Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 20

Project Titles and key words

- Improving treatment and prevention of Toxoplasma gondii infection

  Toxoplasma, blindness, stillbirth, abortion encephalitis

- Antiviral activity of DI influenza viruses

  Influenza, antiviral, respiratory disease

- Cardiac medical device development

  Heart; development; regulatory; service

- Experimental therapies for neuromuscular diseases

  Muscular dystrophy, amyotrophic lateral sclerosis, mdx mouse, SOD1 mouse

- Towards improved control of poultry infectious disease

  Eimeria, Chicken, Vector, Vaccines, Microbiota

- Learning and memory across the lifespan

  Brain, behaviour, environmental enrichment, cognition, ageing

- Safety of Biological Materials

  Pharmaceutical, biological, infection, safety

- Mouse Models of Human Birth Defects

  Heart Defect; Mouse Mutant; Zebrafish Mutant; Embryological Development

- The genetic basis of disorders of the brain and pituitary gland

  Brain, pituitary, congenital disorders

- Mechanisms of NKT cell activation

  Lymphocyte, infection, inflammation, imaging

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Improving treatment and prevention of Toxoplasma gondii infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Toxoplasma, blindness, stillbirth, abortion encephalitis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></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[2]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Toxoplasmosis a disease caused by the parasite Toxoplasma is a devastating disease that can cause abortion, congenital defects, blindness and potentially mental illness in humans. There is no effective vaccine for humans and the treatment which is only partially effective has significant side effects. How to make a vaccine and better drugs have been hampered through lack of understanding of essential parasitic processes and how the human responds to infection. This project will increase our understanding of these things, i.e. how disease occurs and how we can prevent it through vaccination and/or better drugs to treat 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>The benefits of the project are mainly for human health and could prevent abortion, congenital defects, blindness and mental illness in humans. Additional benefits are economical as Toxoplasma is also a pathogen of farm animals which could also benefit from an effective vaccine.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately 9,000 mice are required to achieve the goals of this project.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>Most of the animals used in the project will undergo moderate procedures that will cause a febrile illness 5-14 days following infection that the vast majority are expected to recover from or they will be euthanised before this time if they reach specific identified end points. The health of some animals can decline with time in studies that last longer and these animals will where possible be euthanised as their health declines. However, a few animals deteriorate in health or even die</td></tr>
</table>

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

	unexpectedly in spite of best efforts to prevent this. Therefore the limits for some protocols are set as severe although approximately 1% of animals on these protocols are likely to experience severe symptoms.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have replaced some animals by the use of tissue culture methods where possible. However, the humans response especially to infectious diseases, is complex and relies not only on multiple cell types moving dynamically to and from different parts of the body, influenced by messenger molecules, but also on blood pressure and host metabolism which are as yet impossible to replicate in tissue culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed new techniques that allow us to gain much more information from each animal at multiple time-points in each study meaning that we are required to use less animals overall. We have also worked with colleagues who are expert statisticians to design experiments to make sure we use the minimal amount of animals to get a statistically valid answer to the questions we study. Whenever possible we store parasites in cryopreservation rather than maintaining them in infected animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have many similarities with humans and are widely used in the type of studies we are performing as they have similar immune systems and metabolism to humans. Mice live in a temperature controlled environment with environmental enrichment including bedding to make nests. They are given soft palatable food when they are experiencing febrile illness. Animals are monitored regularly and euthanised when their health declines to point agreed with the NACWO & NVS

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Antiviral activity of DI influenza viruses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Influenza, antiviral, respiratory disease</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)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td></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 project will investigate the application of influenza defective interfering viruses as antiviral agents to protect against respiratory virus disease.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Respiratory disease is responsible for significant mortality and morbidity throughout the world. Many types of domestic livestock such as domestic birds also succumb to respiratory virus disease. There is a great and growing need for new antiviral compounds which is being exacerbated by the appearance of strains resistant to the currently available drugs. The project is investigating a new class of antiviral which protects against influenza and other respiratory virus disease. To date the approach has focussed primarily on influenza A virus and the project will explore the possibility of extending the approach to influenza B virus. The project will identify the mode of action of the new antiviral and will identify key aspects with a view to advancing the study to clinical trials. This will significantly increase the possibility of prevention of respiratory disease in humans and animals.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The study will involve the use of mice and embryonated eggs. It is expected that over the five year period of the project no more than 12000 animals will be used.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>All animals will be checked visually following recovery from anaesthesia and daily thereafter. No adverse effects of anaesthesia are anticipated. As</td></tr>
</table>

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

level of severity? What will happen to the animals at the end?	the work will investigate approaches to treat influenza virus disease it requires the production of adverse responses in mice. Following infection with influenza virus all animals will be checked daily for the presence of symptoms of disease. It is expected that only those infected animals not receiving treatment with antiviral compounds will present with significant symptoms of disease. The progress of influenza virus infection in mice is well understood and can be monitored by external signs which include poor coat condition, reduced activity and increased rates of breathing. A combination of these external signs of respiratory disease reflects the potential outcome of the infection and can be used to identify, in advance, any animals likely to succumb to the infection. With some strains of mice it is possible to monitor weight loss in conjunction with the external signs of disease and this additional measurement will be carried out when suitable. Any animals observed to display signs of distress or becoming very sick in the days following virus infection will be killed immediately using a schedule 1 method to prevent further suffering. When using weight measurements, a daily measurement of the weight of the groups of animals will be recorded and if any groups of mice show a weight loss exceeding 25% of the original weight they will be immediately killed by a schedule 1 method. While every effort will be made to cull mice in the late stages of disease it is not possible to completely eliminate death and no analgesia is available. For these reasons the severity is assessed as severe. Based on experience and refinement of a clinical score system used to assess the severity of infection over the last 5 years, fewer than 10% of the animals used in the studies will succumb to infection. At the end of each experiment all animals will be killed using a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project seeks to investigate the protection from respiratory virus disease. Assessment of the onset and progression of disease is only possible in whole animals which can display the full extent of the pathogenic process. No *in vitro* surrogates for protection have been identified.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals necessary to provide statistically robust data will be used. This will eliminate the need for repetition of experiments, each of which would require the use of control animals. We will use a single strain of mice wherever

	possible to reduce experimental variation. We have carried out power calculations to identify the minimum number of animals per group required to provide statistically robust datasets and will use this to inform experimental design.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most accessible small animal model system for respiratory virus disease. A considerable body of literature has been generated using mouse infection with respiratory virus and this will provide substantial dataset with which to compare the results of the project. All experimental animals including uninfected controls will be monitored daily for signs of infection and the severity of the disease scored using a standard protocol. Mice showing the most severe symptoms will be culled using a schedule 1 method. Additionally, all groups of animals will be weighed daily and if the group weight falls below 25% of the weight at the beginning of the experiment they will be culled.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Cardiac medical device development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart; development; regulatory; service</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>
<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[6]</td><td>~~Yes~~</td><td>~~No~~</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of this project is to provide a service to test new medical devices in-vivo. This may be non-regulatory early development tests for proof of concept or regulatory pre-clinical studies.</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 aim of this project is to support the development of safe, new medical devices for treatment of cardiovascular disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Cattle up to 4 over duration of licence</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>Unclassified – non-recovery</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>Relevant in-vitro studies on hydrodynamic testing, durability testing, functional testing and tests in cadaveric material are expected to have been performed prior to embarking on in-vitro studies. However, none of these can fulfil the in-situ</td></tr>
</table>

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

	measurements that can be obtained from using animal models, in terms of delivery and functionality.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Non-survival studies to observe device anchoring, functionality and retrieval will normally use 2-4 animals. Regulatory tests are set-up to meet the relevant guidelines for testing and these normally state the minimum numbers of animals to be used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In general, the choice of an animal model is based on the following criteria; • The anatomy and function of the heart • The size of the heart and the total body weight • The use of human diagnostic tools (e.g. echo- Doppler equipment) • The expertise of the investigators with a certain type of animal model • Similarity of physiological processes between the animal and human in terms of coagulation, calcification, infection resistance and wound healing All procedures are to be performed are non-recovery and performed under general anaesthesia.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Experimental therapies for neuromuscular diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Muscular dystrophy, amyotrophic lateral sclerosis, mdx mouse, SOD1 mouse.</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)[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>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>There are currently no treatments for neuromuscular diseases that stop or reverse the decline in muscle function. Using mouse models we aim to develop clinically applicable therapies for these conditions via gene therapy or the use of drugs targeting disease symptoms.</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>Neuromuscular diseases are an area of high unmet medical need that affect not only the individual patient but also their relatives and others involved in caring for the patient. Many cases of Duchenne muscular dystrophy (DMD) and amyotrophic lateral sclerosis (ALS) appear spontaneously with no family history, hence they cannot be effectively controlled by genetic counselling. Both conditions are debilitating and fatal and reducing or stopping the progression of the disease would be life-changing for patients.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>All work will be done in cell culture or in mice. Many of the assays in mice will be carried out under terminal anaesthesia or post-mortem. We expect to use less than 5,000 mice over the 5 project period.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The overall aim of the project is to develop clinically applicable therapies. Thus we seek to be as minimally invasive as possible. Most of the studies involve injections or addition of potentially therapeutic drugs to food or water and no surgical procedures except under terminal general anaesthesia (when the mouse does not feel pain or</td></tr>
</table>

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

	wake up at the end). As such the procedures are mostly of only mild severity. However for the final testing of therapies for ALS, the relevant human measure is survival from diagnosis. Thus we will conduct a few limited tests in the mouse model of ALS where we assess the effect of drug treatment on time to the humane end point, the loss of the ability to self-right in under 20 seconds. Such a survival study is categorised as being of substantial severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the vast majority of cases the drugs and genetic constructs under test will have been evaluated in cell culture. However, it is not possible to fully evaluate the treatment effects without testing in an intact whole animal with functional nervous and hormonal regulation of cellular processes and the complex inter-relationship of the muscle or brain and spinal cord and the blood supply which may act to limit drug effectiveness.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the recently developed standard operating procedures (currently located on the treat-NMD website: http://www.treat-nmd.eu/research/preclinical/preclinical-efficacy-standards/). We will also use best practice as defined for our two main models (mdx mouse and the G93A SOD1 mouse). We have considerable experience with each model which provides knowledge of the variation in each measure and therefore accurate calculations of the required sample size. Experiments will use a randomised block design in most cases where mice in the same litter are assigned to different treatments at random to compensate for any litter to litter effects. Where the effect of a specific intervention is unpredictable and the potential variation is uncertain, pilot trials using 3 animals per group with a limited number of groups will be used to assess the value of a larger scale experiment using a range of doses with group sizes determined using power calculations. In some cases it may be possible to use a special method (factorial design) that reduces group sizes to 3-4 per group.
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	All experiments will be conducted in mice as this species has been the most widely used for genetic manipulation and has the greatest number of spontaneous and induced mutants and genetically modified strains. It is also the lowest vertebrate group for which there are models of the most common neuromuscular disorders.

(harms) to the animals.	Two mouse models will be used for the majority of the studies: the mdx mouse model of DMD is a relatively mild model that shows no obvious signs of the disease; in contrast the G93A SOD1 mouse model of ALS is much more severely affected but in most cases the mice will be killed when they start to show clinical signs to reduce the level of harm. Most of the studies involve injections or addition of potentially therapeutic drugs to food or water and no surgical procedures except under terminal general anaesthesia (when the mouse does not feel pain). We will use anaesthesia and appropriate analgesia where a procedure may cause pain.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Towards improved control of poultry infectious disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Eimeria, Chicken, Vector, Vaccines, Microbiota</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></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>Yes</td><td></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>
</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 ultimate objectives of the work proposed here are to improve control of Eimeria species parasites, enhancing economic and welfare-friendly production of poultry, and increase knowledge of host/parasite biology. A series of scientific challenges will be addressed through four work packages:

1. A vaccine vector based on transgenic Eimeria
Live Eimeria parasites have been used to vaccinate a small proportion of the global poultry flock for more than 50 years. The development of approaches to genetically complement Eimeria now provides opportunities to use these parasites as vaccine vectors, supporting vaccination against many other pathogens with a single dose. These studies are designed to test Eimeria as a vaccine vector and expand the transgenic parasite toolbox.

2. Identification of target antigens for recombinant vaccine development
The development of vaccines based upon small numbers of defined antigens requires knowledge of the ‘correct’ antigens. Using host/parasite genetics and scrutiny of panels of rationally selected candidates we have identified a small number of plausible candidates. These studies are designed to extend this work, incorporating data defining host/parasite interactions to identify and validate additional candidates.

3. The genetic basis of susceptibility/resistance to coccidiosis</td></tr>
</table>

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

	Individual chickens and certain chicken lines/breeds have long been recognized to present variable levels of susceptibility to pathogens such as *Eimeria*. Advances in genomic technologies now allow the genetic basis of susceptibility to be investigated, supporting the identification of causative elements (e.g. specific genes or controlling elements). In this work package we aim to identify the genetic basis of resistance/susceptibility and the strain-specificity of immunity induced by some natural infections, informing on host-parasite interactions and improving future poultry breeding strategy. 4. Understanding the consequences of pathogen co-interaction *Eimeria* can cause severe damage within the chicken intestine including haemorrhage and a mucoid enteritis. Such gross pathology inevitably has a profound effect on the intestinal bacteria, etc. Following a series of pilot studies we aim to investigate the impact of *Eimeria* infection on bacteria within the gut to improve understanding of bacterial colonisation and the outcome of vaccination using live *Eimeria*.
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 new vaccine vector that can immunise against multiple pathogens and can be administered safely (orally) at day of hatch, offers benefits to poultry (fewer vaccinations/injections; better disease protection; less handling; wider uptake of vaccines), to farmers (cost of control, ease of administration) and to the environment (reduction in use of antimicrobials and anticoccidials). Identifying panels of *Eimeria* antigens that induce immune protection is critical for development of a new coccidiosis vaccine. Commercial development of such a vaccine would have many benefits (fewer birds used for vaccine production, cheaper vaccines and wider uptake). A prototype next-generation vaccine will ensure that the UK animal health industry has a solid foundation from which to retain a leading position on coccidiosis control, contributing to overall wealth creation. Breeding for disease resistance requires understanding of host genetics. Identifying sections of the chicken genome linked to inherent resistance and improved responses to vaccination will facilitate downstream development of tools to inform future breeding strategies towards the production of naturally parasite tolerant birds.

What species and approximate numbers of animals do you expect to use over what period of time?	Chicken: Up to 6,300 in total over five years. Rodents (mouse, rat): Up to 900 in total over five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Methods for infection of chickens and rodents with *Eimeria* have been refined over many years and are carried out with a minimum of stress to the animals. We minimise animal suffering by having well defined end points and carefully controlling doses of parasites administered. For the vast majority (~95% of animals used) it is expected that suffering will be within a mild severity band. Nonetheless, we require a moderate severity limit because we cannot rule out rare occasions where animals may show clinical symptoms of coccidiosis (e.g. intestinal discomfort, diarrhoea). At the end of each protocol the animals will be humanely terminated using an appropriate Schedule 1 method
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of living animals is essential because *Eimeria* parasites only grow productively in live animals in an absolutely host-specific manner. For this reason replacement has been focused on identifying approaches for complimentary studies, increasing the use of cell and parasite culture in predictive screens for the most effective tests before working with live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise overall numbers the research group operates a pooled resource so that each parasite batch is utilised efficiently, with minimal wastage. Key factors include the use of statistical power calculations to identify the minimum number of animals required for a valid outcome and detailed parasite knowledge to optimise parasite production per animal without compromising welfare.
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.	Chickens and rodents are the natural hosts and the data generated is of direct relevance for development of improved control strategies. Methods for infection of chickens and rodents have been refined over many years and are carried out with a minimum of stress to the animals. We minimise animal suffering by having well defined end points and carefully controlling doses of parasites administered.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Learning and memory across the lifespan</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain, behaviour, environmental enrichment, cognition, ageing</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>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[12]</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>We wish to understand the genomic, molecular and cellular basis of the positive influence of environmental enrichment on cognitive function across the lifespan. To do this we will expose wild-type and genetically-modified rodents from birth or at various ages during their lifespan, to either standard laboratory cages or larger cages with toys, running wheels and a greater number of rodents to facilitate social interactions. Behavioural analysis in learning and memory tasks will indicate whether enrichment had improved cognitive function and whether it can halt or even reverse ageing-related cognitive decline, whilst in vitro studies will allow us to probe the genomic, molecular and cellular basis of the effects of enrichment on the brain.</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 human brain, like other organs, is affected by ageing. This can lead to reduced concentration, forgetfulness, and confusion when confronted with novel or unexpected situations. In more severe cases this puts the person at risk of harm and jeopardises independent living, placing heavy burdens on families and society. There is therefore a great need to both understand the normal brain ageing process, and to develop strategies to limit the impact of ageing on brain function. Similarly, we know that early life experience can have profound effects on subsequent human health, behaviour and indeed longevity. An understanding of the factors and mechanisms that influence the positive development of the post-natal mammalian brain will have impact on the care of children vulnerable through social circumstance or congenital</td></tr>
</table>

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

	neurodevelopmental disorders. The intersection of these two themes, early childhood development and the ageing process arises through environmental enrichment, which has benefits for both groups. Mechanistic insight into the influence of enrichment on brain development and ageing provided through this project will reinforce – and explain - the importance of positive rearing and potentially identify targets for “enviromimetic” drugs that tap into the cellular and molecular processes activated by environmental enrichment.
What species and approximate numbers of animals do you expect to use over what period of time?	900 mice (plus 3000 for breeding) and 120 rats 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?	The learning of a new behavioural task may initially be moderately stressful as the animal will be exposed to a novel, unfamiliar environment. After the behavioural testing animals will be killed humanely and brain tissue will be used for various in vitro analyses. The introduction of telemetric or drug delivery devices will involve surgical procedures that can carry some risk of adverse effects such as infection and post operative pain. These will be managed via good sterile techniques, antibiotics and pre and post operative analgesia as appropriate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no way to mimic the ageing process or environmental enrichment in the test tube. Animals need to be allowed to age and be exposed to the sensory and social stimuli associated with an enriched environment
2. Reduction Explain how you will assure the use of minimum numbers of animals	We can base our sample size calculations on both our prior experience and that of others. This will allow us to generate robust, statistically significant data upon which to draw firm conclusions and in doing so both advance the field and iteratively adjust the sample size of future studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats are mammals and have highly developed brains with which they respond to their environments and learn from their experiences, in much the same way that humans do. Furthermore, the plethora of behavioural tests, the vast literature, the ease with which they can be maintained, trained and tested, together with the ability to genetically modify mice makes mice and rats the mammals of choice for the majority of behavioural studies. Harm to experimental subjects will be minimised through careful handling and acclimation to behavioural tests and an awareness, through visual inspection, of signs of distress or untoward or prolonged anxiety. Should such unexpected behaviour be

	observed, animals will be killed humanely.

Safety of Biological Materials
Pharmaceutical, biological, infection, safety

- Summarise your project (1-2 sentences)

  The aim of this project is to provide general safety data on biological materials (used as pharmaceutical products or in the manufacturing/testing of pharmaceutical products) to demonstrate freedom from contamination or infection.

- 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.

  Current government regulations protecting man and animals require the testing of pharmaceutical materials, and the submission of animal-based data. Testing requirements are established by legislation. Guidelines produced by, for example, the European Medicines Agency (EMA), provide information on the way such assessments should be conducted, such that the data generated may be submitted to Regulatory Authorities responsible for approving marketing authorisation and establishing the safe use of a pharmaceutical product. Where the use of a material is as a cosmetic or as a cosmetic ingredient, then it will not be tested under the authority of this licence. These tests are provided as a service to companies developing and selling pharmaceutical products.

- Outline the general project plan.

  One or more tests, including animal and non-animal tests, may be conducted for the assessment of a particular material. Studies are conducted under contract for sponsors, and the project licensee is typically asked to conduct only a subset of the required regulatory studies. The range of studies conducted under this licence includes those to assess any contamination of animal-derived materials with infectious organisms.

- 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 materials to be tested are injected into animals (by a variety of well established routes) and the animals are then examined for any adverse clinical signs that may indicate that the materials were contaminated for a defined period of time (weeks). At the end of this period the animals are humanely sacrificed. In some cases blood samples are taken from the animals and tested in the laboratory for signs of potential contamination. In some cases, in order to increase the sensitivity of the test, tissues from animals injected with materials are processed and reinjected into a second set of animals.

  The majority of animals are expected to experience no or only mild and transient adverse effects (for example, discomfort during restraint and dosing procedures, transient inappetance, or reduced bodyweight growth). A small percentage of animals may show further adverse effects and in such cases animals will be removed from study or humanely sacrificed under veterinary guidance as necessary.

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

  The materials tested are of benefit to man or animal, such as novel vaccines for prevention of illness in man or animals. However the main benefit is the collection of data allowing assessment of risk to man or animals from exposure to the test material, and enabling establishment of safe conditions of use. Such testing must be completed if the material is to be registered and authorised under current legislation.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

  Our estimate of the maximum number of animals used for this project is as follows:

Mouse & Rat (including neonates), Guinea Pig, Hamster	30,500
Chicken (embryonic)	10300

  These species have been chosen as they are appropriate for detecting potential contaminants and as such are specified in legislation. All studies within this project will follow the principles of Good Laboratory Practice or Good Manufacturing Practice which should increase the quality of the work and reliability of the results, resulting in an overall minimisation of animal use.

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

  Some types of potential contaminants of pharmaceutical products can be tested by *in vitro* means (e.g. using cultured animal cells) and these tests are also carried out on test materials. However, certain types of contaminants (e.g. specific types of viruses) can only be detected in animals as there are no *in vitro* methods available.

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

  The majority of animals are expected to experience no or only mild and transient adverse effects (for example, discomfort during restraint and dosing procedures, transient inappetance, or reduced bodyweight growth). A small percentage of animals may show further adverse effects and in such cases animals will be removed from study or humanely killed under veterinary guidance as necessary.

Project Title (max. 50 characters)	Mouse Models of Human Birth Defects
Key Words (max. 5 words)	Heart Defect; Mouse Mutant; Zebrafish Mutant; Embryological Development
Expected duration of the project (yrs)	5
Purpose of the project (as in Article 5)[13]	

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[14]	Yes	No

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

Clinical unknown.

Birth defects affect 1 in 33 (3%) of all pregnancies world wide and, therefore, represent an enormous burden on society. Many children with birth defects are handicapped, physically, mentally or both, and require life long medical treatment. For example, children with hole in the heart or defective blood vessels require surgery, often repeated as the child growth. Mental capacity might be affected, or metabolism leading to diabetes or obesity.

Background to research.

Despite the importance of birth defects, medical science has not advanced to any great degree in being able to prevent birth defects from arising, except through prenatal diagnosis and termination of pregnancy. The use of folic acid to prevent some cases of spina bifida is a rare example of primary preventive therapy in this area. If we can understand the pathway affected by gene mutation perhaps other small molecules could be used to alleviate diseases. A new challenge is to use the information we gain from studying disordered development, to direct the regeneration of diseased tissue using directed development of replacement cells and tissues (sometimes called regenerative medicine).

Purpose of the project.

Therefore, this project uses genetically altered mouse strains, fish and chick models to determine

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

	the processes in the embryo and fetus that predispose to, or cause, birth defects. The work plan consists of: (i) identifying the genes that cause birth defects; (ii) investigating the embryonic and fetal processes that lead from gene defect to birth defect; (iii) discovering new methods or pathways for preventing birth defects by 'correcting' development in the embryo or fetus.
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 benefits include: (i) increased understanding of embryonic development, both normal and abnormal leading to birth defects; (ii) improved methods of genetic diagnosis and genetic counselling, which should follow from discovery of genes that cause birth defects in mice, provided the findings are confirmed in human studies; (iii) identification of new pathways that might be amenable to drug treatment (iv) identification of pathways of development that might be recapitulated in a later “repair” scenario, in other words using “generation” to inform “re-generation”.
What species and approximate numbers of animals do you expect to use over what period of time?	This programme, which is comprised of several independently funded projects has used approximately 6250 mice per year, and approximately 1600 fish per year. Chick use has fallen off, but we anticipate using 1000 eggs over the duration of the project. For culture of mouse embryos, approximately 100 rats will be required over the 5 year term.
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?	In this project, embryos will normally be studied at an early stage of development, before pain or other sensations have been acquired. These embryos are killed almost the moment they are taken, so there is minimal potential for suffering in any case. Some experiments will be done using embryos cultured in a test tube. This minimises the number of pregnant mice that need to be used, since embryos from a single female can act as both 'experimental' and 'control' treatments. Moreover, use of culture studies minimises the number of procedures that need to be carried out on pregnant females. Where mutant mice have a deleterious phenotype we have defined points at which experiments will be ended to avoid suffering. As less developed organisms, the chance of suffering is even lower, and fish and chicks will be used where such analyses are appropriate. Some protocols will require the use of a general anaesthetic in order to conduct surgical procedures. These are essential techniques during the creation of new strains of mice. This potentially involves

	infection, pain or general distress as risks to the animal. To obviate these issues, we will use careful aseptic technique, supported by the use of antibiotics if necessary. Each experimental has given endpoints at which time mice are killed, or the animal moved to another experiment which has such an end point. Where animals are suffering they will be killed humanely. As the creation of new mutations might produce unexpected results, we will ask advice from the home office inspector whenever this occurs. Our animal house staff are observant and contact a team member if an animal appears to eb suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research into birth defects concerns the processes by which the embryo and fetus develop their specific shape and function. We have chosen to study a mammalian species, the mouse, so that the principles emerging from our research have the greatest chance of applying to the human situation. Mice have hearts, vessels, kidneys and other organs very similar to humans. Chicks are quite similar and fish different in many respects, but many of the developmental pathways are present in all three species. Embryonic development is a four-dimensional process (i.e. varying in space and time), and it therefore requires the analysis of whole developing embryos. Direct genetic studies of embryonic humans are difficult practically, and only descriptive analysis is possible, with experiments ruled out on ethical grounds. Tissue culture systems, although they can provide useful information on certain molecular or cellular phenomena, cannot mimic the complexity of functioning organs, let alone the developing embryo. Computer simulations can be valuable in extending theoretical approaches to embryonic development, but cannot tell us about real biological situations, such as those occurring in the embryo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have several years' experience of designing animal experiments. Numbers are reduced by using the most appropriate breeding scheme to produce as many embryos with the required genotype as possible. This is agreed in discussion between the principle investigator and his research staff. As many strains are required in multiple projects regular meetings are chaired by the PI to co-ordinate the maintenance of the lines so that individuals are not separately maintaining lines and

	therefore using excessive numbers. In addition, wherever possible we maintain conditional alleles (which have no phenotype) as homozygotes, and if compatible with the experiments we maintain two alleles in single mice of breeding colonies. Moreover, we maintain mice on C57BL6. While this does result in smaller litter sizes, using a single inbred background reduces experiment to experiment and intra-litter variability so potentially reducing numbers when a statistically significant result is required. Mouse numbers are reduced by pilot experiments in tissue culture cells, and in zebrafish.
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 is the most appropriate mammalian system that can be used for birth defects research, because of the vast amount of work that has already been done on this species, which makes it the best understood mammal, in genetic terms (with the exception of humans). Mouse genetics is coming to play an ever more important role in the 'Post-Genomic Era', which is now underway, following the decoding of the genetic material in the Human and Mouse Genome Projects. Genetically altered mice enable the construction of animal models of human biology and/or disease that can be used in numerous ways to improve our understanding of disease processes and to develop new methods for diagnosis and approaches to therapy. Zebrafish are catching up fast and have advantages (easily seen down a microscope, fast breeding times, cheaper) and disadvantages (not the same anatomy as mammals) versus mouse. This fish are the second most important organism for us. Many studies of embryonic development employ other sub-mammalian vertebrates (e.g. frogs, chick) or even invertebrate species (e.g. fruit fly). While each of these model systems has its advantages, the over-riding benefit of mouse studies is the relatively straight forward extrapolation of results to humans, and therefore to clinical disease. It is for this reason that genetic modification in mice forms the main basis of this research programme. Fish and chick experiments will be used wherever possible to reduce the number of mice examined. Where we anticipate animals might suffer pain appropriate anaesthesia will be given pre-operatively. Animals are also monitored (e.g. post-operatively) to assess the need for (further) pain relief, or antibiotic treatment where infection may

	be an issue. Each experimental has given endpoints at which time mice are killed, or the animal moved to another experiment which has such an end point. Where animals are suffering they will be killed humanely. As the creation of new mutations might produce unexpected results, we will ask advice from the home office inspector whenever this occurs. In addition new techniques reduce the severity of interventions, For instance, it is now possible to transfer embryos to recipient adult females by non-surgical techniques and we will make use of this advance.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The genetic basis of disorders of the brain and pituitary gland</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain, pituitary, congenital disorders</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project uses normal and genetically modified mice to understand the normal development of the brain and pituitary gland as well as the pathological conditions associated with these organs (hypopituitarism and tumours). This is important to elucidate the mechanisms that control normal development and to learn how defective gene function lead to human disease. In particular, this project aims to understand conditions affecting children.

Hypopituitarism and brain tumours have a significant prevalence in humans and are associated with severe symptoms that negatively affect the quality of life of the patients and often can lead to death. We aim to understand these conditions with the goal of improving patient management and care.</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>Brain defects, blindness, hypopituitarism and brain tumours are important conditions in humans. The proposed research will help understand the causes and development of the disease. This will lead to the development of novel diagnostic tools and improved treatment. Specific benefits may include:
• Increased understanding of human prenatal development. Because we are studying a mammalian system, i.e. mice, there is a high likelihood that the principles emerging from our research will be also applicable to the human situation. Moreover, our research can be directly transferred into studies of human embryonic development through use of the Human</td></tr>
</table>

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

	Developmental Biology Resource, which provides human foetal material for studies of gene expression in relation to congenital disease. • **Novel methods for genetic diagnosis and genetic counselling.** Families carrying mutations in genes that are causative or predispose to disease are informed by my clinician collaborators. This genetic counselling is important for the families and patients as they have a better understanding of the symptoms and can balance the risks of having more pregnancies. In addition, genetic diagnosis can also be performed to assess the presence of the mutation in the developing foetus. • **Novel treatments for childhood craniopharyngioma.** Our research aims to test specific inhibitors in mouse models for these devastating childhood tumours. Some of the chosen inhibitors are already in phase 3 clinical trials in humans and are being used for treatment of other human conditions, including brain tumours. Therefore, the data obtained from the pre-clinical studies will be translated swiftly into humans, and we have the contacts and resources to do this, through our links with neuro-oncologists at the Children Hospital.
What species and approximate numbers of animals do you expect to use over what period of time?	Species: Mice Numbers: Between adult mice and foetal forms, 15,000.
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?	This research project makes extensive use of genetically modified mouse strains. In most studies, living mice are only mildly affected, so post-natal mice in general will not suffer from abnormalities. Our analysis of birth defects will be confined in the great majority of cases to embryos, which are killed at a developmental stage before the onset of pain sensation. In the small number of experiments involving living mice (moderate severity protocols), appropriate protocols for anaesthesia and post-operative pain-control will be used to avoid suffering. We do not intend to pursue <u>any</u> substantial severity protocols. The greatest potential source of suffering is the studies on mice that are predisposed to generate tumours. However, animal suffering is minimised by limiting tumour size to 15mm maximal diameter. Mouse models for human craniopharyngioma tolerate well the tumours for the first 3-4 months with no signs of pain, and they are fertile. Mice are

	culled humanely at the first sign of health deterioration (eg, severe weight loss or abnormal behaviour).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research into birth defects concerns the mechanisms by which the embryo/foetus develops its specific shape and associated function. Hence, an understanding of normal and abnormal development that leads to birth defects, requires analysis of whole animal embryos. Tumours also interact with the host and these interactions are difficult to mimic in vitro. Genetic manipulation studies on humans are neither ethical nor practically possible. The use of mice is required, but measures are put in place to reduce numbers and potential suffering of mice. No other animals are used in this licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Methods for minimising numbers of animals in this project include: • ***In vitro whole embryo culture***. Embryos are removed from a pregnant female and allocated to experimental and control groups. Hence, we do not need to allocate different dams to different groups (reducing numbers of mice used), and there is less need to manipulate pregnant females. • ***Careful experimental design***. For experiments with **qualitative** outcome (e.g. pattern of gene expression), 15-20 embryos (from 3-4 pregnant females) are examined per group to ensure reproducibility. Where an outcome is **quantitative** (e.g. phenotype frequency), professional statistical advice is sought where needed. This is is particularly important for the design of the pre-clinical trials. Our Institution's statistical service provides experimental statistical advice for both human and animal clinical studies
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.	Genetically altered mice offer the most incisive approach to the analysis of birth defects mechanisms and oncogenesis because: • Mouse genetics is understood almost as well as in humans, offering the best possible means for genetic analysis in a mammal. • Birth defects and tumours in genetically-predisposed mice closely resemble those in humans, providing excellent models for analysis. • Technologies to generate genetically modified mice offer a sophisticated route towards studying the effects of genes in

	particular tissues, or at specific stages. • Mice are broadly considered less sentient than larger animals such as primates, cats and dogs, which probably decreases perception of pain.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms of NKT cell activation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>lymphocyte, infection, inflammation, imaging</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)[17]</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[18]</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 examine the function of a specific population of immune cells, so called NKT cells, during infectious and inflammatory diseases.

Immune cells constantly patrol the body in search for invading pathogens such as virus or bacteria. When immune cells encounter these infectious agents, they will respond by using different means to fight the infection. This battle against invading pathogens will require a highly coordinated cooperation among different types of immune cells and the productivity of these cellular communications can dictate the successful or unsuccessful outcome of immune responses. However, although our immune cells are central for protection, when their functions are deregulated they can cause inflammatory and autoimmune diseases such as arthritis, inflammatory bowel disease, psoriasis or diabetes.

Among the different populations of immune cells, NKT cells are known to be involved in the immune responses against different types of virus, bacteria and inflammatory processes. However, the mechanisms that determine their function in vivo are incompletely understood consequently limiting their use in clinical therapies.</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>We fully anticipate that conclusions directly drawn from these studies will prove important not only for dissecting NKT cell functions but to identify the cellular interactions involved in the initiation of a variety of diseases. Unravelling the mechanisms of NKT cell activation is not only fundamental for</td></tr>
</table>

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

	understanding NKT cell biology but such information may provide an additional clue as how to effectively regulate immune responses associated with NKT-cell activation and thus improve the clinical usefulness of NKT-cell agonists. Our studies can identify interactions that regulate activation of different cell types at specific times in specific tissues, and thereby promote the development of highly localized, targeted therapies. This line of research will be useful in terms of the design of vaccinations for numerous infectious and potentially cancerous diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Genetically altered mouse strains which will be used in this study include: (a) knock out mice that lack particular effector molecules or cell types of the immune system; (b) transgenic mice that offer the opportunity to study particular aspect of responses that otherwise could not be monitored due to the low frequency of antigen-specific cells (e.g. T cell receptor-transgenic mice); and (c) reporter mice that make it possible to study particular cellular processes overcoming the suboptimal detection threshold of alternative assays.
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?	Our protocols have been designed to minimise suffering to the mice. We have avoided techniques that might cause unnecessary discomfort to provide the information required. Animals will be anaesthetized for procedures expected to cause temporary pain. Animals will be carefully monitored during and after experiments. No serious adverse effects are anticipated and no animals will be allowed to become seriously unwell as a result of any procedure or experimental induction of inflammation. Animals will be humanely killed before they suffer significant discomfort.
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 complex network of different cell types that interact at specific locations such as the inflamed tissue and in secondary lymphoid organs. These cellular interactions can only be explored to a very limited extent in vitro, since the tissue/organ environment greatly influences their outcome. Therefore, the induction of immune responses with its spatial and temporal requirements can only be explored in its entirety in an intact organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be minimised by careful experimental design (power calculations); limitation of other variables (e.g. use of inbred strains in specific pathogen-free conditions); optimised methods for the analysis of small amounts of material; longitudinal monitoring; and by the

	employment of routinely-used protocols that work reproducibly
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 is the ideal organism for these investigations for a variety of reasons: (a) the parallels between mouse and human immune system are well understood; (b) mouse models of immune diseases are well established and widely used; (c) specific reagents are widely available. All mouse models used will be assessed such that we use the minimum severity in terms of infection or inflammation burden. Commonly, these protocols are already well established and they use challenges of minimal pathophysiologic stress. These models are generally well tolerated in most mouse strains but in all cases clear end points will be set so that any mouse displaying more than moderate discomfort will be killed.