Home Office

Animals (Scientific Procedures) Act 1986

Project summaries granted during 2013

Volume 53

Project Titles and key words

- Blood Products

  Antibodies; blood; complement; immunology

- Cannabinoid function in appetite and body weight regulation

- The function of key protein in T cell signalling

- Nervous system modelling, protection and repair

  Culture models, neuroscience, repair, cell biology, regeneration

- Drug discovery in a model of temporal lobe epilepsy

  Epilepsy, anti-epileptic drugs

- Induction of an acute temporal lobe seizure/status epilepticus and assessment of test compound effects

  Seizure, epilepsy, anti-epileptic drugs

- Drug discovery in partial seizures

  Seizure, epilepsy, anti-epileptic drugs

- Drug discovery in generalised seizures

  Seizure, epilepsy, anti-epileptic drugs

- Nutrient and mineral metabolism in ruminants

  Nutrient, mineral metabolism, ruminants

- Preventing neonatal Escherichia coli K1 meningitis

  Meningitis, bacterial infection, gastrointestinal tract, colonization, prevention

- Heart transplantation using Circulatory Determined Death donors (DCD)

  Heart, Transplant, Circulatory Determined Death

- Analysing Gene Function in Xenopus Development

  Xenopus, nervous system, embryo

- Effects of antibody on fixed tissue calcification

  heart valve, anti-Gal, calcification

Blood Products
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 suffereing.

Cannabinoid function in appetite and body weight regulation

This project will explore the role of the cannabinoid system in the control of feeding and body weight regulation. Eating is a basic physiological need and understanding the mechanisms guiding what, when and how much we eat are fundamentally important issues. In modern societies, our susceptibility to the sight, taste and thought of food drives us to over-consume, when matched with the easy availability of food and reduced energy expenditure of our largely sedentary lifestyles, leads to the development of overweight individuals and obesity.

On the other hand, patients suffering from cancers, various diseases and those recovering from surgery often display anorexia, which may have serious consequences on their chances of survival. Effective strategies to help these people increase their appetite and energy intake are therefore imperative.

In this project we will investigate the acute and chronic effects of cannabinoids on feeding behaviour, ascertaining the particular aspects of feeding behaviors that are modified, the brain areas responsible for these effects, and any interactions the cannabinoid system may have with other neurochemical systems involved in feeding behaviour. Importantly we will also investigate the therapeutic potential of cannabinoids in the treatment of obesity or in the alleviation of cachexia and wasting. As feeding requires the integration of many complex physiological and psychological mechanisms, it is not possible to predict with any certainty the outcome of drugs on feeding behaviour using isolated tissues and therefore, we require the use of intact, conscious animals, and it is well established that basic mechanisms regulating feeding are comparable in rats and man.

The drugs and the doses used will be based on knowledge from previous animal studies, from human studies or by extrapolation from in vitro studies. It is essential that drug doses are not too high, as they may produce abnormal behavioral effects (e.g. drowsiness or stereotypy) resulting in non-specific effects on feeding. It is also important that animals are in good health and not stressed (i.e, stressed rat will not exhibit normal feeding) so we will minimize stress and discomfort in our animals prior to the start of any experiment by handling them and giving them training sessions to acclimatize them to the experiments. In some cases, surgical procedures, with recovery, will have to be carried out on the rats to implant cannulae for central injections or catheters for intravenous injections or blood sampling. Surgery will be carried out under anaesthesia and the animals will be given analgesic drugs post-surgery to minimize post-operative pain.

Our studies have been designed to minimize the number of animals used, while maintaining good scientific practice to obtain statistically valid results and minimizing stress to the experimental animals. We estimate that the maximum number of animals that may be over the 5 year period of the project will be 4000 rats. In the majority of experiments involving food intake, Latin-square designs will be used in which each animal will receive all drug treatments and serve as its own control, resulting in fewer animals being used. In our chronic studies, it will be necessary to use an alternative design in which each animal is used once and only given doses of drug repeatedly, either by injection, via drinking water or by osmotic mini-pump. Dose response curves will be produced between rat rather than within rat to minimize the number of repeated tests.

Findings from this project will further our understanding of the mechanisms underpinning the regulation of food intake and will critical for the development of treatments for clinical disorders such as obesity and anorexia in man.

The function of key protein in T cell signalling

- Summarise your project (1-2 sentences)

To identify the role of key proteins in the immune system, identifying targets for the treatment of immunological diseases.

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

The main objective of this application is to study the function of proteins in the immune system by generating genetically modified mice. Identifying which proteins play a role in the different stages of disease progression such as diabetes, arthritis, AIDs and cancer is crucial in determining therapeutic targets. Many available drugs are not target-specific and can suppress the whole immune system leading to other infections which can be fatal.
The pathways behind these diseases are complex, but by using immunological reagents and genetically modified animals to investigate specific proteins, we hope to identify which proteins are up-regulated or down-regulated during disease progression and therefore lead to drug development.

- Outline the general project plan.

This project continues our existing program of research and builds on our previous findings. It involves using diseases such as arthritis and cancer, as well as viral and bacterial infection. We will use genetically modified mice to determine which proteins are involved in these diseases. Interventions such as treatment with immunological reagents or drugs will be used to test well-defined hypotheses. Outcomes will be measured by determining the progression of disease in treated animals compared to non-treated. Blood sampling and imaging studies will provide essential information, alongside *in vitro* tests at the end of each study which will be performed using tissue samples.

- 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 majority of animals will make a full recovery in most protocols; those used for tumour induction will indeed grow tumours up to 12mm in diameter but *are not expected to show signs of adverse effects that impact materially on their general well-being*.

In rare cases, moderate clinical signs *such as weight loss, stary coat, hunched posture and poor appetite may be observed.*

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

The primary benefit of this research would be to obtain a more complete understanding of the function of certain key proteins in T cell signalling. An understanding of the nature of these molecules that regulate the immune response will be of key importance in the design of new treatments for autoimmune diseases (i.e. AIDS, rheumatoid arthritis) and

organ transplantation.

- 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.

Mice provide the best animal model to study immune function that is very similar to the human immune system and provides a system in which genes can be readily manipulated or deactivated. In addition, most antibodies for studying immune cell function are available for murine immune cells.
The main objective of this project is to study the function of key proteins by creating genetically modified mice deficient in the proteins being studied. Such mutant mice will then be analysed in a variety of ways. Initially, we will examine the development of the immune system and cell numbers in immune organs by flow cytometric analysis of different cellular populations. The different types of cells will be analysed for their ability to respond to various signals such as growth factors, chemokines and antigen receptor stimuli. Mice will be immunised with factors known to give rise to an immune response and the immune system will be analysed.
The minimum numbers of animals will be used that will still provide a statistically valid study. The use of pilot studies will help to assess animal numbers and how best to design the main study in order to gain maximum information.

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

The need for animal models has arisen from extensive previous studies in the lab where the use of cell lines produced contradictory results that could only be resolved or confirmed with the use of an animal model.
Further to this, *in vitro* work has given rise to possible candidate genes as potential anti-cancer/viral drug targets and it is essential to validating these genes *in vivo* and to analyse their function in tumour/viral development. Where possible in vitro work using cell lines will be performed and only extended into animal studies where absolutely necessary. The aim of our project is to identify key proteins in disease which will be initially sought in vitro, but the final aim will be to look at possible treatments using these proteins as targets and therefore will mostly result in the use of animal models for therapeutic purposes.
To maximise the information from a single animal, we will aim to collect tissue samples from multiple body sites and provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments.
We have reduced the number of animals used in the previous project throughout the tumour study by injecting the mice with tumour cells in two sites allowing a control tumour to develop in the same animal as the test tumour and hence eliminating the need for subsequent control.

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

Animals will be housed in groups with suitable environmental enrichment. They will be checked daily and regularly handled. When the animals are on study, the frequency of handling and checking may be increased to ensure that the animals are not suffering. The animals will have access to food and water. Blood samples may be taken at regular intervals and other samples e.g. tissues at the end of the study. Blood

sampling volumes will be kept to the minimum required to obtain information for this study.

When generating transgenic mice in which a harmful phenotype may be displayed, extra care will be taken in monitoring these mice to minimise suffering and where possible inducible constructs will be used, so that the phenotype is only displayed when the gene expression or deletion is induced.

Throughout the protocols there are several routes of administration given this is so that the mice may receive the least intrusive method but yet give the optimal effect. I.e. Intranasal infection of some viruses is the least intrusive method and gives rise to optimal levels of infection, however, other viruses require different routes to give optimal levels. The least intrusive methods will be used where possible.

Nervous system modelling, protection and repair

Culture models, neuroscience, repair, cell biology, regeneration

5

Basic research	Yes	
Translational and applied research	Yes	
Regulatory use and routine production		No
Protection of the natural environment in the interests of the health or welfare of humans or animals		No
Preservation of species		No
Higher education or training		No
Forensic enquiries		No
Maintenance of colonies of genetically altered animals[1]	Yes	

The aim is to develop improved methods for the repair of the nervous system. This involves developing conduits for peripheral nerve repair and creating advanced cell culture models to enhance research into cellular responses in the peripheral and central nervous systems

This project will improve the understanding and treatment of nervous system damage and disease in humans and animals. It will generate new implantable conduits to help with the surgical reconstruction of the damaged nervous system, and will provide new cell culture models for the research community to use as alternatives to live animal models in many experiments.

Rats. Approximately 200 per year, mostly for breeding and cell culture.

1. Models of peripheral nerve repair (moderate severity):
The nerve which runs down the back leg of a rat will be cut under carefully monitored anaesthetic. A conduit will be used to repair this nerve and the animal will be allowed to recover under close supervision to ensure that no discomfort occurs. After the recovery period the animal will be killed painlessly and the repaired nerve removed and examined to assess the extent of repair. These experiments affect one hind leg of the animal for a short time until repair has taken place.
2. Cell culture models (mild severity): This involves painlessly killing young rats in order to obtain brain cells for cell culture.

Cell culture models that allow most stages of peripheral nerve studies to be conducted without using live animals will be used wherever possible. For peripheral nerve repair, before new therapies can be used in clinical trials they must be assessed in a living mammal.
For culture models, alternatives to primary rat cells include primary human cells, cell lines and various stem cell sources, which will be used wherever possible. In some cases these options are not suitable in which case a reliable source of primary animal cells is essential

The number of animals used in peripheral nerve repair studies will be minimised through the use of established control datasets and refined measurements to assess repair post mortem.
Cell cultures will be prepared only when specific experiments are to be undertaken and animal numbers can be calculated based on the number of cells required.

The standard animal model for peripheral nerve study is the rat, whose nerves can be damaged and repaired in the same way as a human. Welfare costs will be minimised by

[1] At least one additional purpose must be selected with this option.

restricting procedures to one hind limb, which does not interfere with the ability of an operated animal to feed and drink.
Rat CNS cells have been characterised thoroughly and show a similar response to damage as the human.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Drug discovery in a model of temporal lobe epilepsy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Epilepsy, anti-epileptic drugs</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)[2]</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[3]</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>Epilepsy is a chronic neurological disorder that affects 0.5-1% of the population and for which there is no cure. ~30% of all epilepsy patients receive little or no benefit from existing anti-epileptic drugs.

We have spent a number of years investigating the effects of new drug compounds upon epileptiform (‘epilepsy-like’) activity in acute brain slices and acute whole animal models of seizure.

This work has identified a number of drugs that exert potent anti-epileptiform and anti-seizure effects which must now be studied in models of the disease itself, epilepsy, in order to meet clinical and regulatory requirements to justify human clinical trials.</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 evidence produced by this project will determine whether new compounds hold sufficient promise for testing in human clinical trials. Drugs successful in human clinical trials will have a significant beneficial effect upon the >30% of people living with poorly controlled epilepsy.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats; 1000; 5 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td>Depending on the efficacy of the test compounds being studied and once rendered epileptic, animals will experience spontaneous seizures. Whilst</td></tr>
</table>

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

effects and the likely/expected level of severity? What will happen to the animals at the end?	behaviourally dramatic, consciousness is lost during seizures and amnesia (in humans) is very common. Adverse events include death from seizure and, very rarely in this model, biting of the tongue during seizure. Some animals will be implanted with screws in the skull in order to monitor brain activity. They will experience some pain during recovery from the screw implantation process that can be controlled with analgesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epilepsy is a disorder of the whole body (brain and periphery) and, as such, is too complex for current *in vitro* approaches to properly model it. Moreover, regulatory (EMA) requirements demand testing in whole animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiment sizes will be determined by power calculations. We will use established quantitative scoring systems to assess seizures induced. A professional statistician will be consulted when required.
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 majority of existing, initial *in vivo* anticonvulsant testing to date has been conducted in rats, providing a large body of evidence with which to compare our results and reduce the need to replicate some investigations for which results are already available. Mouse models of seizure are less discriminatory and predictive than rat models since mice exhibit different seizure circuits in brain than rats and humans. The model is the gold standard animal model of spontaneous recurrent seizure (epilepsy). Anaesthetised models are unsuitable since involvement of the periphery is eliminated and so renders the model less predictive.

Induction of an acute temporal lobe seizure/*status epilepticus* and assessment of test compound effects

Seizure, epilepsy, anti-epileptic drugs

5

Basic research	Yes	No
Translational and applied research	**Yes**	No
Regulatory use and routine production	**Yes**	No
Protection of the natural environment in the interests of the health or welfare of humans or animals	Yes	No
Preservation of species	Yes	No
Higher education or training	Yes	No
Forensic enquiries	Yes	No
Maintenance of colonies of genetically altered animals[4]	Yes	No

Epilepsy is a chronic neurological disorder that affects 0.5-1% of the population and for which there is no cure. ~30% of all epilepsy patients receive little or no benefit from existing anti-epileptic drugs.

We have spent a number of years investigating the effects of new drug compounds upon epileptiform ('epilepsy-like') activity in acute brain slices and acute whole animal models of seizure.

This work has identified a number of drugs that exert potent anti-epileptiform effects which must now be studied in acute models of the principal symptom, seizure in order to meet clinical and regulatory requirements to justify human clinical trials.

The evidence produced by this project will determine whether new compounds hold sufficient promise for testing in rodent models of epilepsy and, subsequently, human clinical trials. Drugs successful in human clinical trials will have a significant beneficial effect upon the >30% of people living with poorly controlled epilepsy.

Rats; 6000; 5 years

Depending on the efficacy of the test compounds being studied, a small number (vehicle control group) or larger number (if the drug is ineffective) of animals in each experimental group will experience seizures. Whilst behaviourally dramatic, consciousness is lost during seizures and amnesia (in humans) is very common. Adverse events include death from seizure and, rarely, biting of the tongue during seizure. Animals are euthanized at the end of the procedure (maximum two hours after induction of seizure).

Epilepsy is a disorder of the whole body (brain and periphery) and, as such, is too complex for current *in vitro* approaches to properly model it. Moreover, regulatory (EMA) requirements demand testing in whole animal models.

Experiment sizes will be determined by power calculations. We will use established quantitative scoring systems to assess seizures induced. A professional statistician will be consulted when required.

The majority of existing, initial *in vivo* anticonvulsant testing to date has been conducted in rats, providing a large body of evidence with which to compare our results and reduce the need to replicate some investigations for which results are

[4] At least one additional purpose must be selected with this option.

already available. Mouse models of seizure are less discriminatory and predictive than rat models since mice exhibit different seizure circuits in brain than rats and humans. The model is the only validated model of acute, temporal lobe seizure available. Anaesthetised models are unsuitable since involvement of the periphery is eliminated and so renders the model less predictive.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Drug discovery in partial seizures</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Seizure, epilepsy, anti-epileptic drugs</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>
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<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[6]</td><td>Yes</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>Epilepsy is a chronic neurological disorder that affects 0.5-1% of the population and for which there is no cure. ~30% of all epilepsy patients receive little or no benefit from existing anti-epileptic drugs.

We have spent a number of years investigating the effects of new drug compounds upon epileptiform (‘epilepsy-like’) activity in acute brain slices and acute whole animal models of seizure.

This work has identified a number of drugs that exert potent anti-epileptiform effects which must now be studied in acute models of the principal symptom, seizure in order to meet clinical and regulatory requirements to justify human clinical trials.</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 evidence produced by this project will determine whether new compounds hold sufficient promise for testing in rodent models of epilepsy and, subsequently, human clinical trials. Drugs successful in human clinical trials will have a significant beneficial effect upon the >30% of people living with poorly controlled epilepsy.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats; 2000; 5 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td>Depending on the efficacy of the test compounds being studied, a small number (vehicle control group) or larger number (if the drug is ineffective) of</td></tr>
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[6] At least one additional purpose must be selected with this option.

effects and the likely/expected level of severity? What will happen to the animals at the end?	animals in each experimental group will experience seizures. Whilst behaviourally dramatic, consciousness is lost during seizures and amnesia (in humans) is very common. Adverse events include death from seizure and, rarely, biting of the tongue during seizure. Animals are euthanized at the end of the procedure (maximum two hours after induction of seizure).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epilepsy is a disorder of the whole body (brain and periphery) and, as such, is too complex for current *in vitro* approaches to properly model it. Moreover, regulatory (EMA) requirements demand testing in whole animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiment sizes will be determined by power calculations. We will use established quantitative scoring systems to assess seizures induced. A professional statistician will be consulted when required.
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 majority of existing, initial *in vivo* anticonvulsant testing to date has been conducted in rats, providing a large body of evidence with which to compare our results and reduce the need to replicate some investigations for which results are already available. Mouse models of seizure are less discriminatory and predictive than rat models since mice exhibit different seizure circuits in brain than rats and humans. The model is one of only few validated models of acute partial seizure available. Anaesthetised models are unsuitable since involvement of the periphery is eliminated and so renders the model less predictive.

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We have spent a number of years investigating the effects of new drug compounds upon epileptiform (‘epilepsy-like’) activity in acute brain slices and acute whole animal models of seizure.

This work has identified a number of drugs that exert potent anti-epileptiform effects which must now be studied in acute models of the principal symptom, seizure in order to meet clinical and regulatory requirements to justify human clinical trials.</td></tr>
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Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epilepsy is a disorder of the whole body (brain and periphery) and, as such, is too complex for current *in vitro* approaches to properly model it. Moreover, regulatory (EMA) requirements demand testing in whole animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiment sizes will be determined by power calculations. We will use established quantitative scoring systems to assess seizures induced. A professional statistician will be consulted when required.
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 majority of existing, initial *in vivo* anticonvulsant testing to date has been conducted in rats, providing a large body of evidence with which to compare our results and reduce the need to replicate some investigations for which results are already available. Mouse models of seizure are less discriminatory and predictive than rat models since mice exhibit different seizure circuits in brain than rats and humans. The model is one of only few validated rat models of acute generalised seizure available. It also can be predictive of efficacy againist absence seizures.Anaesthetised models are unsuitable since involvement of the periphery is eliminated and so renders the model less predictive.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Nutrient and mineral metabolism in ruminants</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Nutrient, mineral metabolism, ruminants</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)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The extent to which nutrients in feed are converted into milk and meat by ruminants has a direct impact on the environmental impact of animal agriculture, animal health and welfare, and the nutritional quality of milk and meat from ruminants. This project will conduct studies that will form the basis of improved feeding strategies that optimise nutrient supply and utilisation for the production of milk and meat with minimal environmental impact and optimal composition for consumers.</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 results of this work will contribute to the more efficient use of feedstuffs in ruminant production by improving the balance between nutrient supply and animal requirements whilst reducing nutrient losses and end-products (e.g. methane and nitrogen) to the environment. The consumer will gain from the increased availability of animal products of improved nutritional specification and from being able to purchase animal products (meat and milk) that have been produced under enhanced welfare systems. Finally the livestock producer will benefit through improved feeding efficiency and lower production costs. The longer term benefit of the research will be to provide the fundamental information which can be integrated into mathematical simulation models which can be used to predict performance accurately.</td></tr>
<tr><td>What species and approximate numbers of</td><td>Cattle 1700;
Sheep 400;
Goats 150.</td></tr>
</table>

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

animals do you expect to use over what period of time?	Over the five year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The surgical modification of animals is expected to cause moderate pain and discomfort for not more than 48 hours. We have reduced the time animals are without feed and water pre-operatively to the minimum. Pain during surgery will be controlled by anaesthesia and after surgery by the use of analgesics, pre- and post-operatively for as long as required. The risk of infection will be minimised by using sterile techniques and broad spectrum antibiotics pre- and post-surgery. The short-term distress of surgery is a one-off adverse effect, but subsequent procedures cause minimal distress and a mild level of severity e.g. variation in diet composition. At the end of procedures, surgically modified animals will be euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The work under this project is principally concerned with the whole animal system from food to milk/meat and as such there is rarely any opportunity to exploit alternatives to the use of live animals. While every effort will be made to develop and utilise *in vitro* and *in silico* techniques, they cannot adequately represent the complex interactions between digestion, metabolism and the endocrine regulatory systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental designs used are chosen to optimise statistical power whilst minimising the number of animals used, typically through the use of change-over designs. The use of change-over designs testing effects of treatments within animals increases precision and scientific rigour, whilst reducing the number of animals required.
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.	Digestive physiology in ruminants is unique and therefore models based on other species are not applicable. The animal models chosen are the commercially important species for the UK livestock and food supply industries and they are the major sources of environmental pollution from livestock farming. Data for the proposed species has been widely reported in the scientific literature and use of similar species will allow direct comparison of our results and reduce the need to replicate some investigations for which data already exist. Animals are provided with all needs to achieve their full biological potential and housed in appropriate facilities with environments to promote natural behaviours and animal interactions. Animal health

	and welfare is monitored daily and any animal exhibiting any adverse effects will be removed from the study and veterinary advice sought. The longevity of animals in our facility is more than double the average longevity of similar animals on farm.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Preventing neonatal Escherichia coli K1 meningitis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>meningitis, bacterial infection, gastrointestinal tract, colonization, prevention</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>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>Newborn infants may contract meningitis caused by the Escherichia coli K1 because this bacterium can colonize the infant gut following acquisition from the mother and then relocate to the blood stream. The project is concerned with preventing this translocation by understanding in greater detail the factors that govern susceptibility and by investigating the potential of therapeutic proteins to accelerate maturation of the gut tissues to enhance the barriers to infection.</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>If these experiments show that dosing of the proteins prevents translocation of the bacteria into the blood, they could be rapidly developed as agents to ensure that the critical event in E. coli neonatal meningitis does not occur. Such measures would reduce the mortality and morbidity of this dangerous disease in Great Britain and elsewhere.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats; 2,600 neonates from 250 adult females over five 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>Neonates will be fed a culture of E. coli; in most cases animals will be culled before the onset of symptoms due to blood and tissue infection, as I am studying the very early stages of infection. Some animals will receive doses of protein, either orally or by injection, with no expected additional adverse effects. Some animals may show signs of infection, such as raised temperature, weight loss, high respiration rate, depressed behaviour,</td></tr>
</table>

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

	hunched posture or high sensitivity to light; if regular monitoring of the wellbeing of the animals identifies any of these symptoms, they will be immediately culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Host-pathogen interactions are a complex interplay of factors, with specific interactions between the pathogen and host tissues triggering systemic responses and this complexity cannot be replicated *in vitro*. Thus, this research mandates the use of *in vivo* experiments as there is currently no *in vitro* system which could generate meaningful data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The neonatal rat has been extensively used to characterise *E. coli* K1 pathogenesis and to demonstrate the efficacy of novel pathogen-targeted therapeutic strategies; it has been employed in my laboratory for fourteen years and has been refined to a point where there is practically no redundancy with respect to animal wastage. The strain employed colonizes 100% of neonates and in infection-susceptible animals 100% will develop systemic infection.
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 neonatal rat model is justified on the basis that it reflects the key features of infection in the human neonate, in particular the strong age-dependency. The method involves oral inoculation of the infective dose, which is the natural route of infection in humans, and colonization of the GI tract, as occurs in the natural history of human neonatal infection with *E. coli* K1. Minimization of suffering will be assured through the use of rigorous health scoring sheets and frequent monitoring, especially during the critical phase of the studies. The study is primarily concerned with GI tract colonization and translocation from the gut lumen to the blood circulation and these events occur prior to the appearance of any symptoms of infection. In experiments involving therapeutics, the primary endpoint will be the detection of bacteria in the blood circulation; animals will be culled as soon as these endpoints have been attained. Any animal showing symptoms of infection will be immediately culled and tissue and blood samples taken. All colonized animals will be regularly observed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Heart transplantation using Circulatory Determined Death donors (DCD)</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart, Transplant, Circulatory Determined Death</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</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>The aim of the project is to determine the optimum technique to restore function, transport and transplant the heart from a circulatory determined death (DCD) donor. Currently the scientific unknown is which method of transportation and assessment of the DCD heart is best prior to successful transplantation.</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 number of heart transplants performed in the UK has declined significantly over the last ten years due to a shortage of brains stem dead donors (BSD). Despite this decline the number of patients on the waiting list continues to grow. This increasing supply and demand mismatch results in approximately 10% of patients dying whilst waiting for a heart with only 43% of listed patients ever being transplanted. We are currently investigating a relatively new type of donor called the Circulatory Determined Death donor (DCD). These are donors who’s hearts have stopped before their organ have been procured. This new type of donor has successfully been used in liver, kidney and lung transplants. Our previous work reveals that some of these donors may also be suitable as heart donors. We suspect that if we manage to use 10% of the current number of DCD donors as heart donors then the number of heart transplants in the UK will double.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately 600 rats over a five year period.</td></tr>
</table>

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All the experiments will be undertaken under anaesthesia from which the animals will not be allowed to recover.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to prove that it is possible to transplant and use DCD hearts we must show that they have restored sufficient function to be able to support the circulation. For this animals have to be used because of the complexity of the circulatory system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have consulted a statistician who has given us the minimum number of animal to be used in order for our experiments to remain valid.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen the rat model due to the scale involved. In order to undertake a heart transplant micro surgical techniques have to be undertaken to join the blood vessels as well as supporting the animal on cardiopulmonary bypass whilst the transplant takes place. Unfortunately this is not possible in the mouse model. The technique involved in a transplanting a rodent heart is exactly the same as a human heart transplant. In order to minimise welfare costs all experiments will be undertaken under general anaesthesia in order to reduce suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Analysing Gene Function in Xenopus Development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Xenopus, nervous system, embryo</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></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[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>The aim of this project is to understand how the vertebrate nervous system is formed, using the frog Xenopus laevis as our model organism. Development of the nervous system begins during the first 24 hours of frog development, as a flat plate that subsequently folds into a tube, forming the brain and spinal cord. The brain is subsequently divided into its main regions and the mechanisms responsible for this are the subject of intensive research. Cells from the roof of the neural tube will subsequently migrate throughout the body to form the peripheral nervous system, as well as pigment cells of the skin and bones of the face and neck. Defects in the development of the nervous system are amongst the most common causes of congenital abnormalities in humans. In this project we aim to study the molecular mechanisms responsible for establishing different regions of the brain, as well mechanisms controlling the migration of cells from the neural tube.</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 project will increase our understanding of fundamental questions of how adult anatomy is formed during embryonic development and how defects in this process can result in congenital abnormalities. Since embryonic development is very similar in all vertebrates the knowledge gained in this project will be applicable to humans. This project will also help us understand diseases such as cancer, which share many properties with embryonic cells. In addition to increasing our scientific knowledge this project may therefore have</td></tr>
</table>

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

	future health benefits for humans.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use the South African clawed frog *Xenopus laevis* as our model organism. Approximately 300 female frogs will be required over the 5 year duration of this project, with females being reused no less than 3 months after previous injection.
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?	Female frogs will be injected subcutaneously with chorionic gonadotrophin, which causes them to lay eggs approximately 12 hours later. Eggs are collected, fertilized with sperm and the embryos used to study how the nervous system is formed. The injection procedure is very mild and usually results in no adverse effects, although it will occassionally cause redness of skin and small sores on the nose. These frogs usually recover within a few days.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Embryonic development is a complex process requiring multiple tissue interactions. These cannot be reproduced using cultured cells. We therefore require male and female frogs to provide the embryos required by this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Female frogs lay thousands of eggs, which are sufficient for many experiments. We therefore organise our work schedules so that as many as 6-7 scientists may use these eggs. Sharing resources minimizes the number of animals we need to inject.
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.	Amphibians have been used in embryonic studies for more than 100 years because they have numerous advantages over other model organisms. They produce large numbers of eggs that can be observed at all stages of development. The large embryos are easy to manipulate, making them organisms of choice for transplantation studies (along with the chick embryo). As a consequence, amphibians were instrumental in demonstrating the key roles of cell-cell signalling in vertebrate development and in identifying the relevant signalling molecules. *Xenopus* is the amphibian of choice because of the ease with which they can be maintained in the laboratory and because they can be induced to ovulate at all times of the year. Genome and RNA sequencing data means it is easy to design reagents for disrupting gene function and these reagents are easily injected into the large

	egg. Since large aspects of development is conserved in all vertebrate embryos, data obtained in this project will be applicable to other vertebrates, including humans. The injection procedure causes no more than mild stress in the majority of cases and animals show no long term adverse effects. Handling is kept to a minimum and animals are returned to the holding tanks as soon as possible. Animals displaying adverse effects receive veterinary care and if necessary killed by terminal anaesthesia.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Effects of antibody on fixed tissue calcification.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>heart valve, anti-Gal, calcification</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3</td></tr>
<tr><td>Purpose of the project (as in Article 5)[17]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>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[18]</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>Bioprosthetic heart valves (BHVs) have been used for over 40 years to replacement heart valves damaged due to infection, birth defect and age. The most common replace valves are made from chemically processed human or animal tissues. Overtime BHVs wear out, becoming brittle, leaky, and non-functional. This degeneration process occurs more rapidly in young adults compared to older patients. Researchers think that BHVs rapidly wear out in young adults because these patients have more active immune system that causes injury to the BHV. A prominent target of this immune response is to a sugar called alpha-Gal which is present in animal tissue but not in humans. This study looks at the potential for the immune system to damage replacement heart valves and tests whether tissues from genetically modified animals which are less immunogenic may be used to create new valves with greater durability in young adults. The experiments will compare the level of calcification and the degree of immune stimulation to the amount of alpha-Gal sugar on tissue from regular pigs and from pigs engineered not to produce the alpha-Gal sugar.</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>Bioprosthetic heart valves (BHVs) are life saving devices used to replace damaged heart valves. A limitation of BHVs is that they rapidly wear out in some patients. In patients < 35 years of age up to 100% of BHVs will wear out within 5 years. Because of this premature wear juvenile and young adult patients are treated with mechanical heart valves which require lifetime anticoagulation. Anticoagulation places these patients at risk for life-</td></tr>
</table>

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

	threatening bleeding and clotting complications and significantly restrictions their life choices for employment and for having children. In developing nations where the incidence of heart valve disease due to infection may be high, young adults may not have access to heart valve replacement therapy unless effective anticoagulation management is available. The primary benefit of this research is to gain a greater understanding about the processes which affect BHV degeneration, specifically the role of antibody in BHV calcification. Publication of these results in peer reviewed scientific journals will sensitize clinicians to the potential impacts of immune mediated injury in BHV degeneration. This research supports the development of more durable BHVs which could eventually advance the standard of care by eliminating the need for anticoagulation and extending access to this therapy to more young adult patients.
What species and approximate numbers of animals do you expect to use over what period of time?	This research is performed in genetically modified mice. Over a 3 year period we expect to use up to 600 mice.
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?	BHV tissue is implanted under the skin of the mice. This is a very simple procedure with low expectations of complications. The surgery is performed under anaesthesia. Sterile instruments are used to prevent infection. Drugs to relieve pain are given before and after the surgery.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying a systemic immune response that can not be replicated using laboratory tests.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A pilot experiment is performed to define the time frame in which substantive calcification of tissue occurs in these mouse implant tests. This insures that subsequent larger experiments will collect tissue at the appropriate time, with measurable calcification, so that meaningful comparisons between groups can be made. The pilot experiment also provides an estimate of the variability in calcium levels. This variability will be used to define the scale of subsequent experiments so that meaningful statistically significant comparisons between treatment groups can be reliably made. The experiments are organized into study groups.

	Each group addresses a specific experimental condition and both positive and negative controls are used. This organization insures that interpretable and meaningful data will be produced from each experiment. The size of each experimental group is based on previous research experience with a related model measuring tissue calcification and on statistical estimates to insure that sufficient data is collected to detect significant differences in calcification between groups.
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.	Anti-Gal antibody is only made naturally by humans and Old World primates. The GTKO strain of mice is a genetic model which, because of the alpha-galactosyltransferase mutation, spontaneously makes anti-Gal antibody and thereby mimics the human immune system with respect to this antigen. This genetic modification makes the GTKO mouse the appropriate model for these studies and a convenient small animal model for studies which otherwise would require nonhuman primates.