Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 7

Project Titles and key words

- Determination of acute chemical toxicity and its mitigation

  Acute, Toxicity, Countermeasures

- Pathophysiology of corneal angiogenesis and corneal transplantation

  Corneal transplantation, corneal vascular disease, anti-angiogenic therapeutics

- In vivo analysis of 2-oxogluterate and iron-dependent dioxygenases

  Oxoglutarate; oxygen; iron; enzyme; mouse

- Assessing and alleviating pain and distress

  Pain Distress Analgesia

- Schizophrenia: mechanisms and potential therapies

  Schizophrenia, DISC1, GABAergic interneurons

- Immunology of viral and allergic airway disease

  Respiratory viruses, bronchiolitis, asthma, immunology

- Safety Testing Using Dogs and Minipigs

  Regulatory Safety Assessment Dogs Minipigs

- Neural development, plasticity and repair

  transgenic mice, CNS, myelin, neural stem cells

- Craniofacial development and associated birth defects

  Cleft palate, submucous cleft palate, TBX22, craniofacial development

- Genetic control of fertility and development

  Infertility, genetics, epigenetics, chromosomes, development

Determination of acute chemical toxicity and its mitigation
Acute, Toxicity, Countermeasures

- Summarise your project (1-2 sentences)

This licence allows determination of the toxic properties of chemicals that might be deployed against either UK Armed Forces or the civilian population as an act of war or terrorism. The information will be used to develop protection, determine and develop appropriate detection levels, aid in computer modelling and test medical countermeasures against the threats.

- 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.

  Toxicities determined are used to develop protection, detection, computer modelling and medical countermeasures against the threats. The license comprises a series of standard toxicity test that may be used together or individually to determine the toxicity of threat compounds.

- Outline the general project plan.

  Objectives
  1 The assessment of the toxic hazard arising from industrial and agricultural chemicals and known or potential chemical warfare agents, their precursors, and compounds arising from structural modification of these molecules.
  2 A variety of toxicological techniques is available under the licence and the method(s) selected will be based upon the level of knowledge required about any particular compound.

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

  Most animals undergoing this acute toxicity testing are used to find the lethal dose of the test material. As such some of them will where necessary be allowed to die as a result of chemical poisoning. No attempt will be made to interfere with the progression of the toxic effects since it has been shown by experience that some animals may proceed almost to termination and then recover to normality

  The exceptions to this rule are:

  1. If an animal is found to be suffering severe pain or distress which will not reverse, it will be humanely killed by a Schedule 1 method.

  2. Should an animal be unable to eat or drink, or suffers some toxic effect such that it loses more than 25% of its body weight (measured at the time of administration of the test substance), it will be humanely killed by a Schedule 1 method. During extended observation periods of longer than 24 hours the animals will be weighed and, if implanted, have their body temperatures recorded at least daily, commencing at least three days before exposure.

Most of the animals undergoing Protocol 2 skin sensitivity will show some degree of skin irritation. Depending on the dose applied, these signs could include reddening of the skin

(erythema), swelling (oedema), and skin loss (desquamation) with eschar formation.

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

  This licence provides a capability for the investigation of potentially toxic chemicals that may be used against UK Armed Forces or UK civilians in either war fighting or acts of terrorism. The information determined from these studies will allow the impact of the use of such compounds on military or civilian populations to be assessed and will inform future protection strategies.

- 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.

  Animal numbers have been set at rats 6150, mice 4750, guinea pigs 7050 and rabbits 150 over the 5 year term of the licence. These are maximum values in order to provide a capability. Previous experience indicates animal usage is likely to be far below this maximum.

  The quality of the toxicity data dictates the type of acute toxicity protocol that is performed. Care will be taken to ensure that the most appropriate methodology be used to give the answer required. This will result not only in the reduction of the number of animals required but also in the overall suffering.

- 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.

Given the current technology available, accurate assessment of toxicological properties requires the use of animal models. Without intact animals it is not possible to take into account distribution of the chemical in the body, its interaction with other body systems or its breakdown to less toxic products. Indeed modification of molecules to more toxic material in the body may also occur. While indicators of the toxicity of some compound types can be assessed using *in vitro* models it is not yet considered possible to accurately predict the toxicity of compounds in intact animals due to the absence of metabolic elements and other potential target organs and systems.

While there have been developments in testing for skin irritancy to progress towards the use of *in vitro* rather than *in vivo* models these are not sufficiently mature or refined to replace the *in vivo* requirements for legislative testing. Developments in the field of skin irritancy will be monitored throughout the life of the licence and modifications put into place with regard to this protocol should developments reach sufficient maturity.

To restrict the number of animals used the various tests are designed to minimise the animal numbers while still providing the level of detail and accuracy required.

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

  Protocols will be carried out by experience highly trained staff to minimise the stress from their handling and compound administration and subsequent care. Most animals

undergoing this procedure are used to find the lethal dose of the test material. As such approximately 50 % of them where necessary will be allowed to die as a result of chemical poisoning. No attempt will be made to interfere with the progression of the toxic effects unless an animal is found to be suffering severe pain or distress, unable to eat or drink or lose more than 25 % of its starting body weight at the time of dosing. In these cases the animal will be immediately and humanely killed.

Pathophysiology of corneal angiogenesis and corneal transplantation
Corneal transplantation, corneal vascular disease, anti-angiogenic therapeutics

- Summarise your project (1-2 sentences)

  This project will select potential therapeutic agents from cell and tissue culture studies to test whether they can inhibit or promote new corneal vessel growth in rodents. This will ultimately benefit patients with blinding diseases caused by the formation of new corneal vessels following inflammation or infection or rejected corneal grafts.

- 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 development of new corneal vessels often occurs post trauma, inflammation and infection. They are not only blinding but also dramatically increase the chance of corneal graft rejection. Currently there is lack of clinical protocols directly addressed for treating corneal vessels. In this project, we aim to enhance the understanding of corneal avascularity as well as develop new therapeutic agents for treating corneal vascular diseases and promoting corneal graft survival.

- Outline the general project plan.

  The homeostatic regulation for maintenance of the avascularity and transparency of cornea will be investigated on a variety of angiogenic (new vessel forming) related proteins using established corneal angiogenesis rodent models including suture or trauma induced corneal vessels, as well as corneal transplantation. We will also use these models to test the anti-angiogenic effects of identified therapeutic agents, initially tested in cell and tissue cultures. These agents will then be evaluated as to whether they will increase corneal transplant survival in pre-vascularised recipients.

- 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.

  In this project, a range of methods will be used to induce corneal vessels i.e. corneal suture placement, “high risk” corneal transplantation (involving pre-placement of corneal sutures, followed by a corneal transplant), epithelial debridement or topical application of chemical agents, e.g. sodium hydroxide. The area of a burn to the cornea will be restricted by applying an appropriate absorbent substance (e.g. filter paper) soaked in the agent to the central cornea for a fixed time period. Mice will receive immuno-modulating or anti-angiogenic substances systemically or locally before or after induction of angiogenesis (as described above) or corneal transplantation.

  From our past experiences, the protocols proposed in this project do not have any adverse systemic effects on rodents in terms of normal health and behaviour. Potential local side effects such pain and infection can be minimised by appropriate handling, aseptic techniques, the use where indicated of anaesthesia and analgesics and careful monitoring.

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

The outcome will potentially identify new therapeutic approach for treatment of corneal vascular diseases and prevention of corneal graft rejection. Firstly via pathways of engaging natural pathways and secondly by testing blockers or agonists as preclinical proof of concept. The project will ultimately promote visual outcome and life quality of patients.

- 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 will be used in this project with the estimate number of 5000 over 5 years. The mice are used because immune responses in rodents are well understood and genetically altered mice will enable us to define pathways and mechanisms more exquisitely, generating more rapid progress of work whilst minimising animal usage.

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

To reduce animal use to a minimum, emphasis of this project is placed on developing *in vitro* assays to elucidate in vivo observations and test our hypotheses. Although non-animal models such as cell culture can minimise animal use, they cannot represent the complexity of the cellular processes that govern inflammatory responses and blood and lymphatic vessel growth. Therefore animal studies are currently essential to achieve our objectives. However, non-animal studies will be performed alongside and, wherever possible, in preference to animal experiments to minimise animal use. For example, cell culture will be used to examine one important vascular promoting protein called VEGF in leukocytes and agents to be used in animal will be tested and elected through measuring aortic vessel outgrowth.

Our previous experience of corneal vascularisation responses suggest that blood and lymphatic vessel growth will be fairly uniform within each model and therefore 6-8 animals per group are considered sufficient for desired power to achieve statistically reliable data. For disease experiments, the null hypothesis that there is no difference in angiogenic disease incidence, mean maximum severity, type of immune cells involved or total disease burden between groups (i.e. no effect), will be tested taking p value < 0.05 as significant. Animal numbers are assessed such that the experiments are powered to a probability of 80% that a statistically significant result will be observed. Experimental careful consideration is given to the minimal number of animals required to provide a statistically significant result, and this will vary between experiments. A stepped approach in which an initial small experiment allows us to estimate the necessary size of a second experiment that has the power to give an 80% chance of demonstrating significance.

Treatments to induce neovessels have been selected based on previous experience of minimal trauma necessary to induce the required vessel growth. Ocular pain will be minimised by frequent monitoring of animals, if necessary under sedation or anaesthesia and by use of topical as well as systemic anaesthesia during surgery. The

genetic modifications of mouse strains that may be used do not elicit any observable effects on well being under laboratory conditions.

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

The proposed experimental designs and statistical analysis of results have been discussed but further advice is available from colleagues within the faculty, thus ensuring adequate power to achieve significant results whilst minimising animal use. In parallel much preparatory work of interrogating underlying mechanisms can be carried out using macrophage cell lines or primary cell lines derived from rodent species. Again with such testing of macrophage behaviour, results can help us to refine and reduce animal requirements further. Mice are used, as we and others have established excellent clinico-pathological modelling that represents well changes observed in man. The use of mice allows interrogation via gene modification, to more exquisitely provide an ability to define cellular and signalling pathways. Each model generates corneal vessels that do not result in significant morbidity or weight loss or significant behavioural change. Procedures are performed under appropriate anaesthesia to provide a non-suffering and adequate analgesia.

In vivo analysis of 2-oxogluterate and iron-dependent dioxygenases
Oxoglutarate; oxygen; iron; enzyme; mouse

- Summarise your project (1-2 sentences)

  We are using carefully designed experiments in animals to understand the roles of catalysts from a family of enzymes known as the 2-oxoglutarate- and iron-dependent dioxygenases in mammals. These enzymes have been shown in cellular and biochemical experiments to catalyse reactions of fundamental importance in biology and are thus thought both to be involved in affecting disease susceptibility and to be potential drug targets, of use in treating diseases in humans and animals.

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

  We will learn about the function of individual enzymes from this family in mammalian physiology and establish their role in contributing to disease susceptibility. We will then study what the consequences are of altering enzyme activity by both physiological and pharmacological means in both normal health and disease models. Finally, as a step towards disease therapy we will investigate the preliminary efficacy and potential side effects of drugs directed at inhibiting members of this class of enzymes.

- Outline the general project plan

  For each enzyme under study we will first establish where and when during the life of animals the enzyme is expressed. We will then use genetic techniques to specifically modulate the expression of the particular enzyme and assess the consequences for the normal physiology of the animal. We know that the activity of this class of enzymes is often affected by the availability of iron or oxygen so we will investigate the effects of manipulating these parameters in animals. In parallel we will investigate the effects of drugs designed to target these enzymes, which generally inhibit their activity. Depending on the results seen we may then combine manipulation of the enzymes expression or activity with manoeuvres that normally stimulate a disease process to see whether altering the function of the enzyme diminishes or exacerbates the disease process (within humane limits). When testing these drug prototypes we will watch out for any deleterious side effects and if these are observed use this information to guide the design of future agents.

- 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.

  Animals will be bred in which the level of expression of a particular gene is either normal or altered by genetic means (e.g. reduced or completely ablated). If the gene has a function critical in animal development it is likely that no viable offspring will be born, but analysis of the events in pregnancy leading to the deaths will be important in establishing enzyme function. In mice the death of the foetus in utero is generally associated with reabsorption, which has little, if any, effect on the health of the mother. If viable animals are born we will assess the effects of alteration of enzyme activity on important medical process including the growth of tumours, the response to altered oxygen availability, manipulation of metabolism, the function of the heart, and the ability to form new blood vessels and heal wounds. In many cases little effect will be

seen, but it is possible that deleterious effects will occur. These include increased growth of tumour cells, reduced tolerance of low oxygen levels, increased obesity, a tendency to develop diabetes or perhaps low blood sugar levels, problems with heart development or function or reduced collateral vessel development and wound healing.
If effects are seen in preliminary experiments we will generally try, where possible, to manipulate enzyme activity in subsequent experiments in the direction which lessens the severity of the animals response to the stimulus rather than increases it.
Finally we will test drugs designed to influence enzyme activity. These may have unexpected side effects. We will always test these first in biochemical and cellular assays and only proceed to animal testing of compounds that appear non-toxic and start our animal experiments by using low doses first, and only increase the dose when we know that side effects are minimal or can be ameliorated.

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

  Several members of this family are already being targeted for treatment of diseases including heart attack and cancer. We will increase overall knowledge about the functions of enzymes in this family and hope to extend the range of therapeutic targets. Given that drugs sometimes cross react within a family of related enzymes it is also important that we establish which enzymes of this family must not be inhibited by drug therapy, so that this is taken into account in future drug design.

- 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.

  We anticipate using up to 3000 mice for these experiments and perhaps 500 rats. Mice are chosen for their study because we know a lot about their physiology and disease susceptibility, there is a well trodden pathway to using genetic techniques to manipulate gene expression in this species and good knowledge about techniques to minimise suffering. Rats will be used either when their larger size makes something technically feasible that cannot be performed successfully in a mouse, or to confirm results in a second species.

  Experiments will be designed with statistical advice to maximise the likelihood of producing meaningful results whilst minimising the numbers of animals used to reach those conclusions.

- 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.

  Our experiments need to use animals because we are investigating functions in which the overall integrated response of the whole organism needs to be assessed. However, our choice of enzyme to study, animal model to use and prototype drug to test will all be based on existing knowledge from biochemical or cell culture work or parallel experiments performed in vitro.

  In addition to our in vitro approaches we have also previously used lower organisms (yeast, trichoplax, nematode worms and fruit flies) to investigate the function of some enzymes of this family, and where appropriate we will continue these ‘replacement‘ activities.

We always try to perform our experiments in the most 'refined' ways possible, learning better ways of doing things from the scientific literature, the experiences of our colleagues and our own experimental results. We often work collaboratively with scientists experienced in particular techniques to ensure best practice.

We pay particular attention to careful experimental design to maximise the likelihood of producing meaningful results whilst minimising the numbers of animals used to reach those conclusions.

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

  Experiments performed to date by this group under previous licences, and those reported in the literature, indicate that the vast majority of animals subjected to the types of protocols described in this licence have a very mild overall experience and suffer very few, if any, adverse effects. Nonetheless, it is not only important from an ethical standpoint to minimise any suffering experienced by animals involved in these experiments but it is also important from a scientific standpoint that the animals are in the most normal condition possible at the time of experiments and thus able to show a full range of responses. We therefore have in place a number of general procedures to minimise any suffering, starting from maintaining the animals in a high quality environment, including regular inspection of the animals, very clear action plans if animals are unwell and seeking the advice of veterinary surgeons if symptoms persist. Any animals judged to be suffering too much will be humanely killed.
  Many of the animals we use are genetically modified. In many cases we already know that the genetic modification is not associated with any adverse phenotypes, but where new genetic modifications are being assessed extra monitoring is put in place to ensure any adverse effects are identified and mitigated at the earliest possible time.
  Some of our experiments involve surgery; appropriate anaesthesia and analgesia are provided, care is taken to ensure the animals do not become cold, aseptic technique is used throughout the procedure and animals are monitored closely post-operatively to ensure good recovery. Wounds are carefully and regularly inspected; in the unlikely event of breakdown or infection they may be repaired once or treated once but if this is not appropriate the animal will be killed. Other experiments may result in the induction of tumours; if these arise animals will be closely monitored and if their health is impaired as a result of either tumour size or the consequences of tumour biology then we have strict endpoints to ensure early intervention. In experiments involving changes in the gases that animals breathe exposures are either very brief, allowing simple assessment of the animals' responses, or where the exposure is longer term our protocols are designed to allow appropriate acclimatisation. Again animals are closely monitored during the induction of hypoxia and the gas composition can be rapidly made more like room air if adverse effects are observed. Metabolic experiments may include alterations to the animals' diet but again the changes made will be the minimum compatible with the scientific aims of the experiments and where weight gain or loss occurs we have strict endpoints to limit any suffering. In some of these experiments animals will be fasted, but this will mainly be during the time the animals would normally be asleep and thus not eating anyway. Experiments to look at heart function involve state of the art equipment designed to minimise any suffering. Immunisation schedules have been designed to provoke a good immune response

whilst minimising inflammation; again the animals are very carefully monitored and killed if suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Assessing and alleviating pain and distress</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pain Distress Analgesia</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)[1]</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[2]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of the project is to develop better means of assessing, preventing and alleviating pain and distress in animals. A major goal is to assess whether animals have similar emotional components to pain and distress that humans experience.

Because we have a relatively limited ability to assess pain and distress in animals, we are often unable to prevent or alleviate these conditions. We are also currently unable implement effectively the current UK and EU legislation that requires research workers both to limit the effects of procedures on animals, and to assess the cumulative suffering that may be caused when animals undergo a series of procedures (for example multiple injections of substances, combined with surgical or other procedures). Finally, without methods of assessing how much pain and distress animals experience, we cannot evaluate the value of particular “refinements” of research procedures.

This project will continue the development, evaluation and application of methods for the assessment and alleviation of pain and distress in animals, using a range of methods including analysis of their behaviour, studying their facial expressions and looking for changes in their responses to mild painful and other stimuli.

When assessing the value of different methods of pain alleviation, we will investigate the</td></tr>
</table>

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

	significance of any undesirable side-effects associated with analgesic or other therapy, and develop means of preventing or ameliorating these effects. This will include assessment of whether use of analgesics could interfere with the outcomes of particular research projects – for example those studying cancer treatments. We will also continue our work to investigate the nature of pain and distress in animals by using techniques that give us an insight into how animals may be feeling, and whether they are aware of any pain and distress that they experience
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)?	Developing easily applied, objective methods of assessing pain and distress will allow us to evaluate the degree of pain and distress associated with particular procedures, and the value of refinements (methods of reducing pain or distress) of those procedures. Developing better methods of treating pain, and showing when and how these methods can be used without affecting the outcome of different research projects will greatly benefit the welfare of large numbers of laboratory animals. Developing methods of assessing emotional states in animals will let us apply humane end-points to studies based on objective and animal-centred criteria (in other words, based on how the animal feels). Improving our understanding of the nature of pain and distress in animals will greatly advance our ability to improve the welfare of animal in many different circumstances, and may also contribute to the use of more appropriate animal models for the development of therapies in man. It will also assist in developing assessments of the “severity” of procedures, and the cumulative effects of different procedures carried out in the same animal, something that is now a legal requirement throughout Europe.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 975 rats, 1125 mice, 96 rats and 72 guinea pigs during the 5 years of this project.
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	Some studies will use animals that are undergoing potentially painful procedures on other, unrelated project. However, when this done, we need to know the effects of the anaesthetics and analgesics used. To do this we need to give animals the same anaesthetic and

end?	analgesic as animals used in other, unrelated projects, so that we can determine the effects of these drugs on their normal behaviour. This will allow use to identify changes in behaviour in animals undergoing surgery or other painful procedures that are due to pain, and not to the effects of anaesthesia or analgesia. The effects of anaesthesia and analgesia should be very minor and should not cause clinically significant side effects. Some animals will receive analgesics and the effects of the drug will be assessed by applying a brief, painful stimulus. The animal can move away to avoid the stimulus. Some of these animals may be blood sampled to assess the levels of analgesic in the blood. This will cause minor discomfort. These studies help us to determine whether a particular analgesic could be useful for treating pain in animals, and in particular will let use assess how long the drug is likely to last. Analgesics that may be useful as improved treatments will be assessed in animals undergoing surgery and other painful procedures. For some studies it is not possible to use animals being used on other procedures, so some animals will undergo surgery (for example opening then closing the abdomen) or other painful procedures (for example production of inflammation of a joint) solely for the purpose of this project and not all will receive pain relief. It is planned to use as few animals as possible in these particular studies. Some rats undergoing surgery will also have intravenous catheters implanted, so we can see if they will give themselves pain relieving or other drugs. The degree of post-operative pain will be assessed using our existing scoring systems, to try to limit the degree of pain that these animals may experience. Some animals will develop cancer or other diseases, either specifically for study on this project, or as part of other, unrelated, research projects. The diseases may cause some pain and distress, but currently used humane endpoints, and the results of detailed behavioural assessments will be applied to limit this. Some of these animals will receive pain relief, and this should have positive effects. We will carry out a range of behavioural tests, and in some of these animals may be given medicines that will cause anxiety, and may also be given

	stimuli they find unpleasant such as a puff of air, or a very mild footshock. They will also be given medicines such as anti-depressants and anti-anxiety agents, and rewards such as preferred treats. In order to study whether the way we keep animals can change how much pain and distress they experience when used in research, some animals will have mild stress induced by being housed in less comfortable environment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because we are trying to develop pain and distress assessment methods that can be used to prevent and alleviate pain and distress more effectively in animals in research, we need to conduct research on these animals. Some of the animals we study as part of this project will be being used as a necessary part of other, unrelated projects, but this is not always possible. We have considered the need to use animals specifically to develop better methods of pain and distress management carefully. We believe that since so many animals currently receive inadequate or inappropriate pain relief, and that in some circumstance (eg cancer research) we simply do not know if the animals experience pain or distress, that this limited use of animals is justified as the results of this project can benefit very large number of animals (both in research and in veterinary clinical practice). To make sure the results are publicised as widely as possible (and so benefit as many animals as possible) we will continue not only to publish results in scientific journals but also to run workshops and develop web-based training using video-material to teach people how to recognise pain and distress in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of animals used on this project by, whenever possible, studying animals that are used in other, unrelated projects. When we use animals specifically for this project, we will design our studies carefully so that the minimum number are used to obtain the information we need. We will also use animals of different strains (breeds) and of both sexes, so that our results can be applied as widely as possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	We will use mainly rats and mice, but some rabbits and guinea pigs in this project. We have chosen to use these particular species as they are the most widely used mammals in research, and we currently have a very limited ability to assess pain in these

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	species. Many of the methods we use cause only mild effects on the animals, but in other studies, some animals will experience pain or distress. To limit the degree of pain or distress that may be caused, all of the animals used will be regularly monitored, at a minimum of once a day. During some studies, the animals will be video-taped at regular intervals (once or twice a day) and during this period they will be monitored closely. During anaesthesia and surgery, animals will be monitored continuously. They will also be examined every few minutes until they recover from anaesthesia, and frequently (typically every 1h for 6-8h) until they are considered unlikely to need additional pain relief. To limit the pain and discomfort that could occur, all the animals that might experience pain will be examined regularly by a veterinarian, who will not know what treatment they have received. If they think the animal is experiencing more than mild/moderate pain, then the animal will given additional pain relief.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Schizophrenia: mechanisms and potential therapies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Schizophrenia, DISC1, GABAergic interneurons</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></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[4]</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Schizophrenia is a complex, highly heterogeneous and debilitating brain disorder with a lifetime risk of around 1%. It is among the top 10 causes of human disability worldwide. Although heritability is estimated between 60-80%, the genetic architecture and the molecular causes of schizophrenia remain poorly understood. Current treatments are essentially palliative and do not alter overall prognosis.</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>Up to date, only very few susceptibility genes have been implicated definitively in schizophrenia among which the gene DISC1 stands as one of the best described candidate. Previously, we have created an unique transgenic Disc1 mouse line to mimic the genetics of Scottish Schizophrenia family. We will address how Disc1 regulates cell signalling pathways and cell-cell communication by using both in vitro and in vivo approaches. This project will allow us to understand what is different in the brains of people with mental disorders and start to work out ways to make them work more synergically and efficiently.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We expect to use around 3000 mice over a period of 5 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>This project involves mainly tissue sampling under terminal anaesthesia and the use of standardized protocols. In addition behavioural tests and a small amount of work involving inducing minute changes</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?	within the brains of embryos whilst in the uterus will be carried out. The behaviour tests do not have any negative impact on the welfare of the animals, while the work involving surgery may have some mild or moderate adverse effects which will be controlled by the appropriate use of anaesthetics, analgesics; aseptic techniques and other peri operative care measures. The full time veterinary surgeon will be on site to advise on anaesthesia and other aspects of animal health and welfare. The animals will be killed at the end of the studies and tissues analysed
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	More than 50% of our research will be carried out in the laboratory by using in-vitro systems to complement the animal work. However in-vitro assays cannot adequately model the complete array of molecular, cellular, physiological and behavioural interactions including the expressing patterns of specific receptors, subsets of neurons, neurite arborization and neuropathology etc, which are essential to fully understand how genetic modifications result in normal or abnormal processes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of multiple samples for single animals will allow us to reduce the total number of animals used. We will plan the mouse breeding meticulously and regularly check the breeding colonies, in order to meet the experimental requirement with miminal mouse numbers. We will conduct multiple staining/hybridization with the sections which could significantly reduce the number of used animals. We will consult a statistician as required and will continue to obtain meaningful data with minimum number of mice in each experiment. Our collaborations with another university and their ongoing supply of Disc1tr mice will reduce the number of mice by avoiding the duplication of breeding colonies. If there is no foreseeable funding to continue to work for long, or if there are no major research experiments planned on any particular mouse strain for long, sperms, fertilised eggs and/or embryos may be cryo-preserved.
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 species for our projects. Firstly, as a mammal, the information we obtain from the research will be closely relevant to human conditions. Secondly, it has small body size and relatively short life cycle, which enables us to obtain maximal knowledge within minimal duration. Thirdly, extensive knowledge of mouse genetics and the availability of the entire mouse genomic sequence enable us to design constructs accurately and quickly. Fourthly, a comprehensive system of

	technologies has enabled us to change mouse genome makeup and assess the resulting phenotypic changes. Peri-operative and anaesthesia care measures as recommended by the Named Veterinary Surgeon including analgesia, heat loss prevention, aseptic measurements and the prevention/control of bleeding, will be followed. Anaesthetics will be administered and maintained by experienced personnel and will be suitable for the species and the duration of the procedures. The behavioural tests proposed are all standard tests and widely used in the current research community. We will use ear biopsy as the most frequently used method for sampling for genetic analysis when required.

Immunology of viral and allergic airway disease
Respiratory viruses, bronchiolitis, asthma, immunology

- Summarise your project (1-2 sentences)

Using mouse models of respiratory viral infections and allergic asthma, we will study the immune and inflammatory responses involved in virus induced bronchiolitis and asthma attacks. We will use this knowledge to modify these responses, in order to prevent, reduce, treat and shorten these conditions.

- 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.

Respiratory virus infections can cause a severe lung disease in infants called bronchiolitis, which can predispose to asthma, and they can trigger severe asthma attacks. There is no prevention or effective disease-modifying treatment available for bronchiolitis and most virus-induced asthma attacks. Excessive inflammation is thought to underlie both of these conditions. However, the immune mechanisms that lead to this inflammation and that underlie the increased asthma risk after viral bronchiolitis are not sufficiently understood to develop immune modifying treatments to prevent, reduce and/ or shorten excessive inflammation and consequent disease after respiratory viral infections.

- Outline the general project plan.

Mouse models of respiratory syncytial virus (RSV) bronchiolitis and of allergic asthma will be used individually and in combination to investigate immune mechanisms that lead to or prevent excessive inflammation and disease following viral infection in “normal” and “allergic” individuals. We will also investigate effects of immune modifying interventions, e.g. by depleting “harmful” immune cells with antibodies. To assess disease, lung function and body weight will be monitored. In some cases immune cells will be studied in live mice by labelling them with light emitting markers that can be used for live-imaging using an ultra-sensitive camera. In most instances immune cells, soluble immune substances and inflammation will be studied after mice have been killed assessing organ structure and inflammation by histology, counting of individual immune cell populations, and detecting immune substances and activation of their genes.

- 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.

Mice will be infected with RSV by applying droplets to the nose or the trachea under light anaesthesia. RSV infection usually does not result in disease, but in some cases can cause transient weight loss from which mice recover after 2-3 days.
In the asthma model, mice will be sensitised to an allergen usually by intraperitoneal injection followed by allergen challenges to the airways by aerosol inhalation or application of droplets to the nose or the trachea under light anaesthesia. Allergen sensitisation and challenges usually do not cause disease.

Changes in lung function (without clinical disease) can be detected after RSV infection and in the asthma model. Lung function will assessed in a chamber, in which the mouse can freely move, using minimal changes in chamber air pressure caused by breathing.

Agents provoking short lasting deterioration of lung function will be aerosolised into the chamber, resulting in short lasting discomfort, as experienced by patients during similar lung function measurements. In some mice lung function will also be measured invasively under deep surgical anaesthesia without recovery.

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

This project will advance science by increasing our understanding of how immune cells and immune substances work to cause lung inflammation in viral infection and asthma. This will provide targets and ideas for future development of immune based therapy and prevention of viral bronchiolitis and virus-induced asthma attacks.

- 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.

We will use up to 20,000 mice over the 5 year project. Although the disease processes are not completely identical to those in man, mice offer the best available model system in which a variety of relevant immunological, genetic and molecular tools are available to study viral and allergic inflammatory lung disease.
All experiments are designed to use the minimum number of animals to give statistically significant results or to obtain sufficient numbers of immune cells for ex-vivo analysis. To that end all groups and controls of an experiment will be run in parallel, all organs of interest will be used simultaneously from each individual and animals will be identified individually. Where appropriate we will use statistical tests to calculate the number of animals we will require based on how variable we expect the results of our studies to be and how big a difference we are looking for between groups. Where such calculations are not possible, e.g. in experiments to generate primed immune cells or organs for histology, we will use our previous experience with similar experiment and published data to determine minimal numbers of mice required.

- 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 mouse models outlined above are an integral part of my research programme, which uses laboratory based experimentation and clinical studies in humans wherever possible. We will assess lung secretions from infants ventilated due to viral bronchiolitis for immune cells. In parallel, we will study effects of respiratory viral infections on human immune cells generated *in-vitro.*
However, there is currently no laboratory based system available that allows us to study the complexity of immune interactions, within and between different organs, and the lung function changes in inflammatory lung disease induced by respiratory viruses and allergen sensitisation. We therefore have to use animal models of disease.

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

All work will be carried out by experienced trained researchers or under close supervision. Wellbeing of mice will be regularly monitored. Following any procedure mice will be monitored continuously until they have fully recovered. Any painful procedure will only be performed under appropriate anaesthesia. If required supportive treatment will be

provided (e.g warming, oxygen application) and humane endpoint have been defined at which mice will be killed to avoid distress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Safety Testing Using Dogs and Minipigs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Regulatory Safety Assessment Dogs Minipigs</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Humans are exposed to xenobiotic materials as patients, consumers and workers. In order to allow sound regulatory decisions regarding safe human exposure levels to xenobiotics, it is essential to conduct a risk assessment by relating the intrinsic hazard profile of the material to the desired or likely exposure in man.
This project licence authorises the conduct of in-vivo studies in laboratory dogs and minipigs to evaluate the hazard profile of xenobiotics in terms of general toxicity, and toxicokinetics.</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 principal benefit of this project is the provision of safety data to facilitate sound regulatory decisions regarding human exposure to xenobiotics.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Over the 5 year life of this Project Licence, it is estimated that 5,200 dogs and 1,050 minipigs 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 level of severity? What will happen to the animals at the end?</td><td>The majority of animals on shorter term studies are expected to have mild adverse effects such as slight weight loss or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more significant adverse effects. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects.</td></tr>
</table>

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

	The majority of animals will be humanely killed at the end of a study; investigations may include sampling of various organs and tissues followed by microscopy to evaluate potential changes.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present there are no scientific and legally acceptable evaluations of systemic toxicity that will satisfy regulatory requirements other than use of animals, though validated *in vitro* tests for specific organs are used wherever possible. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies. Where available, sensitive analytical techniques (eg Dried Blood Spot analysis) may be used to reduce animal numbers. Wherever practicable, the combination of endpoints eg general toxicity, safety pharmacology, mutagenicity etc in studies is considered, to reduce overall animal usage.
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.	Species choice and use of specific animal models is determined by the need to generate regulatorily-acceptable data. Where a choice of species is possible, care is taken to select the most biologically appropriate species, and the species which most closely relates to man. Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Neural development, plasticity and repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">transgenic mice, CNS, myelin, neural stem cells</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3"></td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>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[8]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The brain is made up of neurons, which communicate with one another via electrical impulses, and glial cells, which support neuronal function in various ways. For example, glial cells known as oligodendrocytes (OLs) wrap around and insulate axons, the long projections of neurons that conduct nerve impulses. This lipid-rich insulation, known as myelin, can break down in demyelinating diseases (e.g. multiple sclerosis, MS) causing electrical communication to break down, with serious consequences. We will study the basic biology of OLs and myelin - how OLs develop, why they continue to be made into adulthood (e.g. are they involved in learning?), what causes OLs to degenerate during demyelinating disease and how we might stimulate myelin regeneration. We also study astrocytes and interneurons, attempting to define functional roles for these cells in the normal CNS.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Our work is basic and translational neuroscience that will add to our understanding of normal brain cells and what can go wrong if their functions are compromised. Our work on oligodendrocyte (OL) generation during healthy adulthood is directly relevant to multiple sclerosis (MS) because the spontaneous regeneration of OLs and myelin that occurs in the early stages of MS is likely to be a special case of normal adult myelination. Understanding what limits normal myelin generation will help explain why remyelination fails in chronic progressive MS. In addition, we are investigating the role of new myelin synthesis in motor skills learning. Progress on this front might lead to a</td></tr>
</table>

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

	deeper understanding of the role of OLs in learning and memory, and how myelin loss in old age might contribute to age-related cognitive decline. Understanding these things could suggest ways of improving human performance in healthy young adults as well as slowing later cognitive decline.
What species and approximate numbers of animals do you expect to use over what period of time?	Generating genetically altered (transgenic) mice is one of the key skills of our laboratory; apart from our own research we provide them to many labs worldwide for a great variety of studies, greatly increasing the scientific impact of our work. Because mice breed prolifically we will generate large numbers of mice during the Project (~10,000 mice, most of which will be culled soon after weaning at 2-3 weeks because they do not inherit the required genetic alterations). We will also use relatively small numbers of chick embryos (~100 over 5 years) for developmental studies.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of our genetic alterations are designed to label particular cell populations in the brain with fluorescent proteins, allowing us to identify and study them more easily, and have no noticeable effect on their general health or fertility. In some cases, we will interfere with the production of new glial cells or neurons in order to understand the function of those cells in the normal brain. Possible effects include demyelination, leading to defects in motor learning or, in more extreme cases, muscle weakness and tremor. If any mouse appears to be in distress it will be immediately killed by a humane method, or injected with a lethal dose of anaesthetic prior to fixing the animals for histology and microscopical analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We study the function of the mammalian central nervous system (brain + spinal cord + retina) and its behavioural outputs; this is a complex organ system that can only be investigated meaningfully in the intact, living state in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will design our mouse breeding strategies carefully to ensure that we can obtain the required number of genetically altered animals with the least number of unmodified littermates.
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 only mammals that can be genetically modified routinely, and for which brain anatomy, physiology and function is well documented. Most of our manipulations are designed to mark specific cell populations with a fluorescent label, allowing easy identification of cells without harming the animal. If a procedure causes visible distress the animal will be killed humanely.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Craniofacial development and associated birth defects</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cleft palate, submucous cleft palate, TBX22, craniofacial development</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></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[10]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Orofacial clefts such as cleft lip and or cleft palate are among the most common birth defects worldwide and have a serious impact on quality of life. This project focuses specifically on submucous cleft palate, which causes many of the same problems as an open cleft palate but where surgical treatment is much less effective. We will use mouse models with submucous cleft palate to better understand the relationship between the developing palate muscle and bone formation.</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 intend to investigate the potential to develop novel therapies that could improve palate muscle function and complement what is currently achievable with surgery alone.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will use wildtype and mutant strains of mice that carry mutations that can result in cleft palate. We expect to use less than 500 over the course of the study.</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>We will maintain mouse colonies that are not adversely affected by the mutations they carry. Colonies are maintained in animals that carry mutations but do not manifest the deleterious phenotypes. Any animal whose general health is determined to be inappropriate will be killed by a schedule 1.

To test the efficacy of new substances in treating</td></tr>
</table>

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

	the birth defects under investigation, we will use the technique of embryo culture. Embryos will be cultured ex vivo to investigate palate and midfacial development. In this way, embryos can be maintained and treated after schedule 1 killing.
Application of the 3Rs	
1. Replacement **State why you need to use animals and why you cannot use non-animal alternatives**	Research into orofacial birth defects requires analysis of whole animal embryos to model complex spatio-temporal events. In vitro systems and human tissues will be used where possible but are not tractable to experimental manipulation whilst replicating embryonic development.
2. Reduction **Explain how you will assure the use of minimum numbers of animals**	The outcome of many experiments is qualitative and we will use the minimum number of animals to ensure reproducibility of findings. For quantitative measurements, we perform power calculations to ensure that a minimum sample size sufficient to detect statistical significance is achieved.
3. Refinement **Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.**	Genetically altered mice offer the most incisive approach to the analysis of mechanism underlying birth defects. We will maintain mouse colonies of mice that are not adversely affected by the deleterious effects of the mutations. Phenotypically affected animals will only be studied prenatally. We strive to use the minimal amount of animals needed to maintain the colony and perform the planned work. To test the efficacy of new substances in treating the defects under investigation, we will use the technique of ex utero embryo culture. This technique can be employed following schedule 1 killing to monitor key events during development, whilst imparting no suffering to the animals under investigation.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Genetic control of fertility and development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Infertility, genetics, epigenetics, chromosomes, development</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>There are three main objectives to our study.

1) Understanding infertility. Infertility is common in humans, affecting one third of couples. In at least one quarter of cases genetic defects are likely to be important. Patients with chromosome abnormalities (too few or too many chromosomes), especially those affecting the sex chromosomes, are particularly susceptible to this condition. We aim to understand how these chromosome abnormalities cause infertility and why the sex chromosomes play such a specialised role in the formation of male and female germ cells (sperm and eggs).

2) Deciphering the origins of chromosome abnormalities in offspring. During germ cell development, chromosomes interact with each other and swap genetic information, creating novel combinations of genes that, at fertilisation, give embryo that are genetically unique. When this process of “gene swapping” goes awry, this leads to the formation of eggs and sperm with the wrong number of chromosomes, and the consequence of this is that offspring are chromosomally abnormal, e.g. Down syndrome. We aim to understand how these problems in genetic swapping arise.

3) Understanding how the dose of genes on the sex chromosome is regulated. Males and females differ in many fundamental ways, but one of the most profound is their genetic make-up: females have two X chromosomes while males</td></tr>
</table>

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

	have only one. To correct for this imbalance, one of the two X chromosomes is “silenced” in each cell in the female. This process, called X chromosome inactivation, is absolutely critical for normal embryonic development and defects in this process are associated with mental retardation and cancer. We aim to understand how X chromosome inactivation takes place within each cell.
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)?	Our work will impact on multiple areas of human health. It will help us to understand the mechanisms that cause infertility in humans, and may ultimately lead to treatments for this condition, or the creation of novel contraceptives. In addition, it will help us understand how chromosome errors arise in newborn children. Defects in X chromosome inactivation cause a number of conditions, including cancer. Our work on this area may therefore impact on the development of anti-cancer therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	We have chosen mice as our main model, because their genetic make-up is similar to humans and they can be genetically modified to test the specific questions outlined above. We also use another mammal; a marsupial called the opossum. Germ cell development and X chromosome inactivation in this animal is similar to that in humans, but its gene content is quite different. This allows us to much more easily tease out exactly which genes are especially important for the processes we are studying. We will use around 2500 mice and 550 opossums for our studies.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most material collected for study will be from animals that have been humanely culled, therefore during their life time these animals will not experience any suffering. On occasions, we may need to perform surgery, under general anaesthesia on our mice, in order for instance to remove one gonad or to implant germ cells into a mouse that has none. However, these and other procedures will be carried out by trained personnel and have been refined in accordance with best practice to cause only minimal, brief, discomfort.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our studies require the use of animals, because currently it is impossible to study germ cell development *in vitro*, i.e. in cells cultured in a dish. Notably, a large part of our project will be devoted to developing an in vitro system for germ cell formation, but in the meantime, animals will be used
2. Reduction Explain how you will assure	**We use a number of approaches to reduce animal numbers. We only carry out experiments**

the use of minimum numbers of animals	**once we have surveyed, in detail, existing published work, and we plan experiments in such a way that the minimal number of animals will be needed to get a statistically significant results. We maintain clear and detailed experimental protocols that ensure success of animal experiments, thereby minimising the numbers of experimental repeats. Furthermore, we archive material from each mouse, so that tissue from a single mouse can be reused to answer many biological questions.** **In instances where we need to create a new genetically modified animal, we use personnel that are highly trained in the required techniques, thereby again reducing the numbers of animals that are needed. The colonies of mice that we keep are as small as possible, so that we do not have additional mice that are not used. We provide material from the animals we keep to a number of research groups with diverse interests. This considerably reduces the total numbers of animals that need to be generated at different institutions.**
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 use mammals (mice and opossums), because non-mammalian animal models carry out germ cell development and X chromosome inactivation in a fundamentally different way. Importantly, the mechanisms underlying germ cell development in mice and laboratory opossums is similar to that in humans, and the gene content of the sex chromosomes of the mouse and the opossum has substantial overlap with that of humans. Thus, the experimental outcomes of experiments in mice and opossums are highly informative for understanding human germ cell development and for designing translational approaches to treating human infertility. Finally, the mouse is the most tractable mammal with respect to genetic manipulation. Most material collected for study will be from animals that have been humanely culled, therefore during their life time these animals will not experience any suffering. Most mice are only subject to regulated procedures in so far as they carry genetic modifications. In the majority of cases, the genetic modifications primarily impact on fertility, and thus do not appear to cause pain or distress. Where possible we use highly trained personnel, e.g. animal staff, **NIMR Procedural Services Department to carry out Protocols with**

	moderate severity limits, e.g. induced ovulation, production of genetically modified founders, germ cell transplantation, in order to keep animal suffering to a minimum. Although it is not possible to fully predict the nature or severity of any potential defect arising from a newly-generated genetic alteration, we take steps to reduce effects. For instance, we make sure that characteristics exhibited by mice carrying novel genetic alterations are collated and regularly reviewed. If an animal exhibits any unexpected or detrimental phenotype, we will take advice from the institute veterinary surgeon and/or the local Home Office Inspector, and where necessary animals will be humanely killed.