Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 15

Project Titles and key words

- Pharmacokinetic studies for research

  Drug Metabolism, Pharmacokinetics, DMPK

- Disregulation of skin homeostasis

  Wound healing, skin, inflammation, diabetes

- Preclinical testing of venoms and antivenoms

  Venom, Antivenom efficacy, Safety, Testing

- Genome involvement in brain function, disease and development.

  Disease, genetics, gene regulation, transgenic, mouse

- Mechanisms of Central Nervous System Development

  Embryo; brain; transcription factor; signalling molecule; mouse

- The modelling and treatment of paediatric cancers.

  Children's cancer, mouse models, therapy,

- Elucidating SCA pathogenesis/treatment strategies

  Ataxia, cerebellum, Purkinje cell, calcium

- Mechanisms underlying abnormal heart rhythm

  Arrhythmia, heart disease, sudden cardiac death

- Reproductive and Juvenile Animal Toxicity Testing

  Reproductive Toxicity Testing

- The Study of Transplant Injury

  Kidney; Immunology; Transplant rejection

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Pharmacokinetic studies for research</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Drug Metabolism, Pharmacokinetics, DMPK</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>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[2]</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 overall aim of the project is to give support to the discovery phase of research projects for external clients, ensuring that potential medicines with suitable drug metabolism and pharmacokinetic (DMPK) properties can be selected for further development to treat human disease effectively. DMPK is investigated by studying how potential medicines are absorbed and distributed in the body as well as how they are broken down (metabolised) and excreted. Typically a medicine that is given orally is dissolved in the gut, absorbed into the blood and then the circulated around the body. It may be metabolised, usually in the liver, and it, and/or its break down products (metabolites) excreted in urine and faeces. Some medicines cannot be given orally: for example, due to poor absorption from, or breakdown in, the gut and another route must be used. Other medicines, such as those given by inhalation, do not need to go into the blood stream in order to elicit the desired pharmacological response and/or are more effective when directly delivered to diseased tissue. Confidence in predicting DMPK properties in man is gained by studying the action of the potential medicine in more than one animal species.
The information gained from studies carried out under this licence will help to;
• Understand and then improve the way the compound is given so that there is sufficient information available to treat the disease effectively.
• Understand and then improve the length of action of the compound so that it is likely to stay in the body long enough when given in a dosing</td></tr>
</table>

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

	routine that is easy for patients to use. Potential clients are required to disclose any previous in vivo or in vitro investigations done on their compounds, to avoid any unjustified duplication of procedures.
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)?	Work under this licence will assist our clients in selecting compounds with an expectation of suitable DMPK properties in man and thus reduce the risk of exposure of human volunteers and patients to compounds that would be unsuitable as therapies. The work will enable clear decisions by our clients to progress or halt compounds at key project milestones. Data generated will assist in designing appropriate regimens and limit unnecessary use of animals for pharmacological and toxicological studies.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and Mice will be used on this licence. Over 5 years, it is estimated that 648 Mice and 540 Rats will be used.
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?	Adverse effects anticipated are those associated with routine routes of administration, sampling and those associated with general anaesthetic. In these cases, the likelihood of occurrence is estimated at <1%. The administration of test compounds/ substances will have the potential to affect all animals. Close monitoring, suitable in vitro screening of potential compounds and use of pilot studies, will help to keep the incidence of adverse effects to a minimum The level of severity for Protocol 1 is Non-recovery, and for Protocols 2 & 3 Moderate. Animals will be humanely killed at the end of all protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no non-animal tests (*in vitro*) that completely mimic and predict many key aspects of DMPK such as biliary clearance, tissue distribution or lung kinetics. These aspects are often the result of the interaction of many individual biological processes and these interactions cannot be reproduced by *in* vitro(non-animal) alternatives. For most organs there are currently no *in vitro* models that predict organ clearance and only an intact animal can provide an adequate integrated system. Consequently, animal testing is essential to achieving the overall objective of this licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	DMPK properties can be studied using a range of in vitro (‘in glass’ or ‘test tube’) and in vivo (‘in life’ or ‘in animals’) studies. It is standard practice to conduct the major proportion of testing in vitro using

	cells and tissues obtained from humans or animals. This strategy makes a vital contribution towards minimizing animal usage. In our experience, fewer than 10% of compounds tested in vitro are progressed to in vivo studies. In addition to in vitro testing, the properties of compounds are also predicted based on knowledge of their structure ('in silico'). Whilst in silico predictions or in vitro studies can provide a wealth of information on individual body systems, eventually studies in living animals are needed. Animals offer suitable models to replicate the interplay between different processes that can influence the disposition (what happens after it is given to the patient) of the potential medicine. Once *in vivo* work is deemed necessary, there are a number of approaches adopted in order to minimise the numbers of animals used; • Typically group size is 3 rats (n=2 for early oral screening). • Refinements in sampling in mice, reducing overall numbers required. • Consideration of statistical analysis, to use the smallest group sizes 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.	Purpose bred, adult free-living animals of assured health and genetic status will be obtained from commercial suppliers or from breeding colonies.Most studies will be conducted in rats; this is supported by *in vitro* testing which shows the relevance of this species to man. It is necessary to use other rodent species, for the purpose of this project mouse, if these species are more relevant to man for a particular research project or if more than one species is needed to build further confidence in predicting to man. Animal suffering will be minimised by the following; • Competent personnel will perform all studies on this project licence and adverse effects resulting from regulated procedures will be minimised by careful handling and the application of good technique. • Guidelines on the limit of volumes of administration of substances and blood sampling will be strictly adhered to. • A refinement in sample analysis has led to the reduction in total blood volumes required, now typically 20µl sample size. Thus reducing the burden further.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Disregulation of skin homeostasis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Wound healing, skin, inflammation, diabetes</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></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[4]</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Skin is the largest organ of the body, providing the primary barrier to prevent infection and dehydration. Following disruption of normal skin homeostasis (e.g. wound, infection) a highly regulated series of events occur, involving many cell types, to return the skin to its normal state. Disregulation of these damage response processes can lead to a wide variety of disease phenotypes such as dermatitis, psoriasis and impaired wound healing. Chronic wounds are a particularly important area of unmet clinical need, costing the NHS £3 billion to treat annually, which is likely to increase with an ageing population. Current therapies are inadequate, focussing on treating secondary symptoms (e.g. infection, necrosis) rather than the underlying cause, and often resulting in amputation. This project aims to use, and develop further models, of skin disregulation such as wound healing and inflammation and use them to preclinically test novel drugs.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This project will aid the development of new compounds for the treatment of non-healing wounds in diabetic and elderly patients.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>4300 mice and 1200 rats over 5 years.</td></tr>
<tr><td>In the context of what you</td><td>This project uses these models to test novel</td></tr>
</table>

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

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?	therapies for wound healing. All wounds will be made under anaesthetic and with post-operative pain relief and monitoring. The delayed type hypersensitivity model is not associated with pain and is therefore mild in nature. There is a possibility that administration of a Sponsor's drug results in side effects. Animals under any procedure will be monitored daily with clear weight loss limits and appropriate action will be taken for any animal presenting with any obvious stress or discomfort. All animals will be culled at the end.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use sophisticated *ex vivo* models, such as human skin explants, which we already use to examine certain aspects of skin biology such as cytotoxicity and response to UV. In tandem with this project we are developing new *ex vivo* models to examine aspects of the response to injury. However, these models are not able to examine the complex interactions, such as the inflammatory response. Thus, while we cannot eliminate the requirement for animal use, the *ex vivo* models will complement and act as screens for potential therapies prior to the use of *in vivo* models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have consulted a statistician to establish the minimal number of animals required. In parallel to the *in vivo* work planned we will also use validated *in vitro* assays where possible to examine individual processes relevant to particular disease states and will use these prior to in vivo work to screen compounds and therefore only select the most promising candidates for in vivo testing. The use of two wounds per animal reduces the number of animals required in a study where a drug being tested remains localised and is not systemically distributed, allowing a vehicle versus drug comparison per animal. This will be employed where possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice and rats are the two most widely used species for skin research. We perform a refinement step entailing the staging of hairs on rodents prior to wounding and only wounding animals when hairs are in telogen. Since the hair cycle influences the rate of wound healing, by performing this step we reduce variability and group sizes within experiments.

Title: Preclinical testing of venoms and antivenoms

Key Words: Venom, Antivenom efficacy, Safety, Testing

Project Purpose: Basic and Translational research
Objectives: To protect the health of antivenom-treated human patients this project will use a mouse model to pre-clinically test the effectiveness and safety of antivenoms to treat snakebite victims.

Potential Benefits: The benefits of this project will be the approval for human use of antivenoms demonstrated to be effective and safe in a proven animal model. The project will also establish the initial dose of antivenoms for human trials, and assist antivenom manufacturers meet regulatory requirements.

It is important that human victims of pathogenic snakebite receive appropriate treatment: the inappropriate distribution in Ghana of antivenom manufactured with venom from Indian snakes resulted in an increase in mortality of antivenom-treated patients from 0.5% to 12%. Over the course of the last project licenses, preclinical testing was involved in a project that provided over 35,000 vials of antivenom to help save the lives Nigerian snakebite victims.

Animal species and numbers: We anticipate being requested to test approximately 35 batches of antivenom over 5 years, and require 1910 mice.

Adverse Effects: Snake venoms cause severe cardiovascular and neurological effects in mice. Humane end points will be used throughout for rapid implementation of schedule 1 killing.

Application of the 3Rs
1 Replacement: The mouse model of envenoming has proved a satisfactorily accurate representation of the effects of envenoming in humans. There is no in vitro alternative assay yet devised that supplant these animal tests because (i) the toxin composition, and therefore toxicity, of venom differs significantly between species and (ii) many distinct types of venom toxins act in coordinated ways on multiple and different physiological pathways to exert the lethal effects.

2. Reduction: The literature and results of previous experiments is closely examined to reduce the range of venom, and therefore the numbers of mice, needed to establish the statistical validity of the assays. To further minimise the numbers of mice required to achieve the objective, preliminary range finding studies are performed that use one mouse rather than five to ascertain the likely range of (i) lethality of the venom and (ii) potency of the antivenom. Statistical analysis is performed on all the results, and the minimum number of mice required for statistical validity is used throughout.

During this project and with NC3R-funding, we will be examining in vitro alternatives to reduce the number of animals required for the animal tests. Thus, we will examine the toxic effects of the venoms on cell lines derived from different human tissues – intending to determine whether the antivenom neutralises this cell cytotoxicity in a manner that correlates with the in vivo test results. Also, we will determine whether the binding of antivenom to the venoms immobilised on a chromatographic column can be used as an accurate efficacy-predicting alternative to the in vivo tests.

3. Refinement: Mice are the physiologically least advanced rodent species that could be used for the preclinical assays.

All the previous preclinical tests on antivenom potency have been performed on mice. It would therefore be illogical to change the animal model species. The physiology of mice has been well characterised and the effects of venom can therefore be accurately determined. The consistent use of mouse genetic strains (eg CD1) reduces independent variability and therefore (i) reduces the number of animals required for statistical validity and (ii) increases the validity of comparing results from different experiments.
To minimise costs to the animals we will:

- reduce the duration of the tests
- maximally implement analgesia
- use existing and develop new less-severe humane end points to reduce pain, harm and distress

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Genome involvement in brain function, disease and development.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Disease, genetics, gene regulation, transgenic, mouse</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 Years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></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>
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<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>We still do not know why a significant proportion of the human population are genetically susceptible to obesity, chronic inflammatory disease, depression or addiction. The most modern genetic evidence suggests that many of these problems do not stem from changes in genes but from changes in the genetic switches that ensure that genes critical to health are turned on in the correct cells, at the correct times and in response to the correct stimuli. However, identifying these switches within the vastness of the human genome has been difficult until now and understanding the effects of genetic or environmentally induced changes on the activity of these switches even more so. The current licence will allow us to generate genetically modified animals that will be used to define the specific cells in which these switches are active. In addition, introducing safe doses of specific drug treatments into these GM animals will allow us to identify the stimuli to which these switches respond. This will be important in the future for the prediction and treatment of disease susceptibility and in the development of personalised medicine. Most intriguingly, the current studies will allow us to determine how environmental stimuli such as maternal diet and early life stress alter the activity of these switches in later life. In this way the interaction of genetic and environmental influences on the activity of these switches will provide an important window into predicting and treating obesity, addiction, depression and chronic pain and to developing treatments tailored to specific individuals. (250)</td></tr>
</table>

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

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)?	Diseases such as obesity, chronic pain, depression and addiction cause untold misery in Western societies whilst greatly affecting their economies and ability to compete globally. The experiments outlined in this licence will permit a greater understanding of the environmental and genetic factors that increase susceptibility to these diseases and will provide novel pathways to allowing their prediction and for the development of personalised treatment. (59)
What species and approximate numbers of animals do you expect to use over what period of time?	I estimate that up to 8700 animals will be used during the course of this licence most of which will be used in the generation, breeding and maintenance of the genetically modified lines to be used. Only a small proportion <1200, will be used for experimental purposes. (47)
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?	None of the DNA sequences under study to be used to make transgenic animals encode protein so it is unlikely that they will have any effects on the animals. The possibility of deleterious effects as a result of random insertion into the genome will be largely negated by maintenance of line as heterozygotes. Surgical procedures- vasectomy and embryo implantation are required in order to generate new GA lines. In feeding tests animals will not be allowed to experience ill health as a result of excessive obesity. Also, in early life stress tests measures such as only removing part of the litter from the mother at any one time and transferring pups in the presence of bedding will greatly reduce the chances of maternal stress. At the end of the studies the mice will be humanely killed and tissues sampled (141)
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cell cultures are generally homogeneous and comprised of one cell type. In addition, many of the tissue culture cells used bare little or no resemblance to the cells found in the human body in terms of their behaviour, their ability to communicate or the way they express the information contained in their DNA. This poses a problem as many of the processes that make us human will affect biological systems that depend on a number of different and highly specific cell types which must communicate with each other in very specific ways in order that the information in their DNA is expressed in the correct manner. Although genes play an important role in these cell specific processes what directs genes are the switch sequences described above which only function normally in the correct cells exposed to the correct communications. Thus, gaining a full understanding of the activity of these switch

	sequences requires their examination in whole living animals by the generation of transgenic animals.(162)
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use statistical analysis of the number of animals to be used to ensure that a minimum number will be used for each experiment. We will use statistical analysis techniques recommended in consultation with Biomathematics and Statistics Scotland (BioSS). Consultations with BIOSS will ensure that as few animals as possible will be used but sufficient to ensure statistical significance of data derived from our experiments. We will also maintain lines for the minimum length of time after which lines will be used to produce, eggs, sperm or embryos which will be stored as frozen. Once successfully frozen lines will be terminated. We will also endeavour to devise experiments involving the use of primary cell lines which do not involve the generation of transgenic lines or the use of live animals. (131)
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 suitable model as it is the least sentient small mammal whose genome can be readily manipulated whilst the majority of its anatomical and physiological features are shared by humans. We have chosen early life stress test and maternal dietary manipulation as they represent proven non–invasive methods to alter the methylation status of the genome. In vivo administration of drugs will be carried out using sub-toxic doses whose concentrations will be determined from the literature in consultation with the named veterinary surgeon (NVS) and experiences animal house staff. The effects of these drugs on the behaviour of the treated animals will be closely monitored by the licensee and animal house staff. Any unexpected side effects will be immediately reported to the NVS and animals will be killed if deemed necessary.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>MECHANISMS OF CENTRAL NERVOUS SYSTEM DEVELOPMENT</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Embryo; brain; transcription factor; signalling molecule; mouse</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></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[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>We do not understand why some people develop neurological or psychiatric diseases such as epilepsy, schizophrenia mental retardation and autism, but there is now compelling evidence that the basis of these disorders lies in defects in the way that the brain develops.

Congenital diseases of the brain linked to mutations of developmentally important genes are relatively common but we do not understand how the mutations cause the defects. We can make stem cells turn into neurons in culture but, if we want to use them to replace brain tissues damaged by disease or trauma, we do not know how to ensure that they become the right types of neurons with the right connections. The reason that these unknowns exist is because our fundamental knowledge of how the brain develops is very poor. We do know that some genes are important for brain development but we do not know why: we do not understand enough about how these genes control the way that the brain produces the right numbers of cells of the right types in the right places and at the right times and then connects them up correctly.

The objective of this project is to increase our fundamental understanding of how genes control brain development in health and 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>This project will increase our fundamental knowledge of the mechanisms that generate the mammalian brain. Current knowledge in this area is relatively very poor due largely to the extreme complexity of the brain. Advances in molecular genetics over the last 10 years have provided new ways to investigate this topic and improvement in</td></tr>
</table>

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

	understanding the mechanisms of brain development are likely to increase our ability to tackle currently incurable diseases of the human brain in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice Approximately 14,400
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?	Animals that have been genetically modified will be made and bred to learn about the consequences of the genetic defects, and hence uncover the biology of the affected genes. To assess the consequences of the genetic modifications, animals may be administered with substances to label specific cells, but 75% of animals will have no invasive procedures carried out while they are alive. The adverse effects will be brain defects resulting from the gene mutations that we are studying; i.e. these defects are the subject of our study and relate to human disease. In a minority of cases, animals may receive compounds that aren't expected to cause any side effects in themselves, but that alter how genes are expressed. These substances may be injected or dosed orally, but in some instances we need to be able to place the substances directly into the brain. Here, the adverse effects will be those associated with the surgical techniques required to access the brain at that stage of life, so for in utero stages this includes accessing foetuses through the womb. Pain killers will be given on veterinary advice to ensure suffering is minimised. The maximum severity is classified as moderate. At the end, the animals will be killed and they or their offspring will be analysed or their tissues will be cultured.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Overall, this research project aims to increase our knowledge of how the brain develops in animals including humans and, as such, can not be done without studying animals. Neuronal development *in vitro* does not recapitulate the patterned development seen *in vivo* and *in vitro* techniques are often inappropriate for our studies. Similarly neuronal cell lines are inappropriate because their immortality means that the very processes that we are striving to understand have been disrupted. The use of in vitro methods will, therefore, be a useful adjunct to our overall approach but can not replace in vivo techniques.
2. Reduction Explain how you will assure	The numbers of animals required will be the least required to give statistically significant results. Any

the use of minimum numbers of animals	less would be a waste since conclusions could not be drawn. The numbers required to give statistical significance depends on the effect size – the smaller the effect, the larger the numbers of animals required. Thus, for any specific experiment, pilot experiments are needed to assess the likely effect size and then numbers can be determined.
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 offers the advantage of being an excellent genetic model but, being a mammal, it has a brain whose structure is closer to humans than non-mammalian species. This is important if we are to learn about mechanisms that relate to human diseases. Much of our work (probably 75%) involves the analysis of post-mortem material and animals are always killed rapidly and humanely. Where surgical methods are required, we carry these out as quickly as possible with attention to the use of appropriate measures to minimize the risk of pain and suffering. Where we see evidence of unreasonable harm, animals are killed humanely.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The modelling and treatment of paediatric cancers.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Children’s cancer, mouse models, therapy,</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>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 overall aim of this project is to develop and study rodent models of childhood cancers to increase understanding of these diseases and to test new medicines for children suffering of cancer. There are around 1600 new cases of childhood cancer in the UK each year including leukaemia and bone tumours. Although many children with these diseases can be cured using medicines, some children cannot be cured. Furthermore, children under treatment suffer serious life-long consequences. . Therefore, there is an urgent need for a better understanding of children’s cancer and for the development of new more efficient and less toxic medicines.
Rodent models for children’s cancers and related adult cancers will be created by introducing human cancer cells from patients or from laboratory cultures into mice without an immune system. These animals go on to develop a human cancer as they are unable to reject the human cells. They will be studied to understand how cancers grow and spread and to determine if new medicines can reach the cancer cells and stop them growing.</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 models of children’s cancer will provide information about how cancers form and spread and lead to the development of new medicines. Any new medicines developed and tested in this project could, if shown to have significant anti-cancer effects, go on to be tested in children with cancer and potentially increase their length and quality of life.</td></tr>
</table>

[9] Delete Yes or No as appropriate.
[10] 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?	No more than 8,000 mice and 100 rats will be used in the proposed project 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?	Cancer cells will be introduced into rodents by injection and this will cause some pain and distress. Mouse models are monitored regularly for signs of cancer, such as the formation of tumours and for ill-health. We aim to carry out our studies on cancer models before the mice are sick and have developed methods to watch small numbers of cancer cells in well animals. Some medicines may be toxic to the animals and make them ill. Animals will be humanely killed if they display signs of ill health. Others may be humanely killed to provide tissue samples for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Currently there are no alternative animal-free systems to study how these cancers develop and spread and how and if medicines are able to reach cancer cells and to block their growth and spread. New ways to stop the cancer growing will first be tested extensively in the laboratory using cultured cancer cells. Only medicines that show anti-cancer effects will be tested on mice or occasionally rats.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to minimise the number of required animals, we will use wherever possible state of the art procedures to monitor disease progression and localisation of medicines in intact animals (i.e. without the need of killing them or performing surgical procedures). The smallest possible minimal group sizes are used to provide meaningful data. Initially the new medicines will be given to a minimal numbers of rodents to determine the best dose to use and any potential side effects. The medicines will then be given to mice with cancer to see if they prevent the spread of the disease.
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.	Rodents without an immune system are kept in clean conditions to prevent them catching infections. The models are well-established in mice compared to other species and allow the human cancer cells to retain their characteristics, which is highly relevant for understanding the disease and the development of medicines. Animals are given pain relieving medicines after surgical procedures to alleviate pain. Models are monitored regularly for signs of cancer, such as the formation of tumours and for ill-health.

	Some medicines could be toxic to the animals and make them ill so they are monitored very closely in these studies.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Elucidating SCA pathogenesis/treatment strategies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ataxia, cerebellum, Purkinje cell, calcium</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>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[12]</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>We aim to understand why a certain type of nerve cell in the cerebellum, which is the region of the brain required for balance and coordinated movement, dies in a group of disorders called spinocerebellar ataxia (SCA) and evaluate potential therapies. SCAs are a group of debilitating diseases, with patients becoming more and more disabled and in some instances the disease is fatal. However there are no treatments that can even modify the disease process. By understanding the mechanisms of cell death we aim to identify potential pathways that could be targeted for pharmacological manipulation. This study will also reveal the feasibility of gene therapy to halt, alleviate or reverse the disease and determine the window of opportunity for treatment to be effective. Realistically it is unlikely that any therapy could be administered to patients prior to the first signs of disease and so this study will determine what kind of recovery is possible after disease symptoms are observed.</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 project will have a range of beneficiaries including the ataxia and broader neurodegenerative disease research communities, through the development of new therapeutic approaches to these diseases, the pharmaceutical industry, through the identification of novel therapeutic targets, the ataxia patient community through the increased knowledge of the development of the disease and the potential for treatments, and clinicians, through the identification of potential new diagnostics of early-stage predictors/risk factors for</td></tr>
</table>

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

	later disease onset.
What species and approximate numbers of animals do you expect to use over what period of time?	We will mainly use mice (about 500 per year) and on occasion rats (about 50 per year)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The main adverse effect is that the animals will be uncoordinated and have poor balance but this will be mild and only visible when the animals are attempting motor tasks. Normal cage behaviour will not be affected and the animals' life span will not be affected. Some animals will undergo surgery but this will be under general anaesthetic and following surgery the animals will be monitored carefully for any sign of discomfort. The animals will be humanely culled at the end.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Transformed cell lines cannot recapitulate the workings of the cerebellum which is a highly complex network of specialized cells and so animals are required to obtain these specific cells and model a human disease so that therapies can be developed and tested prior to clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Initially we will address questions with cells and slices, obtaining the maximum amount of information from each animal as sample replicates can be obtained from individuals. Only when promising strategies are identified will we progress to live animal work and the minimum number of animals that provide a robust statistical power will be used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice lacking the beta-III spectrin protein are a good model to study as they develop symptoms mirroring the human disease, possess quantifiable features allowing therapeutic success to be clearly determined and yet the disease does not affect the general cage life of the mouse. Excellent care for the animals will be provided, specialised equipment used for all the behaviour and surgical procedures and advice from vets sought immediately for any welfare issue.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms underlying abnormal heart rhythm</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Arrhythmia, heart disease, sudden cardiac death</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td></tr>
<tr><td>Regulatory use and routine production</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>No</td></tr>
<tr><td>Preservation of species</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</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>Disorders of the rhythm of the heart are an important cause of death in clinical medicine. For example, sudden death due to ventricular arrhythmia, roughly atypical beating patterns of the heart, may account for up to 11% of unexpected deaths. Our proposal focuses on how disturbances in heart cells lead to cardiac arrhythmias and how systems such as the nervous system or hormones in the body might regulate this pathology in genetically modified rodents.</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 hope our studies will lead to increased understanding of the pathobiology of heart rhythm disorders, animal models of human arrhythmic syndromes and potentially new therapies for those diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Recent technological advances have allowed researchers to manipulate the rodent in particular the genes of proteins in heart cells in increasingly sophisticated ways. This allows us to study how genes can cause disease in the living animal with far greater translational potential than was previously possible. Studies will be conducted using rats and mice including animals that have been bred with genetic alteration that enable us to study the effects of individual genes of interest. We will monitor the heart beat and pattern and how well it contracts. In particular we will use imaging and electrophysiology techniques to understand how loss or too much of these proteins lead to normal and abnormal heart rhythm. In addition, we aim to ask how these arrhythmia causing genes might</td></tr>
</table>

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

	interact with the commonest cause of sudden arrhythmic death for example blockage of the arteries supplying the heart. We anticipate studying 1500 mice and 100 rats\year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	A stepwise approach will be used so that initial studies will be carried out using tissues from animals bred under the licence or will be done under a general anaesthetic from which the animals will not recover. As the lines of enquiry progress studies may involve surgery to, for example, implant devices that allow blood pressure and other measurements to be made. Some of these transmit radio waves to allow recordings to be taken from the animal following its recovery from the surgery without the animal being aware that the measurements are being made. In some studies the animals use wheels or treadmills so that the effects of exercise can be assessed. The protocols which cause the most effect for the animals involve an operation to place a thread around a coronary artery so that the blood supply to part of the heart is restricted to mimic a heart attack or to apply pressure around a major blood vessel in the abdomen to mimic high blood pressure. All surgery is conducted using general anaesthetics and the same types of measures to prevent infection as are used in human operating theatres. The animals will receive pain killers following the surgery. The animals are also monitored very closely and will be euthanased to prevent unnecessary suffering if they develop signs set out in the licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The analysis of cardiac rhythm and associated pathophysiological situations requires the use of intact animals. Complex physiological processes involving the function of a number of interacting body systems are being examined. Heart function requires the in vivo function of several distinct organ systems including a functioning nervous system, vascular and renal function and respiratory function. As such these cannot be reconstituted fully using *in vitro* experiments. Thus such analyses require an in vivo analysis of the integration of the function of different organs and for the results to be extrapolated to human physiology these analyses need to be performed in intact animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals used will be minimised by maximising tissue use from each animal and by designing experiments according to good statistical and scientific principles. Important experimental design features will ensure that the correct physiological conclusions are reached.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Using new genetic technologies we can delete genes in only some organs and\or at only some times during the lifetime of the animal. In general this allows us to reduce the severity of overall impact to the animals' health. The most severe

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	aspects of the proposal are the models of heart attack achieved by tying off the coronary artery and increased blood pressure achieved by constricting the main blood vessel leaving the heart. The ligation of the coronary artery mimics the process of thrombotic vascular occlusion in the large arteries of man during myocardial infarction and constricting the aorta that of hypertension and\or aortic stenosis. There are no real alternatives to mimicking these important pathological processes in a controlled fashion. It should be borne in mind that cardiac arrhythmia is a significant health problem in these settings and results in a significant numbers of patients feeling unwell or even dying. We are developing and using cutting edge technologies that remove the need for surgery during data collection. For example the “ECGenie” which avoids the need to implant telemetry devices and sequential imaging techniques reduce animal numbers as it is possible to watch the progression of pathology in a single animal.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Reproductive and Juvenile Animal Toxicity Testing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Reproductive Toxicity Testing</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></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>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[16]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The primary objective of this work is to make a preliminary assessment of the effects of candidate drugs on reproduction. This includes assessment of effects on fertility, embryofetal development and effects on young animals. Follow up work to characterise or investigate effects on reproduction may also be undertaken.</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 work in this project licence is required by international regulations prior to undertaking Phase III clinical trials. The work provides information that is provided to clinical trial participants during the informed consent process regards whether the candidate drug is likely to affect fertility or harm an unborn child. The studies inform on the the need for birth control in clinical trials.
The information from young animals contributes to the design of paediatric programmes and can influence the age of children enrolled in clinical trials and various aspects of the trial design.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>This licence expects to use up to 100 rabbits, mice and rats 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>All the procedures on this licence are of moderate severity. The animals will receive test compounds at doses which may affect their food consumption and body weight gain. The aim is use doses which cause a minimal but measurable effect. This would typically be a decrement in food consumption or body weight gain in a pregnant animal compared to control animals, or a change in an parameter</td></tr>
</table>

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

	measured in the blood . All animals will be euthanased at the end of the experiment. This is often at the end of gestation to permit a detailed examination of fetal development
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Reproductive toxicity testing (in a rodent and non-rodent species) is a regulatory requirement prior to undertaking large scale clinical trials. The regulations conclude that non-animal alternatives can assist in the reproductive toxicity testing cascade but cannot replace the work in mammals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will be minimised by 1. Wherever possible, postponing this specialist work until there is evidence of successful outcome from Phase I and / or Phase II clinical trials. 1. Following international guidelines for regulatory work 2. Seeking professional statistical advice for novel bespoke study designs.
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.	International guidelines require developmental toxicity testing in a rodent and non-rodent species. The "default" non-rodent species is rabbit. On occasion, due to lack of tolerability, metabolism differences or pharmacological relevance, the rabbit is not suitable and alternative non-rodent species include mini-pig, dog and cynomolgus. Use of laboratory strains of rodents and rabbits is considered more refined than these alternative species. Minipig, dog and primate work will not be done under this licence. The most important general measures taken to minimise welfare effects to the animals will be: • Use of trained staff & adherence to site welfare and procedural guidances. • Careful consideration of dose selection • Use of staggered starts with only a small number of animals initially tested. This allow dose adjustment of subsequent cohorts.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The Study of Transplant Injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Kidney; Immunology; Transplant rejection</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[17]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>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[18]</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 provide insight into mechanisms of how kidney transplants are rejected. Proteins of the immune system, the complement proteins, are known to accelerate rejection of transplanted kidneys. The object of the project is two-fold. Firstly, to understand the way complement proteins direct transplant rejection. Secondly, to test therapeutic inhibitors of complement that will decrease the rate of rejection and prolong the life of a transplanted kidney.</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>Of immediate benefit is the generation of knowledge into the mechanisms by which complement proteins damage a transplanted kidney. This knowledge will be beneficial as it will aid the development of therapeutic agents that can be directed to complement proteins at the time of transplantation. These therapies targeting complement proteins will benefit human transplant recipients by helping to prolong graft survival and therefore provide a better quality of life for patients. In the future, stem cell technology could lead to the generation of organs for transplantation that do not express complement proteins which could be a valuable advance to this programme of work.</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 both mice and rats in this project. In the region of 25,000 animals will be used over the 5years of the project. Most of these will be animals bred to produce or to not produce specific proteins involved in the rejection of transplants, so that the mechanisms for this may be determined. This knowledge may be used to determine new treatment approaches to the avoidance of</td></tr>
</table>

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

	transplant rejection. Of these animals 8000 mice and 3800 rats are used in experiments to determine the function of specific proteins in transplant rejection.
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?	Adverse effects that may be experienced following the transplantation procedure include: lack of appetite, reduction in body temperature (shivering), hunched posture, reduced mobility and piloerection. Rarely, with an incidence of less than 5%, there may be irritation of the face, neck and back that may result in scratch injuries. Adverse effects are expected to be at a mild to moderate level of severity. At the end of a study, the animals will be killed in a humane fashion.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project is designed to study the mechanism of whole organ transplant injury and accordingly, only the use of animals will allow such a study. Initially, before any animals are used, all treatments will first be tested in the laboratory using non-animal methods.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experienced transplantation surgeons achieve good reproducibility of the transplant procedure. Accordingly, we will use small numbers of animals in each experimental group, typically 7-10. For kidney transplants, one donor animal will provide one organ for transplantation into one recipient. Within our department we have a dedicated statistician who provides advice on study design and provides calculations to accurately assess the smallest number of animals required for statistically meaningful results to be 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.	Rodents (mice and rats) have been chosen because of the number of genetically modified strains available to work with (particularly mice). We have some mice that do express complement proteins and some that do not, which will allow us to directly compare the two and dissect the role of complement proteins in kidney transplantation. Rats will also be used for renal transplant studies to further characterize the role of complement as some inhibitors of complement are known to inhibit rat complement more efficiently than mouse complement. In addition, the surgical procedure is shorter in rats. Measures are taken to minimize harm to the animals. Firstly, risk of infection in the animals is minimized by applying strict asepsis throughout all procedures. Secondly, animals will be under anaesthesia for relatively short periods of time to reduce harm to them. We will avoid any post-operative discomfort to the animals by regular

	monitoring and providing pain control in the form of analgesia (peri-operative analgesia as advised by the NVS will be used) and keeping animals comfortable in a special warm environment in which the temperature is maintained at 28ºC for 24 hours post-operatively. Animals showing adverse effects may receive supportive care and treatment as advised by the Named Veterinary Surgeon or Named Animal Care and Welfare Officer or they will be humanely killed if their condition gives particular cause for concern.