an unprecedented opportunity for in-vivo live imaging studies.This allows for the exquisite detail of cell and tissue development to be visualized in a living vertebrate animal, something that is not possible in other vertebrates. Furthermore, daily and regular feeding and observation of fish ensures that they are maintained in the best possible condition.

Function and regulation of the immune system
Autoimmunity, cell signalling, tumours, lymphocyte biology

- Summarise your project (1-2 sentences)

  The overall aim of this project is to identify molecules and cells that influence the development, function and dynamics of the immune system. We aim to gain an understanding of how an efficient and appropriate immune response to infectious organisms is mounted, how immunological memory is established, and how a state of tolerance to self tissue is maintained.

- 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.

Knowledge of the molecules and cell interactions that drive the immune system is vital for informing development and improved efficacy of vaccines. Improvements in vaccination strategy could also benefit human disease in the control of malignancies. Understanding why, in some instances, tumours are not recognised and developing strategies to prime appropriate immune responses against them would offer significant therapeutic benefit. On the other hand an understanding of why the immune system may stop being tolerant of self components resulting in the development of autoimmune diseases would be beneficial for the development of therapeutics to help with these debilitating diseases.

- Outline the general project plan.

For many years we have been interested in how cell signalling molecules influence the responses of white blood cells. Recently genetic screening studies have identified that mutations in a number of the molecules that we study are associated with several autoimmune diseases and disorders of the immune system. By making genetically altered mouse models we can mimic these mutations found in humans and ask how the immune response is affected. Our readouts are to look at the influence of these proteins on the ability of white blood cells to mount responses to infectious organisms or tumours and to initiate autoimmunity. Getting immune responses started frequently can only be done in the context of the intact animal, however subsequent detailed analysis of white blood cell responses will be further studied by culture outside the animal.

- 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.

Most of the procedures used are mild and do not cause stress or pain to the animals. Many of our studies involve using tissues from genetically altered animals and mice are bred and maintained without further procedures and humanely killed to provide organs which we can further study in culture in the laboratory. Sometimes substances that modify the immune system are given by injection but these do not cause adverse effects. In some instances mice will be infected with organisms, like bacteria, so that we can follow immune responses, some infections may cause the animals to lose a little weight transiently but we expect few other adverse effects. In some instances we will be provoking autoimmune disease which are more substantial procedures and in the case of arthritis expect to see paw swelling, while in a model of multiple sclerosis (EAE), that we use infrequently, we expect to see partial paralysis of the tail and hind limbs. Occasionally we follow the immune response to tumours and tumours are induced by injection of tumour cells under the skin. Tumour size is monitored carefully and no adverse effects are generally seen before the experiment is terminated. Blood samples are taken from superficial vessels of conscious animals in amounts that do not harm the health and well-

being of the animal.

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

We aim to identify biochemical pathways and important molecules that can be targeted to modify immune cell behaviour. We want to be able to target pathways that reduce responses in the case of autoimmune disease or alternatively to boost responses, for example to try and fight off tumour cells or infections.

- 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.

Our experimental model uses the mouse as this is the best studied and has an immune system that is similar to that in man, numerous reagents for tracking immune responses are available, and there are advanced techniques for and availability of genetic modifications. An important part of our program requires the production and breeding of genetically modified mice that show little or no ill effect from the altered genes they carry, and form the bulk of the animals we report (max estimated usage 25,000 mice). Analyses of the effects of the genetic modifications are carried out mainly on tissue samples from the mice after they have been humanely killed. By using techniques that have been standardized over the last 20 years and group sizes that are statistically validated, the overall numbers of mice used are kept to a minimum. The numbers will be kept as low as possible by good experimental practice that reduces the need for multiple repeats of experiments, by vigilance of the breeding program, and by information gathered continually from our scientific colleagues.

- 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.

Some of the functions of immune and haematopoietic cells can, to some extent, be reproduced in cell studies; however information from such studies is limited for a number of reasons. Firstly, the cellular interactions involved in the immune system are complex and we do not understand how all the contributors interact so we cannot reproduce this complexity in culture. Secondly, we cannot study how specific genetic mutations influence the workings of a complex immune system as we cannot readily introduce mutations into genes specifically in cell lines. Wherever possible we carry out experiments in culture conditions and we aim to reproduce our findings in human cells as they are most relevant for the processes we are trying to understand.

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

The protocols have been adapted to ensure best practice and the least suffering to the animals as we are trying to recapitulate the workings of the immune response. Undue stress and/or pain would not only harm the animals but would influence how the immune system behaves and potentially provide us with a less representative picture of a normal immune system.

Regulation of breathing by hypoxia and hypercapnia
AMPK, LKB1, hypoxia, breathing, pulmonary

- Summarise your project (1-2 sentences)

This project aims to determine the role of two enzymes, LKB1 and AMP-activated protein kinase, in regulating blood supply to the lungs and breathing patterns, respectively, during hypoxia. These studies will advance our understanding of the mechanisms that underpin hypoxic pulmonary hypertension, sleep apnoea and sudden infant death syndrome.

- 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.

We aim to investigate two key processes by which our bodies adjust to changes in oxygen availability, in order to ensure that we receive the oxygen we need.

At all times blood supply to our lungs is regulated to ensure that the blood flows to areas of the lung that can best provide the oxygen we need. This process is called hypoxic pulmonary vasoconstriction, which drives the closure of blood vessels in areas of the lung where oxygen supply is low in order to divert blood flow to areas of the lung that are rich in oxygen. However, in disease, for example in cystic fibrosis or obstructive airways disease, hypoxia in the lung can be widespread and may thus trigger global hypoxic pulmonary vasoconstriction and pulmonary hypertension. The World Health Organisation's latest estimates suggest that survival time from pulmonary hypertension is between 3 and 6 years

The rate and depth at which we breathe is also highly regulated by oxygen supply. This is controlled by the carotid and aortic bodies, which monitor blood oxygen and signal the brain to increase the depth and rate of breathing when oxygen supply falls. Malfunction of this process has been proposed to trigger hypoventilation and central apnoeas in idiopathic sleep apnoea, altitude sickness, heart failure, preterm birth and polycystic ovary syndrome. Current therapies for such breathing disorder are poor

We will also investigate whether or not similar mechanisms regulate breathing and oxygen supply after birth and determine their role in sudden infant death syndrome (i.e. cot death).

Our studies will define the role of genes that code two key enzymes that our previous investigations suggest may be involved in regulating breathing and blood supply to the lungs. Further work will improve our understanding of the physiology and pathology of these processes. Therefore, there is the potential to identify new drug targets which could lead to new therapeutic strategies. This is important as current therapies are poor.

- Outline the general project plan.

We will delete 3 genes, each alone and in combination, and study the effect of gene deletions on breathing patterns and blood flow to the lungs. The general project plan is to delete our target genes:

1. In the muscle cells that line blood vessels and thus determine whether the enzymes for which these genes code are required to induce blood vessel closure during hypoxia, and thus hypoxic pulmonary vasoconstriction and the development of hypoxic pulmonary hypertension.

2. In cells that regulate the rate and depth of breathing in response to changes in oxygen supply to the body. Thereby we will determine whether or not loss of the enzymes for which the genes code trigger, for example, sleep apnoea.
3. In the cells that produce adrenaline after birth in order to trigger air breathing by the new born. Thereby we may identify the mechanisms responsible for sudden infant death syndrome.

- 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.

We will measure breathing patterns and associated brain function, and also assess blood pressure and blood flow in a low oxygen environment. We have observed no adverse side effects in previous studies. We will continue to closely monitor animals for any signs of harm, and we aim to reduce the disease severity. Therefore the majority of work will be breeding and maintenance of transgenic animals, in order to allow for post-mortem tissue collection and / or studies on animals under anaesthesia from which animals will not recover. There will be a limited number of surgical procedures from which animals will recover, but suffering will be minimised using anaesthesia during surgery and by post-operative analgesics. Surgery will be used to implant monitoring devices to allow for the measurement of the levels of gases of interest (e.g. oxygen) in awake animals that are behaving normally or to allow injection, in a pain free manner, into specific areas of the brain in order to determine how breathing is regulated by changes in measured gases.

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

Our studies will provide new therapeutic strategies for the treatment of sleep apnoea, sudden infant death syndrome and pulmonary hypertension. This is important because in each case current therapies are poor. For example, life expectancy for patients with pulmonary hypertension is between 3 and 6 years. Although less severe, sleep apnoea is a debilitating and progressive disease, with loss of wakefulness during the day and cognitive dysfunction, and at present the only treatment is by bedside CPAP (continuous positive airway pressure) machines.

- 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 6500 transgenic mice will be developed. Approximately 2000 of these will be used in experiments during the course of this project, about 500 mice per year. We are careful to use as few as possible to answer our questions, and aim to get the maximum amount of information from each animal to help us in our research. We do this by asking simple questions with cells/sections of animal tissue initially, and only progressing to live animal work for the most vital research questions that may provide for the development of new therapeutic strategies. Mice are the smallest mammals that are used to model human disease, and many drugs/therapies now in use in humans were first tested in mice and / or other rodents, giving more confidence that if they work in these animals, they may work in humans.

- 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.

Where possible we will study processes (e.g. protein-protein interaction) using cell culture techniques. However, we have to use transgenic mice because there is no non-animal

approach that will allow us to study the effect of gene deletion on breathing patterns, sleep apnoea and blood flow to the lungs and the rest of the body. The physiological systems that control these processes are complex and are arranged in three dimensional structures with many interacting cells, and we cannot model this yet in culture dishes. Only animals can help us study functions with this complexity.

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

The protocols and how they are carried out have been designed to limit suffering. Most studies are of mild to moderate severity. Those that require surgery will not include re-use of animals. We recognise that research experiments do not produce good, reliable and repeatable results if the animals involved are ill or suffering. Therefore, in order to answer important research questions, as well as for personal reasons, we are highly motivated to provide excellent care for our animals. Our experiments use very specialised equipment to limit injury, and provide for fast operations and excellent post-operative care.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Neuronal cell development and survival</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Disease, Motor neuron, Axonal transport, RNA metabolism</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)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our objectives are: 1) To understand how defects in the components of the intraneuronal transport and signalling systems lead to the death of motor neurons in motor neuron diseases. 2) To investigate the underlying mechanisms of the roles of proteins implicated in motor neuron disease in response to DNA damage and to elucidate how defects in these proteins could affect the expression of other genes.</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>Our study will contribute towards our understanding of the mechanisms of motor neuron death caused by defective intraneuronal transport or response to DNA damage. Therefore, our findings will benefit the scientific community with a broad range of interests in neurological conditions. Moreover, working from the mouse models of motor neuron disease to mouse primary cells and neurones derived from reprogrammed mouse skin cells, will aid the understanding of the mechanisms of disease onset and progression. Using this knowledge in human derived fibroblasts and neurones and applying this information back to human conditions and for cross species comparisons at the cellular and neuronal tissue levels will set a paradigm for the effective use of both the mouse and human-derived cells as valuable model systems.

In addition, this research will benefit patients and their families, who have been affected by motor neurone disease, hereditary motor neuropathies, and some cases of intellectual disability; and health professionals, who work with the above mentioned patient groups.

The benefit from the outcomes of this study could be immediate, as our findings could inform the</td></tr>
</table>

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

	beneficiaries about the causes and basic mechanisms of the disease. In the longer term, understanding the relationships between defective axonal transport or DNA repair response with abnormal neuronal cell function and development, will potentially have a significant contribution towards discovering novel drugs and more effective treatment of the above mentioned diseases and perhaps other related disorders. Moreover, this study could provide improved knowledge of prognosis for informing patients and ensuring best possible care planning.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse ~5,500 over five years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	A proportion of the animals used in this study are transgenic mice that start showing signs of a progressive muscle weakness in their limbs at around 120 days of age. The level of severity of the phenotype in these mice is substantial, as this is a progressive condition which leads to paralysis at about 135 – 165 days of age and it is crucial for this research to obtain tissues from all stages of the disease in order to pin point the correct pathway that is impacted by cellular response to DNA damage and stalled gene expression. To minimise the animal suffering we monitor these mice twice a week between 100 – 120 days of age. Mice with signs of paralysis will be given dry mash and gel blocks and their food and water intake will be monitored daily. Mice will be weighed once and checked twice every day till end point (righting reflex within 30s is not observed; or 15% loss of body weight) is established. End-stage mice will be monitored 9am-5pm. If the mouse shows sever symptoms then it will not be kept and will be culled humanely as specified by the Home Office. No mice with severe symptoms will be kept overnight. Another group of mice showing adverse effects in this study exhibit an abnormal gait but have normal feeding behaviour and life span.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Motor neuron disease targets neurons in the brain and spinal cord and thus it is impossible to have access to these tissues during the development of the disease before the post-mortem stage. This would provide us with data about the very late stages of the disease. Although we will be using skin fibroblasts isolated from patients and reprogrammed cells, we will still need mouse

	models to have access to tissues at all stages of life and for culturing primary neurons.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will maintain and breed just enough animals for providing us with required tissues and cells for generation of data which are statistically sound.
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.	Sequencing of the mouse genome has revealed that ~99% of mouse genes have a homologue in the human genome and that for ~80% of mouse genes an analogous (orthologues) gene exists in the human genome. In addition, human and mouse have common biochemical pathways. Because of the above properties several large international mutagenesis programmes have been generating mutant mice that could serve as model systems for late onset human disorders such as motor neuron disease. The mouse clearly does not have the same physiology as humans, but does, largely, share the same biochemical pathways as well as genes. Thus we can work with mutant mouse models of human motor neuron degeneration to highlight and interrogate the proteins and pathways that are involved in motor neuron disease. To minimise the animal suffering we monitor the animals which show signs of muscle weakness or paralysis twice a week between 100 – 120 days of age. Mice with signs of paralysis will be given dry mash and gel blocks and their food and water intake will be monitored daily. Mice will be weighed once and checked twice every day till end point (righting reflex within 30s is not observed; or 15% loss of body weight) is established. End-stage mice will be monitored 9am-5pm. If the mouse shows sever symptoms then it will not be kept and will be culled humanely as specified by the Home Office. No mice with severe symptoms will be kept overnight.

Effects of phytase in farm production
Efficiency, environment, enzyme, pig, nutrients

- Summarise your project (1-2 sentences)

To improve performance and feed efficiency of pigs and chickens through the addition of dietary enzymes.

- 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.

Sustainable pig/poultry production depends on maximising nutrient availability from the diet and feed efficiency whilst reducing nutrient excretion. Excess excreted nutrients and emissions from pig/poultry production are leading to the environmental concerns that intensive pig/poultry production is producing manure that may cause pollution. Dietary manipulation through the addition of enzymes may be a method to alleviate some of the environmental impacts of pig/poultry production whilst improving growth performance, feed efficiency, and reducing dietary costs.

Reducing the level of crude protein in pig/poultry diets has shown to be effective in reducing nitrogen excretion into the environment, however appropriate amounts of amino acids must be maintained in order to prevent any negative effect on growth on performance or well being. Additionally phosphorus excretion is a threat to the environment. Phosphorus is an essential nutrient found in plants as phytate which is poorly available to pigs and poultry resulting in high levels of phosphorus being excreted into the environment. In order to meet the animals phosphorus requirement, inorganic phosphorus must be added to pig/poultry diets which is expensive. However the addition of phytase (enzyme) to pig diets increases phosphorus availability reducing the amount of inorganic phosphorus required and the amount of phosphorus excreted into the environment.

The effects of phytase addition are not limited to increasing phosphorus availability, it has also been shown to increase the availability of other nutrients such as peptides, amino acids, and glucose. The mechanisms behind these 'extra phosphoric' effects are not clear, however it has been suggested that the negative effects of phytate may be due to protein-phytate complex formations and/or compromised intestinal uptake of nutrients (Selle & Ravindran, 2008).

The ability to determine the hormonal and metabolic state of an animal will help in understanding the mode of action of factors that are involved in improving performance and nutrient efficiency in pigs and chickens

- Outline the general project plan.

- Animals (pigs, broilers, and layer hens) will be fed diets that differ in composition (changes in nutrients and or the addition of enzymes).
- Blood samples will be collected from a sub-set of animals. Animals will not be sampled more than once within 3 weeks.
- Animals will be monitored daily as routine until the end of the trial.

- 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.

Alteration in diets: Throughout the trial a number of diets will be used. Diets will not compromise growth below 80% of the rate of growth of contemporary control fed animals and thus no adverse effects are expected for animals fed these diets.

Blood samples will be collected using a sterile syringe. For pigs this will be done from the vena cava at 4 weeks of age and from the jugular vein thereafter. Pigs will be restrained by a trained technician for sample collection at 4 weeks of age and by nasal snaring thereafter. Birds will be restrained by a trained technician and sampled using a sterile syringe from the wing vein. Animals will be monitored daily as routine.

Possible adverse effects include discomfort whilst being restrained and discomfort at the injection site. This is however unlikely as trained technicians will make the pig/bird as comfortable as possible and blood samples will be collected using aseptic techniques.

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

The programme of work aims to improve pig/poultry performance and feed efficiency and to reduce nutrient excretion through dietary manipulation. Elucidation of the mechanism involved in improving feed efficiency and reducing nutrient output will allow the use of the most cost effective and sustainable pig/poultry diets.

- 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.

The aim of the experiment is to improve performance and feed efficiency and thus reduced the environmental impact of pigs/poultry whilst enhancing our understanding of the mechanisms involved in this improvement. In order to do this blood samples will be taken from pigs, broilers and layer hens so that levels of blood hormones, metabolites and nutrients can be measured.

960 pigs in total (approximately 30% of these animals will be blood sampled).

1280 birds, 768 broilers and 512 layers (approximately 30-50% of these animals will be blood sampled).

- 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.

Pig and chickens housed under commercial conditions will be used. Work needs to take place using animals within a farm environment in order to match environments seen on commercial farms allowing the results to be applicable to commercial production.

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

Blood sampling will only be carried out by a trained technician.

The negative control diet will only be used in experiments where it is not possible to get the required information using only a positive control diet.

All animals on the trial will be monitored daily by a trained technician. Any poor/ill health or discomfort will be handled as necessary by a trained technician.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Management of insulin resistance in ponies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Insulin resistance, laminitis, equine metabolic syndrome
Equidae, nutraceutical</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)[15]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</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>Fat ponies tend to be resistant to the metabolic effects of insulin. Both obesity and insulin resistance are risk factors for the painful condition of laminitis which affects the feet. Fat, insulin resistant ponies with laminitis are said to have ‘Equine Metabolic Syndrome’ (EMS). The best way to reduce laminitis risk is to reduce body fat which can also improve insulin sensitivity. However, this can be unacceptably slow where animals have pain; also, there is some debate as to which is the best feedstuff to offer when ponies are being ‘slimmed’. Because insulin resistance may be an important factor in inducing laminitis, it makes sense that ‘diet’ foods should contain fewer sugars and therefore cause minimal disturbance to blood sugar, and hence insulin, levels. It is probable however, that insulin resistant ponies may have abnormal glycaemic responses to feeds.

Therefore, the first aim of this study is to investigate the glycaemic and insulinaemic responses (GIR) to different forages, pasture and a ‘test’ feed in insulin resistant and normal ponies to a) determine which feed types have the least response and would be most suited for feeding to ‘dieting’ ponies b) to work out whether the GIR to the a standardized test diet would be of value as a simple way to determine just how insulin resistant an individual pony might be.

Although weight loss is the best way to limit laminitis risk, this can be difficult for owners to</td></tr>
</table>

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

	manage under some conditions. The second part of this study evaluates the ability of a nutraceutical compound to ameliorate insulin resistance in ponies with EMS who are managed to maintain a constant body mass.
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 study will inform the nutritional management of EMS ponies during controlled weight loss programmes. Further, we will be able to determine any relationships between the GIR to feeds and insulin resistance. If this association is proved, then it may be possible to use the GIR to a standardised test feedstuff as a simple field test to detect and monitor insulin resistant animals. Finally, although all EMS ponies are best managed by corrective management to reduce body fat, this cannot always be achieved immediately or may be too slow where animals have laminitic pain. If the nutraceutical can improve insulin resistance without weight loss, it may offer an immediate method to reduce laminitis severity or risk.
What species and approximate numbers of animals do you expect to use over what period of time?	Ideally, we will need to use 12 ponies in our first study. The ponies will be given one of 5 test feeds and a test to evaluate insulin resistance each week for 6 weeks. The order of tests will be randomized for each pony. The second study will use 12 EMS ponies and will be run over 18 weeks. The first 2 weeks will be an adaptation period – then the ponies will be randomly allocated to one of 2 groups. One group will be given the nutraceutical in their daily feed while the other will be fed the carrier product alone in the same manner. After 6 weeks, no feed supplement will be offered for a 3 week ‘wash out’ period – before the groups are ‘swapped over, with each group receiving the alternative compound for a further 6week period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the various tests, we will need to fast the ponies overnight to standardize results. We don’t expect any adverse reaction to this very mild intervention. All ponies will need to be fitted with uni/bilateral jugular vein catheters for testing. Again, this is a very mild procedure performed under local anaesthesia and we rarely encounter any adverse effects. Very occasionally haematoma or a mild infection can occur in the immediate vicinity of the

	catheter site. These resolve rapidly with appropriate care. It is our intention that the animals will be discharged from the act at the end of the study and re-homed into private domestic settings.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Horses and ponies have a unique metabolism and other animals or isolated tissues cannot therefore be used to derive the information we seek. This work on ponies will provide clear benefits directed towards improving pony welfare.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have a lot of experience in studies of this nature. These studies allow us to gauge the level of response and variation in responsiveness that we are likely to encounter. These data have allowed us to use statistical approaches to determine the number of animals that we need to get clear answers.
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.	Our questions can only be answered by using insulin resistant ponies. Ponies will be tested for insulin resistance shortly after arrival. Those which don't meet our criteria will be immediately re-homed. The work is being performed within the Veterinary Hospital and the people conducting the study are experienced veterinary surgeons. The ponies will be monitored continuously and any issues will be immediately addressed. The staff charged with the day to day care of the animals are experienced equine technicians. All animals will be habituated to the essential handling methods at outset and the ponies will be allowed to exercise at liberty daily with a companion wherever possible