Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 50

Project Titles and key words

- Neuroimmune interactions

  Cytokine, brain, liver, microglia, astrocyte

- Education of Personal Licence Applicants

  Anaesthesia

- Glial transmission in brain plasticity

  EDP, plasticity, LTP, cortex, astrocyte

- Inhibitory neurons in health and disease

  Brain, psychiatric disorders, schizophrenia, interneurons, transgenic mice

- Preclinical cardiovascular evaluation

  Cardiovascular, integrated systems, regional haemodynamics

- Assessing welfare in fish via application of optimal and sub-optimal holding conditions

  Fish, welfare, biomarker, stress, happiness.

- The energetics and biomechanics of flight in homing pigeons.

  Heart rate, accelerometry, data loggers

- Investigation into drug pharmacokinetics

  Malaria, anti-infectives, pharmacodynamics, pharmacokinetics

- Breeding and housing of genetically modified and mutant mice.

  Breeding genetically modified mice

- Cartilage Repair

  Repair of articular cartilage

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Neuroimmune interactions</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cytokine, brain, liver, microglia, astrocyte</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>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></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>It has become clear that events throughout the body play a significant role in controlling the response of the brain to injury and disease. The principal goal of the work described here is now to discover how inflammation both in the brain and in the rest of the body can affect brain integrity and nerve cell function. We will build on our discovery using rats and mice that the injection of immune molecules into the brain resulted in a rapid response by the liver and we were surprised to find that that molecules made by the liver coordinate and control the body’s response to brain injury. In our previous experiments inhibition of the liver response reversed brain injury. Thus targeting events in organs distant from the site of injury can change the outcome of brain disease. Under this licence we will principally use rats and mice to continue to explore how, infection, diet, affect brain disease and how brain disease impact on the body’s ability to cope with 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 results from our preceding in vivo work have been published in at least 30 peer-reviewed articles. Our collaborators, also working under our previous licence, have also published widely. We expect the results obtained to translate to new therapies, and we expect our work to lead to the development of novel contrast agents for use in the clinic. Indeed, we have been awarded a grant application to take our work on novel contrast agents into the clinic.</td></tr>
<tr><td>What species and</td><td>Mice and rats and over the 5-year period. 10 scientists working on this project will expect to use</td></tr>
</table>

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

approximate numbers of animals do you expect to use over what period of time?	approximately 1500 rats and 2000 mice. This is based on our ongoing experience of these experiments.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	It should be noted that we will use pain relief for all surgical procedures and that the use of imaging techniques reduces the overall number of animals required. We have developed a number of models that are clinically silent and we use these wherever possible. However, some animals might exhibit transient hemiplegia after stroke, but otherwise we expect no clinical signs. The animals will all be culled at the end of our experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the past we have established features of the host response to brain injury and disease in whole animals that could not have been predicted from existing knowledge or have been achieved in cell culture experiments. By the nature of this project it is important not to confound our data by using animals that are stressed or ill. Experienced licensees will gently handle animals and behavioural experiments will be performed in conditions where sound, light and heating are controlled. Many of the molecules that we know are important in the brain-immune system communication pathways are not expressed in fish or flies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use state-of-the-art imaging techniques, molecular biological that require less tissue and behavioural experiments to examine the effects of the inflammatory response in the whole body and in the brain on the evolution of pathology in the brain. Wherever possible we will try to sample tissue or image in a serial manner to reduce the number of animals required. We use power analysis to ensure that the most appropriate number of animals are used in our experiments and we use archival material or surplus animals wherever possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The complex regulation of the immune system involves a neural component, which will we also be studying. Rats and mice can be used in our experiments to closely model the human immune responses to brain injury and disease, which is not possible in non-mammalian species. All animals undergoing surgical procedure will receive pain relief and we will use power analysis to ensure that we use no more animals than necessary. Wherever possible, tissue will be retained for further experiments and we will also employ behavioural outcomes and molecular outcomes to ensure the each animals generates multiple outcome measures in situations where we have previously

	shown that there are no confounds associated with this approach.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>EDUCATION OF PERSONAL LICENCE APPLICANTS</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Education, Anaesthesia</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)[3]</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></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[4]</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>Researchers who need to perform regulated procedures are required to complete accredited training courses before being able to obtain a personal licence. The purpose of such training is to ensure that applicants gain sufficient knowledge that their subsequent research work is of the highest standard and complies with both the ethical and legal requirements of the Animals (Scientific Procedures) Act 1986, amended 2012.

The project aims to fulfil the following three broad objectives in order to benefit the welfare of animals under procedures:
• to demonstrate refined and up-to-date techniques for the humane restraint of animals, the prevention and alleviation of pain and the performance of minor procedures
• to demonstrate principles of good anaesthetics practice including routes of anaesthesia, principles of balanced anaesthesia and anaesthetic monitoring.
• to re-inforce in course delegates a culture of care and concern for animals undergoing regulated procedures via the demonstration of best practice. This third objective is an important one that deserves to be stated separately but one that is achieved as part of the other two.</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</td><td>These broad objectives, which encompass over more than 50 learning outcomes and 20 specific objectives, aims to foster two of the three Rs by demonstrating good practice: Refinement, broadly defined as improvements to procedures which</td></tr>
</table>

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

project)?	minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable, and Reduction to minimise animal use and enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals, thereby reducing future use of animals.. This will therefore directly benefit the welfare of animals used in many other projects (refinement and reduction), as well as encouraging the highest standards of experimental work, and more generally, instil a culture of care that is the bedrock of ethical animal research.
What species and approximate numbers of animals do you expect to use over what period of time?	500 mice and 10 rats over the course of 5 years to teach over 1,300 student-modules sessions
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 project involves one non-recovery protocol of induction of anaesthesia carried out by skilled and experienced professionals (usually veterinary surgeons) using highly refined anaesthetic techniques. Therefore the only significant adverse effect experienced by the subject animal will be the momentary discomfort associated with the induction of general anaesthesia
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A principal aim of the training courses is to impart the principles of anaesthetic monitoring in a practical (rather than theoretical) context. This is already addressed by a combination of lectures, video presentations and group discussions. But whilst much can be achieved this way, the art of anaesthetic monitoring is also essentially a practical skill and adequate contextual, rather than theoretical, knowledge will also be imparted by watching live anaesthetised animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project is committed to using surplus animals thus not requiring the breeding of animals for the purpose of this PPL.
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 principal aim of the project is to teach the most refined and appropriate methods for anaesthesia and minor procedures. The high level of veterinary involvement in this project will also help to maintain the highest standards of patient care & support. Mice (which is the main model animal) and rats constitute the majority of species used in the biomedical sciences (and surplus animals are more

	easily available) and allow the demonstration of general principles also suitable to higher species. The animals are asleep throughout and are not woken up.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Glial transmission in brain plasticity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>EDP, plasticity, LTP, cortex, astrocyte</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></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[6]</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>The project will investigate the role of a cell type called astrocytes in controlling how nerve cells in the brain communicate with each other. Sensory information (touch, pain vision) is transmitted to the brain and deciphered by nerve cells in the cortex which signal to each other by connections called synapses. These connections change depending on the sensory information. For instance, a large part of cortex of rodents is devoted to information transmitted from their whiskers, and cutting whiskers causes changes in the connections between nerve cells. Scientists believe that these changes are similar to those that underlie learning and memory and those that happen due to stroke or limb loss in people. It is also known that in Alzheimer’s Disease there is a problem with the way that these plastic changes happen. We aim to study how astrocytes are involved in cortical plasticity, which happen between nerve cells, and also what sort of changes happen to astrocytes.</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>Understanding the mechanisms of plasticity is essential as there is hope that being able to manipulate plasticity when there is damage would help us to treat conditions that affect the brain such as epilepsy, blindness, deafness, phantom limb pain and memory deficits in diseases such as Alzheimer’s.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>About 2000 mice and rats will be used in this project over 5 years</td></tr>
</table>

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The project will involve the expression of light activated proteins in mouse brain. Under deep anaesthesia a small opening will be drilled in the skull and the genetic material injected into the brain. Following surgery, the animal will be given analgesia and following a period when the proteins express in the brain, the animal will be placed under terminal anaesthesia and after death slices will be taken from the brain and experiments conducted. No adverse effects of pain and suffering are expected since procedures are conducted under general anaesthesia. Moderate severity level is expected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	These studies are aimed at understanding the mechanisms underlying plasticity, particularly in the barrel cortex. Because it is complex structures like the cortex that undergo plasticity in the brain, it not possible at present to reconstruct the connections and complex architecture of the brain in culture. Also the aim of the study is find out how plasticity changes when input from the outside world changes. These changes must take place in a living animal before the cells can be investigated. It is therefore necessary to use mice and rats.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have discussed with statisticians and performed calculations to determine the minimum number of experiments required for our experiments. In addition, many brain slices will be taken from the same animal and different methods combined in the same experiments to increase the quantity and validity of the data. The use of viral expression of proteins greatly reduces the number of animals used compared to generating different transgenic mouse colonies expressing different proteins.
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.	Because the studies are aimed at finding out about cortical plasticity they must be conducted in mammals since animal s such as invertebrates do not have such brain structures. The most appropriate species is the mouse because a large amount is known already about plasticity in mouse cortex and there are long established methods for generating plasticity which we will also use. Our results will therefore build upon and advance existing knowledge. All surgical techniques will be conducted with general and local anaesthesia, and following this animal swill be monitored for distress, though in our experience we have never observed distress following procedures.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Inhibitory neurons in health and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain, psychiatric disorders, schizophrenia, interneurons, transgenic mice</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td>Yes</td><td><s>No</s></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our two objectives are to investigate 1) basic function and 2) disease related processes that take place in inhibitory interneurons in the brain, in particular in a region called hippocampus and in areas directly connected to it. Basic function of these neurons is not well known, and their dysfunction has been suggested to contribute to various different types of brain diseases, although this connection is poorly understood.</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 gained knowledge advances understanding basic function of the brain. Identified disease mechanisms may offer novel therapeutic targets.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>In five years, we estimate to breed and maintain maximally 3000 mice with mild phenotype and 3000 mice with a disease genotype. In a year, experimental slice preparation procedures (with or without injections) will require up to 400 mice with mild phenotype, and 400 mice with disease phenotype. In addition we will use 150 mice per year for psychoactive drug treatment experiments in both groups (300 together). We will also use 40 rats per year in slice preparation studies.</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</td><td>a) We breed and maintain transgenic mice for experiments. Animals are either not expected to exhibit any harmful phenotype, or they carry a disease risk gene, which can trigger in them disease symptoms such as hyperactivity, which can</td></tr>
</table>

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

happen to the animals at the end?	be seen as increased spontaneous locomotion in the cage. Animals can also exhibit social withdrawal seen as increased time spent in isolation when housed with other mice. None of these phenotypes have been reported to affect the health of the animals. Animals will be forwarded to step b, c or d below. b) Mice can be genetically manipulated to express optically-excitable opsin molecules in specific neuron subtypes in the brain. This will allow selective activity modulation of their neurons by specific wavelengths of light, applied in form of laser or LED light beams. Opsins can be introduced in the brain by genetic means or via viral injections. The latter requires minor head surgery, which however has very successful and quick recovery. Surgery can cause some adverse effects such as head ache and disorientation, therefore animals will be medicated with painkillers postoperatively. Animals will be used further as described below in steps c or in d. c) Some mice will be treated with psychoactive drugs in order to investigate the mechanisms these substances facilitate development of schizophrenia-like symptoms. Drug doses we use are known to induce short (10-15 min) period of hyperactivity, which can be seen for example as increased spontaneous digging activity in the home cage. The acute effect with these concentrations wears off in 30 min. In some animals with specific genetic background, the treatment can elicit symptoms of the disease including permanent signs of hyperactivity. Animals will be sacrificed as described below in d. d) All animals will be sacrificed under deep anaesthesia. Once terminated, their brain tissue will be used to prepare acute studies of electrical and activity and later anatomical analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research will require use of real brain tissue. It is important in these projects that the status of the cells as well as anatomical properties of the brain tissue is as close to the intact brain as possible. Although neuronal cultures provide a valuable experimental model for certain studies, cell or brain tissue cultures cannot be used here because anatomical and functional properties in these preparations are changed and different from intact brain. Similarly, disease development is a gradual process which involves more than just the affected

	neurons and therefore isolated cell cultures would not yet work in this study. In this work we cannot use computer simulations, because many cellular parameters of these neurons, including plastic behaviour and disease progress, are not known.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be minimised through; I) Well-managed genetic mouse breeding strategies and the use of heterozygote mice as well as both males and females. II) Replacing rat as the main experimental animal with transgenic mice. Because novel transgenic mice allow identification of specific cell types this gives us a better yield of successful experiments per animal. III) Designing optimal control experiments to prevent false results, eg. use of an inactive drug to confirm specificity of action. IV) Using the most appropriate statistical analysis of data which maximise the power of the data and minimise the group size. V) Good surgical methodology and practice to minimise loss of animals by maximising chance of recovery. VI) Literature updates to avoid replication of experiments already published and use of pilot experiments with small number of animals before embarking on full-scale 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.	Rats and mice represent a standard animal model in mechanistic brain research. In addition their brain is close enough to human to make interpretations about how our own brain works. Therefore most commonly used disease models are based on these animals. Some anatomical visualisation techniques can show species specific differences. Rats will be used to test species-specificity of key results. The great majority of our experiments are performed on transgenic mice of unlikely adverse effects. In most cases they carry a harmless transgene. In some cases, expression of a specific disease gene is scientifically necessary in order to understand how the genetic alteration changes the brain. Introduction of opsins in the brain via viral injection requires minor surgery, which has very successful and quick recovery. Studying the acute brain slices harvested from animals under terminal

	anaesthesia is the least severe method available to produce satisfactory scientific results in our project. Animal suffering will be minimised using: I) Good surgical methods to maximise recovery and minimise adverse effects. II) Animals will be given time to recover from surgery, typically at least a week or two, before continuing with any further steps. III) Drugs will be administered in volumes and routes appropriate to mice, which are small animals. For repeated drug treatment, we aim to use oral administration rather than injections when this is scientifically feasible. IV) We routinely monitor health of animals using scoring sheets to detect and intervene early signs of suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Preclinical cardiovascular evaluation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cardiovascular, integrated systems, regional haemodynamics</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></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 main objectives of this study are to look at the effects of drugs on blood flow to different parts of the body and to determine whether these effects could explain some of the clinical vascular events seen in humans. Where possible, this project will also try to understand the ways in which these drugs may affect the cardiovascular system.</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 overall purpose of this project is to examine whether the adverse cardiovascular effects of compounds identified in the late stages of clinical development, could have been predicted preclinically, thereby providing a possible novel preclinical model for safety testing.

The benefits of this project include obtaining a clear understanding of the cardiovascular effects of compounds that failed in clinical trials. The outcomes could lead to this methodology forming an important part of future projects. The potential benefits would ensure protection from cardiovascular adverse events occurring in man in the future. Moreover, finding out that drugs potentially increase cardiovascular risk during early preclinical development could reduced the use of not only rats and mice, but also dogs and monkeys, which are often used in later stages of drug development.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>The rat is the animal of choice, since there is an extensive background literature on cardiovascular regulation in this species, the cardiovascular</td></tr>
</table>

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

over what period of time?	system resembles that of man in many ways, and implantation of the measuring devices is not possible in mice because they are too small. Across the lifetime of this project, it is estimated that a maximum of 675 rats will be used. For a typical rat in the full experimental schedule, surgery to implant flow probes (maximum of 3) around blood vessels, such as the renal and mesenteric arteries and the descending aorta, and to implant catheters in blood vessels will be carried out under general anaesthesia with operative and post-operative analgesia. Once the animals have recovered from the surgical procedure, they are dosed with the drug of interest. The flow probes will detect changes to the animals' cardiovascular parameters. The signals from these sensitive instruments are passed to data recorders allowing very specific interpretation of data and thus increasing knowledge about the effect the drugs have on the circulation.
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 order to minimise animal use, short-term experiments run over a maximum of 4 days, with animals acting as their own controls, and being exposed to more than 1 compound, if appropriate. Possible adverse events include reactions to anaesthesia, issues with wound healing, damage to catheters, or reactions to the drugs. The expected severity level of the procedures is moderate. The health and well-being of animals will be closely monitored, particularly post-surgery, and any concerns raised with the NVS or deputy. As a consequence of discussions with the NVS, or following completion of the experimental protocol, animals will be killed by 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 use of animal models is essential to enhancing our understanding of cardiovascular responses to drug administration and provides a means of replicating elements of the treatable phases of disease to drive forward new therapeutic options. The 3Rs will be implemented where possible to improve the scientific models used in this project. Although *in vitro* work can often replace some aspects of whole animal studies, the aim of the current project is to evaluate the complex cardiovascular effects of compounds in a robust model of integrated, intact systems. *In vitro* studies have been used extensively in earlier-phase preclinical studies, facilitating the early elimination of new drug discovery compounds before the need for *in vivo* evaluation. Indeed one outcome of the project should be a reduction in the need for future

	in vivo work, in animals such as dogs and primates.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We aim to substantially reduce the number of animals wherever possible, by implementing strategies that allow examination of the effects of different pharmacological interventions, in the same surgically prepared animal, on different experimental days. This achieves a reduction in the use of animals requiring surgical preparation without unduly increasing the burden on each animal (other than by extending the time for which the animal is held in the experimental condition), because the effects of the experimental interventions are minor and transient, relative to the burden of surgery. In addition, data obtained from these studies are analysed each week. This rigorous interpretation helps to inform the design of further experiments, ensuring robust experimental design and forward planning which contributes to reduction in overall animal use.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats have been used extensively in preclinical *in vivo* pharmacology experiments, and were chosen over the mouse for this proposed project because the Doppler technology cannot be applied in the mouse, due to size constraints. Moreover, the rat offers a model system that shares a level of commonality in its cardiovascular physiology that is considered a close parallel to that of man. The choice is therefore a rodent model system at the lowest possible neurophysiological sensitivity that is able to produce data of significant physiological significance that may be, as much as realistically possible, considered valid for extrapolation to the living human situation. The methodology used in this study has been refined over many years. The probe size is considerably smaller than other commercially available systems, surgeries are performed by highly trained personal licence holders, and new refinements to techniques (including isolation of vessels and suturing) are implemented on an on-going basis. Animals are very closely monitored during the post-surgical phase (every 15 min) and any concerns are brought to the attention of the NVS or deputy. Subsequently, daily checks ensure that the animals do not experience any additional harm or suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Assessing welfare in fish via application of optimal and sub-optimal holding conditions.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, welfare, biomarker, stress, happiness.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>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[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>The goal of this research is to discover metabolic indicators of good (and poor) welfare in fish. Currently the holding conditions for experimental fish in research establishments are variable and defined empirically; this research will examine whether (or not) environmental enrichment measurably improves fish welfare.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Our approach has the potential to discover novel biomarkers of fish welfare AND identify methods for their routine measurement by care and health officials within and outside the laboratory establishment (e.g. Home Office Inspectors, Named Animal Care and Welfare Officers, Named Veterinary Surgeons, Auditors).
If welfare could be assessed objectively on a routine basis, inappropriate environment and care could be avoided which would:
- improve holding conditions for millions of fish held in research aquaria (breeding, stock and experimental fish)
- reduce the risks of experimental failure (and therefore the number of fish used for scientific purposes),
- reaching wrong conclusions (as meaningful data would be obtained).
ALSO, the role of environmental enrichment in fish welfare merits investigation. Evidence of welfare benefits could shape the way fish are maintained in research establishments (as well as in the aquaculture industry which involves far greater numbers).</td></tr>
<tr><td>What species and</td><td>Common laboratory fish (i.e. rainbow trout, zebrafish, stickleback, etc). The maximum total</td></tr>
</table>

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

approximate numbers of animals do you expect to use over what period of time?	number of fish we will use will be 8,000 over a 5-year period. It should be noted that this number reflects the social behaviour (shoaling) of some species which benefit from being held in groups.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The main focus of the application is to discover markers of positive welfare in fish. The bulk of the fish will be held under improved (⅓) or standard (⅓) holding conditions where adverse effects are not expected. Only ⅓ of the fish will be exposed to a procedure of mild severity: anticipated adverse effects are restricted to disturbance of physiology.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The primary aim of this work is to discover markers of positive welfare which cannot be done without exposing live animals to different holding conditions: in-vitro systems don't have the ability to process perception of environmental conditions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of fish in an experiment is a product of the number in a tank and the number of replicate tanks. We will use a relatively low number of fish per tank where possible (i.e. zebrafish and stickleback which are routinely held in small tanks), but rainbow trout are routinely held at higher densities that promote schooling behaviour. We will need to use several replicates, because the research is very novel and a strong foundation of data will be needed to identify and validate putative welfare indicators.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Our establishment has significant expertise in working with fish. The species proposed for use represent the vast majority of experimental fish worldwide and have been chosen on the basis of literature data and statistics on returns (where available). The procedures which the fish are exposed to are classed as mild, the exposure period is limited to 4 weeks, and fish will be regularly monitored throughout. In the unlikely event that observed effects become more adverse than anticipated, prompt action will be taken (for individuals or tank groups as appropriate). The whole concept behind this licence is about refining the conditions under which experimental fish are held. The identification and development of welfare indicators for fish is the desired outcome of this research, which will facilitate refinement of husbandry for fish (experimental and stock) and improve the quality of scientific data.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The energetics and biomechanics of flight in homing pigeons.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart rate, accelerometry, data loggers</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The energetic costs, benefits and adaptive value of specific animal morphologies and behaviours are of fundamental importance to the successful survival of all animals, whether considered on a day-to-day basis or over evolutionary time scales. However, there is relatively little comparative data on the quantitative energetics and consequences of many of these behaviours, or of specific morphological differences between species, and this is particularly the case with regard to the biology of flying animals such as birds, which are difficult to study. Detailed investigations are required on how energetic and biomechanical costs vary with fundamental variables such as flight velocity, the effects of differences in body mass and/or body/wing/tail morphologies, the effects of climbing flight, the difficulties in circumnavigating geographical barriers and the effects of carrying external and/or internal packages for conservation or scientific purposes. This work will take a modern approach to monitoring behaviour and energetics utilising miniature electronic data loggers that can measure variables such as GPS position and ground speeds, along with 3D-accelerometry and heart rate.</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>Studying free-ranging animals has the benefit that it is often more refined and informative than studying captive animals that are restricted in movement, or which do not express natural behaviours. These studies will inform on changes to the strategic flight behaviour and wing kinematics of birds when carrying extra mass and when flying in different modes and flight speeds. They will also address issues of the evolutionary design of flying animals,</td></tr>
</table>

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

	with respect to both natural and sexual selection processes, and improve our understanding and modelling of the biomechanical costs of flight.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 80 homing pigeons (Columba livia) 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 present project utilises a small (20-60 bird) colony of homing pigeons (*Columba livia*) as an ideal model species with which to study animal flight. Birds are trained to fly up a 12 ft high tower to study take-off and hovering flights or to fly relatively short distances (up to 20 km) from their home loft. The main technique involves the deployment of miniature external data loggers which can record GPS, 3-axis accelerometry and heart rate. A number of the experimental flights use approaches that would normally be considered below the threshold for regulation, with added weights ≤ 3% body mass (utilising a very lightweight elastic harness), while the measurement of heart rate requires the mild protocol of the application of a subcutaneous pin electrode. No major adverse effects are expected from any of these procedures and they are not expected to exceed a Mild level of severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Work on wild animal ecology requires the study of their natural behaviour, while studies of captive animals both informs the interpretation of wild animal studies and also provides valuable insights in itself, while providing opportunities for more detailed and accurate data collection. The study of animal locomotion can be usefully informed by mathematical modelling but is a complex subject that requires real animal experimentation to feedback into the modelling process. Separation of the effects of drag against weight, or the details of the design and shape of a moving wing require direct testing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	While these studies are designed as a continuous series of experiments in which knowledge is gathered and refined from one experiment to the next, the work is initially designed around a single colony of homing pigeons. This varies in size from around 20 to 60 individuals and constitutes a relatively small social colony. Birds will fly as individuals but are also enjoy flying in a group. Previous experience has shown that homing pigeons fly with heart rates or around 640 beats per minute, with a SD of the difference between days of around 10 beats min^{-1}. I feel that it is desirable to

	be able to have a chance of detecting a change in heart rate of around 1% or 7 beats min^{-1}. In a paired test, using a total N of 24 birds would yield a statistical power of around 93%. Thus, I consider a total N of between 20 to 30 birds, to be a reasonable compromise between obtaining a satisfactory experimental resolution of changes in energetic costs and the requirement to reduce the numbers of animals used in experiments. Typical tests will include t-test (usually paired), ANOVA (usually repeated measures), regression and multiple regressions.
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.	Homing pigeons are an ideal species for the study of animal flight. They have been kept as domestic animals for hundreds of years, have a calm temperament and are tolerant of handling. They are derived from the feral rock dove (*Columba livia*) and home to a loft with great precision and motivation and are relatively hardy and easy to keep. They can home from home over great distances (up to around 700 km) and are capable of flying for at least 10 hours without stopping. There is no better domestic species for the study of free-flying animals. Our methods use state-of-the-art miniature electronic data loggers, which are well tolerated by the birds. The latest modules range from around 1.5% up to 5% of body mass and have been shown to be excellent for recording high resolution variables while minimising detrimental impacts on the experimental subjects. A recent review found no evidence of additional predation or mortality as a result of wearing backpacks. We employ daily maintenance by experience animal technician and have many years of experience in pigeon husbandry. We are visited regularly by the Veterinarian and have had little disease or other medical issues. A number of the experimental flights use approaches that would normally be considered below the threshold for regulation, with added weights ≤ 3% body mass. The major technique that requires regulation is the use of subcutaneous electrodes in order to record the electrocardiogram of the heart (ECG) and the addition of added mass and ornamentation to the wings. These are considered to be very mild procedures. Previous experience has shown that gold safety pins can be left in the skin of the birds for many weeks without causing any behavioural effects or evidence of infection. Insertion into the skin of the upper and lower back takes a few seconds and appears to

	cause very little concern to the birds and no evidence of any subsequent effects or suffering.

Investigation into drug pharmacokinetics

- Summarise your project (1-2 sentences)

Investigation into drug pharmacokinetics and pharmacodynamics of anti-infective agents, in particular novel antimalarials, to determine their suitability for follow on human trials.

- 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 intend to discover more effective drugs through translation of promising in vitro observations into optimal in vivo qualities.

Specific objectives include:

1. Assessment of sensitivity of rodent Plasmodium parasite species to antimalarial drugs in vivo paying particular attention to the ability of the parasite to acquire resistance to the drug under investigation.
2. Understanding the pharmacokinetics (the relationship between the body and the drug) and pharmacodynamics (the relationship between the pathogen and the drug) of candidate drugs in vivo with the explicit aim of establishing oral bioavailability and clearance so as to inform clinical decisions on dose size and frequency where any successful candidate is introduced into human trials.
3. Assessment of drug efficacy against novel in vitro test systems based on humanized mouse model.

Progress will be carefully monitored to ensure only the most promising agents are selected and the numbers of experimental procedures minimised.

- Outline the general project plan.

We plan to evaluate the utility of novel chemical entities (NCEs) as potential drugs or drug leads based upon (1) Pharmacodynamics (e.g. drug efficacy) and (2) Pharmacokinetics. Pharmacodynamics of NCEs will be assessed first through a number of in vitro efficacy, ADMET and toxicity screens. Only NCE which possess potent antimalarial (or antiinfective) activity e.g. IC50<1uM will be assessed using the described in vivo models. The in vivo models described will allow the estimation of drug activity against both circulating malaria parasites and liver stage parasites (using refined GM animal models). Using the described models, we will also be able to assess NCEs for which the malaria parasites can readily become resistant to.

The pharmacokinetic features of the NCEs will be assessed using the described protocols in order to (i) triage the best NCEs for clinical development, and (ii) inform human dose ranging studies.

The Target Product Profile for all antimalarial drug discovery work will be aligned with that of the Medicines for Malaria Venture (www.mmv.org).

- 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 design of the protocols is such as to minimise suffering and incorporate consideration of the 3Rs. Lethality is neither an anticipated nor a desired outcome. Nevertheless, animals will be observed as described in the specific protocols and at least twice daily. Advice will be sought from experienced care staff, the NACWO, project Licence holder or deputy or the named veterinary surgeon.

- Adverse effects of inducing Plasmodium infection in mice and rats are expected to be negligible. The infection may manifest itself as temporary piloerection, loss of appetite and reduced inquisitive activity. The incidence will be very low.
- Adverse effects of General Anaesthesia will be controlled using a good choice of technique and dose, and adequate monitoring.
- Surgery may lead to infections; this will be controlled by good aseptic technique and use of antibiotics as advised by a veterinary surgeon.
- Some post-operative pain is inevitable. This will be controlled by use of pre/post operative analgesia.

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

  There are some 0.5 billion malaria infections and nearly 1 million deaths per year, mainly in children under 5 years old. There is no malaria vaccine and drugs are the only viable treatment option. Unfortunately, the malaria parasite has now developed resistance to all but one class of antimalarials and recent reports now indicate drug failures in this last class in parts of SE Asia. Consequently the development of new drugs is urgently required. In vitro assays are present to determine promising antimalarial drug leads but there is no in vitro assay capable of predicting human in vivo efficacy.

  The Liverpool team is an international leader in the field and has previously registered one antimalarial and delivered a further 2 into clinical development. Currently the team

has 2 further antimalarials in pre-clinical development. It is our ambition to take these all the way into a registered drug to deliver a new antimalarial with efficacy against drug resistant malaria parasites in order to reduce the mortality and morbidity associated with malaria and to integrate the roll-out new drugs into malaria control and elimination programmes.

- 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 have taken advice from both statisticians and mathematicians and expect to use 2083 rats and 7275 mice.

- 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 research will require the use of animal models, as current in vitro test systems are poorly predictive of human efficacy and cannot be used to inform clinical development (e.g. human doses).

The project plan clearly describes how the in vivo work is fully integrated into the overall objective of drug development. The attrition of hits via the upstream in vitro efficacy and ADMET assays, as well as the tractability of the NCEs against the TPP, ensure that only a very limited number of the most promising compounds are then tested using the in vivo PD/PK models.

We are committed to methods that ensure that the number of animals used is minimised and that procedures, care routines and husbandry are refined to maximise welfare.

Close monitoring of all animals will be undertaken to respond rapidly to unpredictable responses that may result in suffering. The rodents used in this licence are the least likely to suffer, where suitable models of drug efficacy and drug disposition have been developed.

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

The procedures outlined in Section E that involve sampling without anaesthesia or anaesthesia with recovery have been carefully designed, reviewed to be consistent with

NC3Rs guidelines.

Removal of blood and tissue is always performed under general anaesthesia either by inhalation or injection. Volumes of blood to be removed have been chosen such that they are the minimum necessary for accurate and precise measurement of drugs and metabolites.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Cartilage Repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Repair of articular cartilage</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)[15]</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[16]</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>This project helps to develop new treatments to repair cartilage in damaged hips and knees. The clinical need is for a reliable treatment for cartilage defects that can induce repair or regeneration of stable cartilage and prevent the progression to degenerative and painful joint 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>The new treatments will help patients by relieving pain, restoring function and protecting undamaged joint structures. When compared with older designs of replacement joints, the new treatments will require less invasive surgery, permit shorter hospital stays, allow faster recovery and permit easier repair in the event that a replacement joint gets broken or wears out.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The estimated numbers are likely to be no more than10 athymic mice and athymic rats, 10 rats, 10 goats, 20 rabbits and 50 sheep used per year.</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>Whilst much of the early research work can be carried out in a laboratory or using a computer, once the prototypes/devices have been developed they have to be tested in an animal in order to find out how they behave in a real joint and how the surrounding bone and cartilage tissues will respond to the implant. Each study is peer-reviewed and approved by a group of experts and a layperson. This is to check that the study is absolutely necessary, to minimise the number of animals used and to further refine the protocol where possible. Every attempt is made to minimise the pain and trauma associated with using the implants because pain-free joint repair is one of the key objectives of</td></tr>
</table>

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

	the project. The expected level of severity is moderate. The animals are humanely terminated at the end-points of each study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cell culture and other non-animal types of testing cannot fully replicate the loading, physiological and anatomical conditions required to demonstrate safety and efficacy of novel cartilage repair prototypes/devices, therefore animal studies are necessary in the development of new cartilage therapies. There will be extensive non-animal testing to select and help improve the prototypes but animal studies will be necessary at some point in the development of these devices to show safety and efficacy in comparison to appropriate controls and/or currently approved treatments as required by regulatory authorities. The majority of animals used under this licence will be sheep to evaluate cartilage repair devices / therapies due to their joint size which facilitates surgical procedures of this type, weight bearing and the acceptability of this model to regulatory authorities.
2. Reduction Explain how you will assure the use of minimum numbers of animals	There will be extensive non-animal testing to select and help improve the prototypes but animal studies will be necessary at some point in the development of these devices to show safety and efficacy. Consultation with a biostatistician and other experts at the planning stage will help to optimise study design, minimise the number of animals required, and meet the study objectives.
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.	*Choice of Species* After a thorough review, sheep have been chosen because of their relatively large joint size, weight-bearing nature and their similarities in anatomy and cell/tissue (histology) structures to humans. Rats may be used for other aspects of testing such as biocompatibility. If foreign cells or foreign tissues are used then they may require testing in either athymic mice or athymic rats. Due to a naturally occurring mutation, these animals lack a thymus and therefore have no functional T-cells which mean they are immune-compromised and won't reject implanted foreign cells or tissues. Small animals such as rabbits may be used in some screening studies but for pivotal studies then prototypes/devices will be evaluated in sheep. It is also a requirement of regulatory authorities that they are tested in large animals before they are tried in human clinical trials. For this reason, we have built up an extensive amount of expertise in using sheep for the evaluation of prototypes /devices intended to repair cartilage.

	Minimising suffering Animal suffering is minimised by: (a) consultation with people with expertise in orthopaedic surgery and animal welfare; (b) thorough laboratory testing and refinement of the techniques and equipment before any surgeries take place; (c) starting with pilot studies using small numbers of animals to monitor animal behaviour when a novel type of surgery or prototype is being tried for the first time. This is to ensure that it does not cause suffering before continuing to a larger study; (d) standard veterinary procedures are used to administer anaesthetics and pain-relief before, during and after surgery. (e) each animal is carefully and closely monitored throughout the study and health checked beforehand. After surgery, pain-relief is given until there is no further need. This is to ensure that animals do not suffer.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Breeding and housing of genetically modified and mutant mice.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Breeding genetically modified mice</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)[17]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[18]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The purpose of this Project Licence application is to seek authority from the Home Office to maintain lines of genetically altered and mutant mice in order to provide suitable animals, or materials, for research.

Genetically altered and mutant animals are extremely valuable to scientists. They can provide an insight into fundamental biology and can be used to understand disease mechanisms. The mice bred and housed under this Project Licence will be used for research into addiction, diabetes, atherosclerosis, stem cell research and epilepsy.

Genetically modified mice bred under this Project Licence will be mated and reared normally. The mice are expected to behave and breed in the same way as normal mice.</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>After the mice have been bred they are issued to research scientists either for isolated tissues or transferred onto other Home Office authorised Project Licences. This will allow different researchers and research groups to share resources ultimately leading to a reduction in the numbers of mice bred.</td></tr>
</table>

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

What species and approximate numbers of animals do you expect to use over what period of time?	It has been estimated that up to 20,000 mice may be bred under this licence over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No adverse effects are likely and the likely level of severity will be mild. If any adverse effects from *g*enetically modified and mutant mice breeding were to occur then the mice will be immediately euthanised by an appropriate method suitable for the species.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The authorised scientist that has requested the breeding and maintenance of any strain of GA mice will be asked at an Ethical Review Meeting to discuss the consideration that has been given to the use and development of *invitro* alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Care will be taken to ensure that the numbers of animals produced are at the minimum required. Where ever possible mice not required will be used as sentinels, controls in other projects, or used for the provision of tissues.
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 (GA) mice are the only models available. When taking samples for genotyping only the mildest appropriate method will be used. During tail tipping and blood sampling a suitable local anaesthetic will be used. Tail tipping will usually take place when the mouse is between 21 and 28 days old. The tail tipping of mice over 42 days old will be conducted under a general anaesthetic followed by a suitable analgesic. Immune suppressed mice that have a poor immunity will be provided with sterile supplies. If any adverse effects from genetically modified and mutant mice breeding were to occur then the mice will be immediately euthanised by an appropriate method suitable for the species.