Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 13

Project Titles and key words

- Effects of dysregulated glucocorticoid exposure

  Glucocorticoid, Prednisolone, Dexamethasone, Metabolism, Cognition

- Testing Coronary Stents in a Rabbit Iliac Model

  Rabbit, iliac, coronary, stent, thrombosis

- Production of genetically altered animals

  Genetically altered animals

- Regulation of immune responses to infection

  Innate immunity; macrophages; neutrophils; influenza

- Corneal Graft Rejection in High Risk Recipients

  Intraocular inflammation, corneal graft rejection, immunomodulation, artificial cornea, imaging

- Why do grey squirrels strip the bark off trees?

  Sciurus carolinensis, bark-stripping, calcium, deficiency

- Studies of Peptide Receptors in the Intestine

  Peptide Receptors, Intestine

- The role of RING finger proteins in malignancy

  PML, BRCA1, RING finger, Malignancy, DNA repair

- Strain typing of TSE agents

  Scrapie, prion, strain

- Immunomodulation in poultry

  Immune response; bird; disease resistance; vaccine

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Effects of dysregulated glucocorticoid exposure</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Glucocorticoid
Prednisolone
Dexamethasone
Metabolism
Cognition</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)[1]</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>
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<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Rhythm disturbances in the adrenal ‘stress’ hormone (glucocorticoid hormone, specifically named cortisol in humans and corticosterone in rodents) can occur due to chronic stress conditions, chronic sleep disturbances such as sleep apnea or even due to shift work or jet lag, as well in chronic conditions such as arthritis. In addition, rhythm disturbances can result from clinical treatment with synthetic glucocorticoids such as dexamethasone or prednisolone. These long acting synthetic steroids are widely used in the clinic, and prescribed for a myriad of medical conditions from asthma to ulcerative colitis. The medical literature describes many common adverse health effects in these patients, most notably metabolic related health effects in glucose and fat metabolism, but also cognitive and behavioural-related problems. Unfortunately, it is not fully understood whether the association between the altered pattern of glucocorticoid exposure and the metabolic and cognitive dysfunctions are causal or coincident. Therefore, we aim to investigate how the altered patterns of glucocorticoids will affect the metabolic target liver, and different brain regions known to be important in memory and learning function. It is essential to perform these studies in well-deigned in vivo experiments, where all the parameters can much</td></tr>
</table>

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

	tightly control, to enabling functional relationships to be assessed in a manner that would not be possible in clinical studies. Ultimately, the findings from this programme of work will provide molecular targets for therapeutic intervention in patients, as well as inform as to better dosing regimes for a more favourable effect:side effect ratio for the patient. Our overall objective is to establish how altering the normal physiological glucocorticoid rhythm changes gene regulatory networks in different target tissues and organs throughout the body, and how these molecular changes affect metabolic, cognitive and behavioural parameters. Our objectives are: 1) To establish robust animal models that best model well-characterised glucocorticoid rhythm altering conditions such as chronic stress. To do this, we will infuse defined patterns of corticosterone into animals that have been adrenalectomized to remove their endogenous glucocorticoid secretory patterns. 2) To assess how treatment with a synthetic glucocorticoid analog (eg prednisolone or dexamethasone, both widely used in the clinic) affects/alters the normal pattern of endogenous glucocorticoid secretion and the activity patterns of the intracellular glucocorticoid receptor GR (and in some cases the related receptor MR). 3) To use a novel gene discovery approach to identify genes involved in early changes in dysregulation of normal physiological processes, especially metabolic function, cognitive function and memory and learning processes. 4) To assess metabolic effects on insulin sensitivity, related to altered patterns of natural glucocorticoids or treatment with synthetic glucocorticoids. 5) To assess if altered glucocorticoid rhythms dysregulate normal cognitive function and memory and learning processes.
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)?	A better fundamental understanding of the target genes and pathways identified in the proposed studies - and importantly, their relationship with the development of either metabolic dysfunction or cognitive impairments - will aid in the development of new approaches for treatment with glucocorticoids in the clinic. A successful outcome of this programme of work would be the identification of new targets, which would be then used to develop therapeutic intervention in the treatment of rhythm altering chronic disease and

	stress conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (2000)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected adverse effects are primarily related to the moderate severity surgical procedures that will be performed on the animals. Adrenalectomy, craniotomy and cannulation will be carried out by highly skilled small animal surgeons, and the best quality post-operative care will ensure minimal suffering to the experimental animals. We have worked with the appointed vet and animal care officer to develop these protocols to ensure a minimum amount of suffering. The experiments are mainly done in basal stress-free conditions, and are either minimally stressful (stress-free blood sampling from in-dwelling cannula), non-regulated (behavioural testing), or involve a moderate stress that will be restricted to the shortest effective time (10min noise stress or 30min restraint stress). All procedural animals will be killed by an humane schedule 1 approved method at the end of an experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While cell culture methods will continue to be used to assess intracellular activity, the use of animals is necessary to assess complex functional and physiological effects. There are currently no cell culture assays or computer programmes that can mimic all of the complex interactions that occur in vivo, especially in relation to metabolism and cognitive function and behaviour. We propose to use rats for the majority of the studies, as they are the lowest vertebrate group used for the glucocorticoid infusion and multiple blood sampling methodology. The hypothalamic pituitary adrenal (HPA) axis of the rat has been extensively characterised and many similarities between the HPA axes of rat and man exist, making it a useful model in which to answer questions that cannot be asked in man, for ethical reasons.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that we use the minimum number of animals in well-designed, statistically valid experiments, and use procedures aimed at minimizing potential adverse effects in line with the 3R ideology. Our established methods for painlessly infusing, and obtaining tiny blood samples will reduce the stress on the animals. Use of each animal will be maximised so that one

	group of animals can be used to answer multiple questions. For example, multiple tissues will be collected from all experimental animals for analysis in more than one laboratory-based experiment, where possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats are the lowest vertebrate group on which well-characterised glucocorticoid infusion and multiple blood sampling models have been developed. The rat adapts well to the processes of adrenalectomy, with saline replacement, and chronic cannulation followed by connection to the automated blood-sampling system and/or infusion pump. It is currently not possible to perform similar experiments on animals with a smaller circulating blood volume than that of the rat.

Testing Coronary Stents in a Rabbit Iliac Model
Rabbit, iliac, coronary, stent, thrombosis

- Summarise your project (1-2 sentences)

  Coronary stent thrombosis (blood clot within a stent) is a potentially lethal complication that can occur with coronary angioplasty and stenting. We plan to use a rabbit iliac model to investigate whether novel stents can reduce this risk when compared to "standard" stents.

- 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.

  Coronary artery disease is the leading cause of death worldwide and a common form of treatment is coronary angioplasty and stenting (ballooning and insertion of a stent, or scaffold, to hold the artery open). Despite major advances in coronary stent technology and drug therapy, stent thrombosis remains a serious complication and is associated with a very poor outcome, including heart attack in 70-90% of cases and death in up to 40%. This project aims to identify and validate strategies to reduce the risk of this complication. This includes the use of novel stents made of biocompatible materials.

- Outline the general project plan.

  As part of a large multi-disciplinary European study, novel stent materials and coatings have been developed. These aim to improve biocompatibility of coronary stents and reduce the risk of stent thrombosis. Extensive *in vitro* and *ex vivo* work has been done and novel stent prototypes have been developed that need to be evaluated in appropriate pre-clinical animal models with respect to safety and efficacy. In addition, newer generation coronary stents already in clinical use and balloon angioplasty techniques will be tested.

- 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 propose to use an acute rabbit iliac model to assess early clot formation and a chronic model to look at re-endothelialisation (coverage of stents with vessel wall lining). The acute model will involve measuring flow across iliac arteries implanted with novel and newer generation stents as compared to "standard" control coronary stents. The effect of balloon angioplasty to the stents after deployment will also be looked at. Stented vessels will be removed post mortem and examined for signs of inflammation and clot formation. In the chronic model, animals will be recovered for a period of 14-28 days after stenting. Stented vessels will be removed post mortem and assessed for re-endothelialisation and clot formation. Blood will also be analysed using platelet function tests (testing the effect of anti-platelet drugs) and this will be correlated with the risk of stent thrombosis.

  Other than transient discomfort during administration of substances and blood sampling, we do not expect any adverse effects. Surgery will be performed under general anaesthesia and hence the animal will be insentient throughout this time. During the recovery period, the animal will be observed closely for signs of pain, limb ischaemia, infection or nerve damage, which are rare events.

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

  With nearly 90,000 coronary stent procedures performed in patients every year in the UK alone and with an incidence of between 0.5 and 4%, any strategy that can reduce the risk of stent thrombosis is extremely important. The new and novel stents that we are investigating aim to be more biocompatible, increasing the rate of re-endothelialisation and reducing the risk of stent thrombosis.

- 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 New Zealand White rabbit has been chosen, as it is a well-recognised model for the study of vascular biology and produces responses that are similar to the vascular behaviour after angioplasty in humans. In addition, the size of the iliac arteries is similar to human coronary arteries, enabling direct transfer of the technology to human trials.

  The numbers of animals needed have been determined through careful power calculations based on previous work, and are likely to be approximately 25 for the acute protocol and 25 for the chronic protocol. Wherever possible, the novel and 'control' stent for each experiment will be tested in the same animal. Data will be shared between all groups of the European study consortium in order to avoid duplication and keep numbers to a minimum.

- 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.

  Based on both literature searches and the investigators' experience in vascular biology, there is no *in vitro* or computer model that can adequately replicate the *in vivo* assessment of pathophysiological responses to stenting.

  Use of clinical data and biological samples from human databases and bio-banks will be used wherever possible to replace animal experimentation. Extensive *in vitro* and *ex vivo* work has already been undertaken and is still on-going to develop and test novel stent materials.

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

  The degree of suffering by animals should be minimal. All animals will be housed and cared for in dedicated state of the art facilities. Stress and pain will be minimised by use of good handling techniques, effective pain control, appropriate sedation and anaesthesia, and efficient post-operative nursing care. For recovery experiments, vascular access will be gained through small incisions to reduce post-interventional wound pain.

Production of genetically altered animals

- Summarise your project (1-2 sentences)

  This project licence is required for the production of genetically altered (GA) animals for use in fundamental and applied studies under additional project licence authority.

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

Although the development of alternative techniques such as cell/tissue culture or computer based technologies has expanded greatly in recent years, GA animals continue to play a fundamental and vital role in biological research alongside these laboratory and computer based techniques. Genetically altered animals allow specific manipulation of genes and examination of gene function within a complex physiological environment which cannot be fully replicated in vitro. The study of transgenic, gene-targeted or gene-edited animals is the standard test for confirmation of gene function and furthering our understanding of normal development as well as disease processes. In recent years new techniques for creating GA animals has further developed the potential of these animal models.

- Outline the general project plan.

  Lines of animals will be produced as required by different scientific groups.

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

  The procedures applied to animals are those required for assisted breeding technologies so most often superovulation, embryo transfer followed by breeding and maintenance of genetically altered animals. Adverse effects associated with superovulation/embryo transfer across the species are minimal. We use appropriate anaesthetic and analgesic regimes. There can be unexpected phenotypes with the production of genetically altered animals especially when using new technology. Often these failures will happen prior to the birth of any live animal. All offspring are very closely monitored by experienced staff from birth in order to detect any issues quickly.

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

  Genetically altered animals underpin both fundamental biological research as well as applied studies as models of disease and therapeutics in both man and animals. Most of the animals produced in this licence will either undergo basic analysis contributing to our understanding of basic biological cellular processes, for example immune cell function or will be supplied to other project licences for studies investigating for example neurological disease (e.g. Alzheimers, Parkinsons disease), bone disease, diabetes, failures in growth and development. All of these conditions cause significant pain and suffering and these animal models allow us to understand these disease better as well as investigating potential novel therapeutics.

- 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.

  As this is a production licence for a large research facility that utilises a wide range of transgenic animals there are unavoidably large numbers of animals on this licence. This is driven by scientific demand which can vary through the lifetime of a project licence. Species involved are mice (predominantly) potentially up to 140,000, rats- 6,200, sheep- 1400 and pigs-900. These different species are used to fulfil different scientific functions. Mice and rats may be used more for proof of concept work and in the study of fundamental biological processes as well as some applied disease studies. Sheep and pigs are used more specifically in the development of human models of disease.
  Minimum animal numbers are used through good practice and experienced staff performing techniques. Careful attention to newborn animals , especially large animals minimises losses around birth and close monitoring of animal numbers using a state of the art database allows us to control breeding. In addition for lines that are not being used we will freeze embryos or semen rather than maintaining live lines.

- 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.

  Although cell based in vitro or computer models can be useful in studying genes ultimately the numbers of cell/organ interactions involved in physiological processes mean that the whole animal is required to study normal and abnormal gene function. Success in this project unavoidably necessitates the use of whole animals. It is not possible to use any other approaches. However in working towards this licence application much of the work has been done using in vitro techniques for example, to identify sequence variation in target genes, to establish genetic association with a given disease, establish that altered gene activity is associated with differences in transcriptional signalling. These studies have included in silico analysis and modelling, patient and animal phenotype/genotype cataloguing, biochemistry and cell-based experiments.

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

  Experience has allowed us to refine the techniques, the majority of which come into the mild or moderate severity category. Analgesics are used following embryo transfer surgery and offspring monitored closely so that veterinary intervention or euthanasia where required can take place quickly.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Regulation of immune responses to infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Innate immunity; macrophages; neutrophils; influenza</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td colspan="3"><table>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The objectives of the project are to fully understanding how cell surface receptors expressed on key cells of the immune system are able to regulate inflammatory and immune responses. By using strains of mice that have been bred to either lack individual receptors or express forms of the receptor that don’t work anymore, we can compare their responses to normal mice and work out the function of each receptor. Using this approach we have demonstrated that the receptors are important in controlling immune responses to infectious agents such as flu and in inflammatory responses such as asthma and septic shock. The proposed project will continue this work and will provide additional new information and knowledge that will allow us to understand how the immune system is regulated in health and disease. Not only will this research lead to better understanding of disease processes, but it is also expected to result in better treatments for these important human diseases in the future.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Regulation of inflammatory responses is crucial in human diseases such as asthma, acute lung injury and sepsis. Our work expected to give important insights into the signalling pathways involved which may lead to new therapeutic approaches to treating human disease. We also expect to reveal new insights into how the immune system defends itself against the influenza virus, especially during the first few hours after infection. Therefore this research could also lead to better treatments for</td></tr>
</table>

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

	flu and asthma.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used exclusively. We expect to use approximately 5000 mice over the 5 year period of this project. Only a small proportion of these will undergo any treatment. The majority are used in breeding programmes and humanely killed to produce the tissues such as bone marrow which are a vital source of specialised cell types for in vitro experiments.
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?	Apart from breeding and maintenance, some of the mice will be treated with agents that induce mild inflammatory and immune responses. They will also be inoculated with influenza virus to study how the receptors of interest control the response to infection. In all of the studies of inflammation, asthma and flu infection, the majority of animals will only undergo short-lasting feelings of malaise and possibly mild fever. This is very similar to how we feel when we have a cold. These treatments are not expected to lead to long-lasting harm or suffering of the animals. At the end of experiments or at the end of their useful breeding life, mice are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune system is a highly complex, integrated network of cells, secreted molecules and tissues. Although individual cell types can be isolated and studied *in vitro*, in most cases it is not possible to extrapolate *in vitro* findings to an *in vivo* setting in the whole animal, especially when studying responses which involve multiple parameters such as those invoked during host-pathogen interactions. Therefore, *in vivo* studies are essential if one is to obtain a complete understanding of the role of a given molecule(s) in the immune system. The mouse provides an excellent model system for understanding how the mammalian immune system works and the use of gene targeted mice has greatly increased our knowledge of the functions of specific proteins involved in host immunity. In this project, we propose to use mainly ‘Knock-Out’ and ‘Knock-In’ mice to continue our functional analysis of cell surface receptors involved in these important functions
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed cell culture methods for expanding large numbers of cells from the stem cells present in mouse bone marrow. These include bone marrow-derived macrophage cultures using CSF-1 and dendritic cell cultures using Flt-3 ligand. We are also currently refining expansion of other cell types such as T regulatory cells. These *in vitro* cultured cells mimic their natural counterparts very closely

	and are therefore an effective replacement for animals in biochemical studies. We will continue to exploit immortalised cell lines wherever possible to extend findings made with the *in vivo* mouse models of inflammation and infectious disease. Animal numbers are also minimised by the use of good statistical tests. The number of mice used in this study will be calculated according to four components; 1) the nature of immune response expected in control groups of mice; 2) the anticipated effect of the loss of a particular immune cell/molecule on the immune response 3) the significance level; and 4) the error rate (acceptable false negative) that is judged to be reasonable.
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 mouse provides an excellent model in which to study the relationship between the immune system and disease induction, since mice are well characterised immunologically, their immune systems closely resemble those of humans, and the majority of these models have been extensively studied and have pre-determined correlates of disease regulation. This allows us to reduce the number of unknown factors in any given experiment and increase the probability of obtaining interpretable and meaningful data. In addition, multiple genetically modified mice lacking various immune molecules/cells have been generated, and can provide an very refined approach to performing detailed analyses of the role of receptors in immunological functions. We aim to minimise welfare costs, yet at the same time maximise the output of data from animal experiments, by using sophisticated *in vitro* assays on tissues and cells in order to evaluate how the mice have responded to the various challenges used. This refinement is expected to reduce the severity limit of immune models because the experiments can be terminated before the onset of clinical disease; nevertheless important quantitative scientific data can be readily obtained.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>CORNEAL GRAFT REJECTION IN HIGH RISK RECIPIENTS</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>intraocular inflammation, corneal graft rejection, immunomodulation, artificial cornea, imaging (680 words)</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)[5]</td><td>
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</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Blindness due to corneal disease can only be treated by corneal grafts, but such grafts are often rejected if there is a previous history of infection as is often the case (high risk grafts).
The reasons why some corneal grafts are at “high risk” of failure is not known. Certain factors however are recognised such as the presence of many blood vessels in the cornea. This usually occurs after infection such as Herpes simplex or fungal infection of the cornea, but the blood vessels themselves rather than the infection are thought to be the main factor. However, this has never been properly investigated because of the lack of good animal models. We have now developed such models in which we can investigate the nature of the immune response both the previous infection and to the blood vessel rich cornea itself. We are therefore in a unique position to address this very important world-wide problem.</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 potential benefits therefore for patients with corneal blindness are threefold (a) we will understand the reasons why such grafts fail (b) we will be able to test out new treatments for corneal blindness and (c) we have the exciting possibility of developing an artificial cornea which contains therapeutic agents which may prevent non-acceptance in corneal graft recipients without need to use medication with severe side effects, which are currently used for prevention and treatment of this graft non-acceptance.</td></tr>
</table>

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

What species and approximate numbers of animals do you expect to use over what period of time?	We will be using mice for our studies as they develop clinically identical disease to humans. We are planning to use genetically modified / transgeneic mice, but also wild type mice, hence the estimation of 10000 animals during the duration of the project to ensure sufficient mice of the correct genotype for the study. Once particular experiments have been completed certain genetically modified lines may no longer be required and will be cryo-preserved.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The main procedures to be used are the induction of inflammation in the cornea (herpes simplex and fungal keratitis) followed some time later after corneal healing by healthy donor cornea transplantation. Generation of infective keratitis involves corneal scratching under anaesthesia and application of the infective agent. This would cause moderate distress. The corneal graft procedure involves grafting a donor cornea to a recipient with a single continuous suture under anaesthesia, again involving moderate distress. Similarly the skin graft procedure involves moderate distress. Minimal distress in relation to the handling of the animals while examining under the operating microscope and when administering immunomodulatory treatments. Some animals will undergo more detailed imaging of corneal vessels by scanning laser ophthalmoscope and these animals will be under anaesthesia. All animals will be humanely killed on the end of experiments while tissues will be further processed for detailed immunology investigations.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Corneal graft non-acceptance (rejection) is a clinical entity for which there is no model in dish. Understanding the mechanisms of graft rejection process is not possible from humans samples as they are harvested as end stage opaque samples just prior to the surgery for corneal transplantation, and do not show the reveal the process or mechanism of rejection. Therefore only animal models are able to provide necessary information that will determine future possible treatment for affected individuals. Approximately 10% of the work will be in dish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be planned to incorporate optimal experimental design and to use the minimum number of animals per group consistent while ensuring statistically relevant data to demonstrate

	sensitivity and reproducibility; and provide data for peer review in published papers. We will plan the mouse breeding meticulously and regularly check the breeding colonies, in order to meet the experimental requirement with minimal mouse numbers. As a result of our previous experience in our existing project licence we have already achieved the best possible designs for our standard laboratory protocols particularly corneal grafting procedures.
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 protocols involve procedures performed mainly under general anaesthesia and involve no suffering to the animal. Ocular surgery is routinely performed under the high- quality operating microscope with the finest possible instruments and sutures. The immunomodulatory treatments will be administered without anaesthesia; however these do not cause suffering, only temporary discomfort.

Why do grey squirrels strip the bark off trees?
“Sciurus carolinensis” “bark-stripping” *“*calcium” “deficiency”

- Summarise your project (1-2 sentences)

The working hypothesis of this project is that grey squirrels undergo seasonal periods of calcium deficiency, and that they ameliorate this deficiency by stripping the bark of trees and eating the phloem beneath – thereby damaging trees. The project will initially test this hypothesis by determining the extent to which such a deficiency exists in the wild, and the extent o which grey squirrels can utilise the source of calcium present in the trees – calcium oxalate.

- 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.

Grey squirrel bark stripping tree damage has a negative impact on UK and European woodland biodiversity which can mar woodland conservation efforts, and costs the Forestry Commission an estimated £10 million per year in beech, sycamore and oak timber losses. Currently grey squirrels are controlled lethally through the use of warfarin or through trapping. This project aims to inform the production of humane non-lethal alternatives to grey squirrel control. The underlying causal mechanism is currently unknown as to why grey squirrels strip the bark off trees. The main bark stripping season does however coincide with a period of time in which it is plausible that some or all of a grey squirrel population could be partially deficient in calcium. The phloem of trees contains calcium oxalate crystals which can be utilised as a source of calcium by some rodents, and so bark stripping may putatively be an attempt to obtain this source of calcium.

- Outline the general project plan.

The project is split into four distinct stages. Stage 1 will aim to determine if grey squirrels undergo seasonal periods of calcium deficiency by looking at intra-specific variation in calcium status as determined by bone density. Stage 2 will aim to determine if grey squirrels can utilise calcium oxalate as a source of calcium. Contingent on the positive outcome of Stage 2, Stage 3 will aim to determine if grey squirrels can ‘self-select’ for calcium oxalate as a source of calcium through a preference study. Contingent on the positive outcome of one or both stages 1 or 2, Stage 4 will aim to determine if bark stripping behaviour can be induced by calcium deficiency in the largely natural environment of four large outdoor enclosures. As a by-product of Stage 4 the following aim will be addressed: the production of baseline intra-specific physiological data of a temporal and spatial nature.

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

The project will require a number of regulated procedures to be carried out on grey squirrels. These include housing individuals singly, blood collection for analysis, feeding of a diet partially deficient in one or more nutrients (primarily calcium), and a diet in which

calcium is primarily present only in the form of calcium oxalate crystals. Possible adverse effects are as follows. Due to the fact that all procedures will be carried out by a trained individual and that the modified diets will be designed so as not to produce such a calcium deficiency that results in suffering, there are no expected adverse effects. If an individual does become overly stressed however (i.e. seizures), a competent individual will be on hand to provide a humane end-point using a schedule 1 technique.

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

The most likely benefit of the project is an increased understanding of the causes of this destructive bark stripping behaviour so that more constructive solutions to the problem can be developed. It may allow the development of humane, non-lethal, alternatives to control with poison, therefore conveying an animal welfare benefit to the grey squirrel population itself. If such a solution to the problem can be developed as a result of this work, and the problem of bark stripping be solved, then the likely benefits will be extensive. They will include protecting UK and European woodland biodiversity, aiding woodland conservation efforts, and freeing up funds spent by the Forestry Commission on timber losses and damage control to spend on more worthwhile causes such as red squirrel conservation. If the baseline cholesterol data proves informative, the potential benefits of this study increase exponentially. The prospect of a humane fertility control for grey squirrels that can be orally administered in a species-specific manner, bodes well for the red squirrel, a species nearly extinct in England and Wales, and likely to soon be under threat in the rest of Europe.

- 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.

No other species can be used to replace grey squirrels in this project as it is addressing a species-specific problem and so requires a species-specific solution. The number of individuals to be used is dependent on the results of stage 1 and also a pilot study. From which an idea of variation in calcium status between individuals, and the expected effects of treatments will inform sample size calculations so that the minimum number of individuals will be used.

- 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.

Computer models can not be utilised in the grey squirrels' stead as it is important that results are seated in real-world scenarios. Studies on grey squirrel faeces may also take place in order to determine the presence of calcium oxalate degrading bacteria.

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

Protocols will be carried out by competent individuals and the administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>STUDIES OF PEPTIDE RECEPTORS IN THE INTESTINE</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>PEPTIDE RECEPTORS IN THE INTESTINE</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></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></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>This project aims to elucidate the mechanisms by which peptides present in specific sensing cells or neurons within the gut regulate intestinal function under normal and stressful conditions, and how changes in these mechanisms contribute to gut dysfunction and obesity.
The incidence of obesity and diabetes has increased significantly and the projected NHS costs associated with treating these preventable diseases are estimated to increase by £2 billion/year by 2030. Certain types of gastric bypass reverse diabetes rapidly and reduce weight long term in man, but we do not know how the gut contributes to this apparent cure.</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>These studies will provide urgently needed basic scientific evidence to help explain the mechanisms involved that influence body weight under normal and stressful situations and after a change in diet. This project will also provide a basis for the development of novel therapeutic strategies to treat bowel disorders and metabolic changes associated with stress or altered diet, that lead to obesity.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice and rats, including naturally occurring genetically altered rodents that are accepted models of diabetes and obesity will be used. It is estimated that the total number of animals used over 5 years, including animals whose gut may be used for in vitro experiments, will not exceed 7,000. About 70% of these animals will be used to determine how intestinal function is altered under stressful situations or by altered diet.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td>The naturally occurring genetically altered ZDF rats develop glucose intolerance, hyperinsulinaemia and</td></tr>
</table>

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

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	polyuria, and we will minimise these effects by using rats no older than 20 weeks of age, checking their weight and general condition daily. Adverse effects are not anticipated with the genetically altered animals we propose to use. They breed and behave normally and are robust and healthy. However, animals exhibiting any unexpected harmful phenotypes exceeding mild severity will be killed or, in the case of individual animals or particular interest, advice will be sought from the local HO inspector. No adverse effects have been observed after colonic administration but daily observation of animals will occur looking for signs of diarrhoea, constipation, prolapse or abnormal movement or loss of condition. If an animal shows an abnormal condition then the advise of the NACWO and/or NVS will be sought and if the condition remains for 24h the animal will be killed by a Schedule 1 method. At the end of the experiments the animals will be killed humanely and tissue may be collected for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The structure of the intestine and the nervous system are complex. Peptides are contained in nerves and sensing cells embedded within the cell layer lining the gut lumen, but these are few in number and they do not survive long in tissue culture. There is no non-sentient system that models the mammalian intestine or the peripheral nervous system, or that can replace the use of animals. Instead we use *in vitro* assays where hormone-containing cells and nerves within the gut remain intact, measuring functions in isolated tissues initially. There are significant similarities in certain peptide-receptor mechanisms within the mouse and human gut, but the availability of healthy human tissue is limited. Comparison of responses in mouse, rat and human specimens will continue to be used wherever appropriate and will compliment data from whole animal GI transit experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Previous experience measuring gut transit *in vivo*, indicates that the numbers of animals required for statistical significance to be revealed is n=8-10 per group. We will make efforts to minimise *in vivo* experiments by performing *in vitro* experiments, in order to address basic questions prior to whole animal studies. For example, we measure natural faecal pellet movement *in vitro* prior to colon transit measurements *in vivo*. A smaller number of experiments will be performed in rats, where appropriate, e.g. naturally genetically-modified ZDF rats that are a well-characterised and

	accepted model of diabesity. Also, a smaller number of chronic stress experiments will be performed, for example if gut-specific peptide or receptor loss results in increased resistance to acute stress. Then, a chronic stressor will also be tested.
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 studies have shown the same key peptide-receptors mediate the same functions in mouse and human colon, indicating the mouse gut is the best model for the human intestine. For disease models, the naturally occurring genetically altered ZDF rats that develop diabesity, will be included as they are an accepted model of diabetes and obesity and aspects of their gut activity are similar to those of the mouse and human. Mice with an inducible or a tissue-specific mutation will be especially valuable in future studies allowing peptide or receptor genes to be switched on or off in adult animals. We will compare their gut functions with those from previous studies using germline altered mice, providing refinement and reducing numbers and welfare costs.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The role of RING finger proteins in malignancy.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>PML, BRCA1, RING finger, Malignancy, DNA repair</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>-</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[10]</td><td>-</td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our laboratory has had a longstanding interest in understanding the function of proteins carrying a particular zinc binding region (called the “RING finger domain”). We have focused on two such proteins, namely PML (for ProMyelocytic Leukaemia) and BRCA1 which are both involved in repairing damage to DNA in the cell and play an important role in human cancer. The PML gene is disrupted by a chromosomal rearrangement in a type of leukaemia (Acute Promyelocytic Leukaemia, APL). In addition to being involved in leukaemia, altered expression of PML has been associated with a number of common tumours including those involving the brain, lung and prostate. Alterations in the BRCA1 gene also represent an important healthcare issue, with carriers of mutations having a very high risk of development of early onset breast and ovarian cancer, which are associated with a relatively poor prognosis. Our major objective is to understand the function of PML and BRCA1, considering how loss or alteration of the proteins contributes to altered cell biology and the development of cancer.

With respect to our work on leukaemia, we are using mouse models to establish the role played by PML in normal blood development. We are also using mice to gain more information about the type of bone marrow cells in which the disease arises and establish the relationship between cell of origin and the pattern of mutations found in different populations of leukaemia cells, determining how they affect clinical outcome and response to</td></tr>
</table>

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

	therapy. An important further objective is to decipher the role played by RING finger proteins such as PML and BRCA1 in DNA repair; this will not only help us understand how tumours develop, but may also identify vulnerabilities of particular tumours which can be exploited to improve treatment outcomes.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding the molecular genetic basis of these cancers should lead to measures for early diagnosis, help refine disease diagnosis, improve outcome prediction and development of better treatment approaches.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 7000 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?	For breast cancer studies the adverse effect would be breast cancer development. For the analyses concerning Pml and other genes involved in leukaemia the adverse effect is expected to be onset of blood cancer in some animals. For this project mice are monitored very carefully for signs of illness, with strict criteria adopted when mice are euthanized to ensure that they do not suffer.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have already undertaken extensive studies, based on study of patient samples. While this has been informative, it does not allow us to understand the mechanisms underlying the stepwise progression to leukaemia or other malignant diseases. We need to use animal models to achieve our objectives, which provide primary cells for experimental analysis and allow testing of anti-tumour agents. It is not possible to reliably determine whether particular mutations will induce tumours by use of *in vitro* assays alone, since these do not reliably model the *in vivo* situation in terms of the cellular environment, nor do they take into account the latency period required for tumour development. Moreover, *in vivo* efficacy of therapeutic agents cannot be reliably extrapolated from results of *in vitro* assays.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical analysis will predict the minimal number of animals needed to achieve meaningful results *i.e*. to be sure that any differences are likely to be real rather than due to chance, as well as ensuring that biologically important differences are not

	missed. These statistical estimates take into account different possible outcomes of the experiments performed.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mouse is the most appropriate model for these studies, given the large body of work published by investigators in the field concerning normal blood development in this species, forming a firm basis for comparison with alterations resulting from targeted mutations or expression of leukaemia-associated fusion proteins. Similarly, there is an extensive body of work on mammary gland and breast cancer development in this species, which makes it the most appropriate species for this aspect of the project.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Strain typing of TSE agents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Scrapie, prion, strain</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)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></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></td></tr>
<tr><td>Preservation of species</td><td></td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td></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>The objectives of the project are to compare newly emerging strains of Transmissible Spongiform Encephalopathies (TSEs) with established strains to establish if they are new strains or if they are related to those already known.</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>New TSE strains could represent a danger of infection to humans through the food chain (similarly to BSE) or could be a danger to animal health and welfare. This project will help provide information to allow control of such strains, development of diagnostic tests and an early warning of potential public health risks.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Species: mice
Numbers: a maximum of 2,700 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>Many of the animals will develop TSE clinical signs and the level of severity is moderate. The animals will be killed by approved methods when disease is manifest but not severe. Experience allows accurate recognition of symptoms and euthanasia at a defined stage.</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>At present there are no reliable methods for replacement of mice in this study. Work is underway to develop such methods but none is yet suitable for study of novel field strains of TSE. This project will however produce samples which will</td></tr>
</table>

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

	allow non-animal alternatives to be developed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project plans to use minimum numbers of mice while at the same time using sufficient to allow reliable interpretation of results. The numbers per experiment are continually reviewed in the light of previous experience and statistical advice.
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.	There are no invertebrates or fish models available to study TSEs and non-animal methods will not allow us to study complex interactions between body cells and brain cells. This project will however provide tissue samples which will help in the development of non-animal methods.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Immunomodulation in poultry</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immune response; bird; disease resistance; vaccine</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>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Diseases of the gut and lung are significant problems in poultry produced for eggs and meat. This is a welfare issue for these birds resulting in suffering and death, as well as being a disease risk as some of these conditions are transferrable to humans.
In addition there is a considerable financial impact. he estimated diseases effects and control costs of for example coccidiosis (a disease in the chicken gut) and infectious bronchitis virus (a respiratory disease) in the UK are over £12M and £23M respectively. The costs of zoonotic diseases run into billions per annum, e.g. acute gastroenteritis in humans in the United Kingdom each year at a recurring cost of ca. £2Bn. At present we control these diseases using a variety of vaccination strategies but these are not highly effective.

In this project we want to improve vaccines and disease control in poultry and thereby reduce the risk of human infection. The role of the immune system in birds in relation to vaccination and disease has not been greatly investigated. The major gaps in our knowledge for the development of vaccines include the understanding of (i) how immune responses at different stages of infection work and how the immune response can be sustained for longer, (ii) how pathogens such as bacteria and viruses avoid detection by the bird and (iii) how we can best improve the immune response to defend against disease
The objectives of this PPL are to</td></tr>
</table>

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

	1. Define the interaction between the early and late immune responses after exposure to pathogens such as viruses and bacteria 2. Define how substances produced by viruses and bacteria alter the immune response in the bird 3. Investigate and test substances to improve host protection against infection.
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 aims to study how the immune system of birds functions in order to understand how we can improve poultry health and welfare. Most of the UK flock of 850 million meat chickens and egg laying birds receive 20-30 vaccines throughout a 6 or 78 week lifespan, respectively, and this is seen as preferable to having to use therapeutic drugs to treat infections, which might impact on the human food chain. Several poultry diseases pose a threat to human health and outbreaks of bacterial foodborne diseases and viral pandemics frequently arise. Reducing the prevalence of poultry diseases will improve animal welfare and lower the incidence of human infection. The work under this licence will fill gaps in our knowledge on avian immune responses and pathogen evasion mechanisms and lead to a better understanding of host-pathogen interactions. The findings will steer future work on developing vaccines and identify markers of host immunity to support selective breeding of animals with improved resistance.
What species and approximate numbers of animals do you expect to use over what period of time?	Birds, mostly chickens, but other birds e.g. ducks and turkeys are used for influenza related research. The number of birds will depend on the grants obtained in the near future, and will be not more than 2000 birds per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No adverse effect when using non replicating pathogens and substances. Mild respiratory effects such as wheezing and sneezing when using live respiratory viruses, or after dual infection with bacteria and viruses. Mild symptoms, diarrhoea, after infection with coccidia. The animals will be culled according to schedule 1 at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Use of animals is necessary because it is not feasible to study the complex interactions between pathogens such as bacteria and viruses and the immune system of the host in plastic dishes. There are no relevant surrogate models to be used under this licence because experiments are performed in

	the target animals, i.e. poultry species. To replace the use of animals we have developed assays using cells in plastic dishes to screen for interactions of pathogens or vaccines with host cells before we perform experiments in the birds. Moreover, we are able to keep parts of organs such as rings of windpipes in culture and investigate how pathogens interact with the cells lining the windpipe.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals we will use genetically altered chickens that are resistant or susceptible to certain diseases. Existing knowledge of the genetics of these birds enables us to focus on the complexity of immune responses. The minimal number of birds required will be estimated and studies designed and interpreted in collaboration with Biomathematics & Statistics Scotland.
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.	There are no surrogate models to be used under this licence because experiments are performed in the target animals, i.e. poultry species. Wherever possible we will meet experimental objectives before animals exhibit moderate disease symptoms or humane end-points are reached. Subsequent use of commercial birds reared on site will enable us to perform experiments that are closest to the real world, the poultry industry, as we can thereby ensuring that the translation to the field situation is realistic.