Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 3

Project title and keywords

- Vaccines against bacterial disease

  Vaccine, Clostridium difficile, Clostridium perfringens, Burkholderia pseudomallei

- The role of the calcium receptor, CaSR, in disease

  Calcium, cardiovascular disease, lung disease, kidney disease

- Cancer-induced bone pain

  Pain, bone, cancer Src, neuroscience

- Rederivation and health screening of genetically altered animals

  GA Rodents; Rederivation; health screening; Cryopreservation

- Developmental mechanisms of mammalian cortical development

  Brain, Cerebral cortex, neural progenitors

- Molecular imaging assessment of therapeutic strategies in animal models of disease

  Molecular Imaging, Cancer, Therapy

- Sleep Behaviour of Zebrafish

  Sleep, zebrafish, behaviour

- Epithelial transport in health and disease

  Diabetes, Chronic Renal Disease, Homeostasis, Epithelial Transport, Gut-Renal Interactions

- Tissue-engineered allografts for congenital heart defects

  Stem Cell; Congenital heart disease

- Closed-loop BMIs for upper-limb function

  Motor disorders, neural prosthetics, spinal Cord

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Vaccines against bacterial disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Vaccine, Clostridium difficile, Clostridium perfringens, Burkholderia pseudomallei</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>
</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 aims of this work are to further understand how three different bacteria, Burkholderia pseudomallei, Clostridium difficile and Clostridium perfringens, cause disease. B. pseudomallei causes melioidosis, a disease of humans in tropical and sub-tropical countries which is difficult to treat with antibiotics. C. difficile causes a severe gastrointestinal disease which is frequent in hopsitalised patients and is often difficult to treat. C. perfringens causes a range of diseases in livestock throughout the world. The work is planned in 3 stages: the establishment of murine infection models; the identification of vaccine candidates in murine infection models; protection studies in mice with candidate vaccines.</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>There are no good licensed vaccines for the diseases we are studying and we plan to use the information we obtain to devise vaccines. These vaccines would be used to immunise susceptible humans and animals, thereby protecting them from these life-threatening diseases. In addition, the new information we obtain, describing how these bacteria cause disease, will be of wider importance to scientists and clinicians. It will enable them to advance their work to develop new diagnostics, pre-treatments and therapies for disease caused by these bacteria.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>We plan to use up to 1,500 purpose-bred mice for these studies</td></tr>
</table>

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

over what period of time?	
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 work will involve infecting mice with the pathogens or toxins produced by the bacteria outlined above. Disease caused by *B. pseudomallei* and *C. perfringens* in mice is fatal without intervention. *C. difficile* infection in mice is characterised by the bacteria colonising the gut and without mice showing signs of disease. Therefore, some of the work will be of substantial severity. All animals will be killed using a Schedule 1 (or exsanguinated under terminal anaesthesia) method, either when they shows signs of disease or at the end of each study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Before carrying out the work outlined in this proposal we will, where possible, have used biochemical assays and cell culture systems. Where possible we will use *Galleria mellonella* (waxmoth larvae) to investigate the pathogenesis of disease and the behaviour of mutants. We will use *in vitro* and *in silico* approaches to identify and pre-screen vaccine candidates. To confirm the role of virulence associated genes in disease of humans and mammals and to evaluate vaccines we will use relevant murine models of infection as at present the above models are unsuitable for assessing virulence and vaccine efficacy in the context of host innate and adaptive immune responses.
2. Reduction Explain how you will assure the use of minimum numbers of animals	**We will carry out a power analysis to determine minimum group sizes required consistent with a power of 80% and a significance level (*P*) of 0.05 for comparison of test and control groups. Some studies will involve a side-by-side comparison of the virulence of wild-type and mutants. To reduce the number of animals we will, where possible, test several mutants simultaneously allowing us to reduce the number of control mice used. For the generation of antisera we will immunise groups of 3 mice to ensure that even if one animal fails to respond to the immunisation we will be able to obtain sera from 2 animals which will then be pooled.**
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use commercially available inbred mice for animal studies. Mice have been used widely as infection models for the pathogens we are working with. To minimise suffering we will use humane end points and animals which develop disease will be killed using a schedule 1 method. Refined indicators of protection such as changes in weight and body temperature will allow us to refine humane end points at the early stages of disease.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The role of the calcium receptor, CaSR, in disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Calcium, cardiovascular disease, lung disease, kidney disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>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>Our blood calcium is controlled by a protein called the calcium sensing receptor (CaSR). Mutations in the CaSR cause disorders of calcium metabolism and result in cardiovascular, kidney and lung diseases. The objectives of these studies are to use genetically engineered mice to: 1) understand how loss of CaSR controls the development of the kidney, heart and blood vessels in the womb, and; 2) to determine how loss of CaSR causes adult kidney, cardiovascular and lung 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>Drugs that activate the CaSR are already being used to treat kidney disease and are safe in humans. Our studies will determine whether we can use these drugs to rescue problems of lung and kidney development in the womb, or for cardiovascular disorders, which arise as a consequence of high blood pressure or diabetes.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Genetically modified mice have been chosen because they mimic the human disease. We will delete the CaSR only from the blood vessels, which would be impossible in humans. To ensure that we only use animals that are necessary, we have consulted our University statistician. For objective 1 and 2, we will use no more than 1000 and 2880 mice over 5 years, respectively.</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>Blood pressure will be measured non-invasively by placing a cuff (similar to that used in people) around the tail of the animals. Urine and faeces will be collected by housing animals in cages where they will have free access to food and water. The genetic modification could lead to blood pressure changes, which might lead to cardiovascular deaths (severity level: moderate). To reduce this risk, we</td></tr>
</table>

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

	will keep our breeding colonies only up to 12 months as they are more likely to occur in older animals. Treatment with drugs that activate the CaSR should reduce the risk of cardiovascular death. All animals will be checked every day and, if cardiovascular problems emerge, we will immediately notify the Home Office. All animals will be killed using schedule 1 methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We would not be able to perform the proposed studies in cell lines because we would lose the interaction between the cardiovascular, kidney and lungs. These interactions will be studied independently of the mother's influence and without the confounding effects of changes in Ca^{2+} metabolism, which would occur if we took the receptor away from the whole mouse body. Where possible, we will use human tissue (fetal and adult) to confirm the validity of our studies in mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All the experiments we plan to carry out are fully established in our laboratory and will allow for a significant REDUCTION in the number of experimental animals. All the studies will be carried out in blood vessels, kidneys and lungs from the same animals, which will lead to a significant REDUCTION in the number of experiments. MRI studies will produce images from the whole body, allowing for a significant REDUCTION in the number of animals. Long-term studies using non-invasive measurements of blood pressure and of lung function will inform us on how cardiovascular, kidney and lung diseases develop over time.
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.	All the methods of keeping the colony are approved by our local veterinary and are in line with Home Office regulations. Breeding and housing conditions aim at reducing stress to the minimum and will include enriched environment and gentle handling to reduce anxiety levels. For measurements of blood pressure and of lung function, animals will be allowed to familiarise themselves with the equipment before the experiments. MRI studies will be conducted under general anaesthesia. Blood samples will be collected from the tail vein after application of a local anaesthetic cream.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Cancer-induced bone pain</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pain, bone, cancer Src, neuroscience</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><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[6]</td><td><s>Yes</s></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 on cancer-induced bone pain will test whether the an experimental Src inhibitor has efficacy as an analgesic.</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 primary potential benefit relates to new knowledge about the role of Src in cancer-induced bone pain. A secondary potential benefit relates to the future use of the drug on humans: if the pre-clinical data are supportive we will perform a phase II clinical trial on human cancer patients who have painful bone metastases from their disease to determine if the drug has clinical efficacy.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will use about 300 rats over the 5 years of the licence</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The expected adverse effects are mainly due to the growth of cancer cells in bone, which will cause a dull pain that increases over time. Some limping is also expected, but this will not be allowed to progress significantly. There are no known toxic effects of the drug at the dose that we will be using.
The expected level of severity is moderate.
At the end of the study the animals will be humanely killed.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement
State why you need to use animals and why you cannot</td><td>It is necessary to use animals for this work because it would not be ethical to perform them on humans – we are studying the changes that occur in the</td></tr>
</table>

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

use non-animal alternatives	nervous system in bone cancer pain, which requires invasive surgery and the removal of tissue samples; these procedures could not be done on humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals required was calculated using statistics to estimate how few would be required to be able to detect a sufficiently large effect with confidence.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use laboratory rats because this species is the lowest on the evolutionary tree that displays behaviours that are thought to be similar to those of humans. Pain and suffering are kept as low as possible by: 1. Performing all surgery under general anaesthetic, and giving appropriate pain relief as necessary. 2. Keeping the survival period as short as possible. 3. Having agreed welfare standards so that no animal can suffer un-necessarily.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Rederivation and health screening of genetically altered animals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>GA Rodents; Rederivation; health screening; Cryopreservation</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)[7]</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[8]</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>Our specific objectives are:
1. To rederive infected lines of valuable transgenic mice.
2. To permit the implantation of imported rodent embryos into recipients, so these models can be studied on existing research projects.
3. To permit cryopreservation of rodent embryos and subsequent reimplantation at a later date, in order to reduce the number of live animals maintained for breeding purposes.
4. To monitor the health status of immunocompromised 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>By providing a centralized rederivation and cryopreservation programme, it is possible to ensure that best husbandry conditions are maintained along with detailed record keeping of breeding, health and welfare. Standardisation of procedures will reduce experimental variability, increase the significance of results and reduce the number of animals needed.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately 27,000 laboratory mice will be used over a 5 year period..</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td>Over 5 years approximately 10,000 genetically altered mice will be born. The great majority of these will be examined with mild, non-invasive</td></tr>
</table>

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

effects and the likely/expected level of severity? What will happen to the animals at the end?	techniques. Some may be given injection of substance or via the drinking water, that alter the way the genetic alteration behaves. Some will have blood samples taken and all will ultimately be humanely killed. Up to 10,000 female mice will be humanely killed to donate eggs and embryos after hormone treatment. Another 6,000 mice will be used as surrogate mothers for embryos and where possible non-surgical methods will be used to implant the embryos. Another 90 will be used to make sterile males which are required in the production of genetically altered mice.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The human genome has been sequenced, as have a number of other genomes (partially or completely), notably the mouse. However, the function of many genes is not known or fully understood, either individually or in the ways they interact to produce their intended effects, or how they are dysfunctional in disease. To better understand normal physiological processes and abnormal disease processes requires, when necessary and justified, the use of whole animal models
2. Reduction Explain how you will assure the use of minimum numbers of animals	Regular discussions with scientists take place to avoid animal overproduction. A centralised service is administratively efficient, with breeding controlled to produce batches of animals as needed and any spare can be made available to different scientific projects If the direction of the work changes and GA strains are no longer required, rather than simply carry on breeding, embryos in very early stages of development can be harvested and frozen at very low temperatures to preserve them. This process is known as cryo-preservation.
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.	Rederivation by embryo transfer, compared to caesarean section, decreases the risk of a foster mother rejecting pups. This method eliminates the need for precise timing of caesarean surgery, a common obstacle with transgenic lines in which pregnancy length often varies Any animal undergoing recovery surgery will receive routine analgesia and will checked frequently post operatively. The majority of the genetically altered mice that do have altered biology have only mild symptoms that do not lead to pain of suffering .Mouse passports provide details on strain mutation,

	husbandry and welfare issues. Animals are cared for by a dedicated team of highly trained technologists. Environmental enrichment, including nesting material, is provided to each animal to allow mice to accommodate natural behaviour. Specific areas where refinement is applied include alternative samples to tail tipping for genotyping. The use of non-invasive methods for genotyping will be explored (for example; ear notching, saliva sampling, hair plucking etc).

Developmental mechanisms of mammalian cortical development

Brain, Cerebral cortex, neural progenitors

5 years

Basic research	Yes	
Translational and applied research		No
Regulatory use and routine production		No
Protection of the natural environment in the interests of the health or welfare of humans or animals		No
Preservation of species		No
Higher education or training	Yes	
Forensic enquiries		No
Maintenance of colonies of genetically altered animals[9]	Yes	

The structure of the mammalian cortex is complex containing hundreds of different neuronal types and glial cells, all of which are generated from neural progenitors Our aim is to decode the mechanisms of 1) how the progenitors become neurogenic from expansive, 2) how the progenitors generate various types of neurons and glia, and 3) how the generated neurons establish proper connectivity, which are the crucial to understand how our brains undergo healthy development.

The knowledge from these studies would contribute to the understanding of underlying causes of psychiatric disorders which stem from neurodevelopmental abnormalities, such as schizophrenia, bipolar disorder, and autism. In addition, answering how and when the cortical progenitors lose their competency of generating all types of cortical neurons will greatly contribute to the insights how we could manipulate differentiated cells to reprogram them back to self-renewing/multipotent progenitor cells. This would be useful for regenerative purposes of the brain, which lacks self-renewing ability due to causes such as ischemia or neurodegenerative diseases.

Over the 5 years the maximum number of mouse and rat required for this study is 9000 mice and 500 rats respectively. However we will minimise the number of animals wherever possible by taking alternative approaches.

Most of the procedures *are expected to result in no more than transient discomfort and no lasting harm. In procedures employing the surgical techniques, Animals are expected to make a rapid and unremarkable recovery from this surgical procedure. Any animals showing any deviation from normal health or well-being will be killed.*

Our studies focus on the development of the cortex, which is a unique anatomical structure in the mammalian species. Therefore the use of animals which have the "cortex" is absolutely essential for this study.

The proposed research is, however, preceded, informed and complemented by *in vitro* work including dissociation cell culture, slice brain culture as well as the usage of iPS/ES cell delivered neural progenitors. In this bottom-up approach, experiments on neuronal and ES/iPS cultures will be used to plan the most crucial and effective experiments to perform *in vivo*, and to provide effect size and variability estimates to allow power analyses for minimal group size calculations. We will also continue to provide quantitative data to computational neuroscientists interested in modelling our results, with the aim

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

of producing accurate mathematical models of neuronal development in the future.

A number of measures will be taken to ensure the minimum number of animals will be used in the proposed research. We will employ the following experimental guidelines to ensure maximum data quality from every animal:

1. High quality animal welfare to minimise suffering and distress, reducing inter-animal variability. This includes refinement of surgical techniques where possible
2. Randomised and blind group experimental design
3. Analysis blind to experimental group
4. Appropriate statistical analysis

Our initial experiments will be pilot studies, informed by our in vitro data as to expected effects, variability, and group size by power analysis. The results of these pilot experiments will then be used to inform and refine future factorial experimental designs.

Most of the proposed animal experiments will use mice, the simplest available mammalian model with the cortical structure. Using mice will enable us to build on a decent bank of existing knowledge concerning cortical development and cell connectivity and furthermore to include well characterised genetically altered lines.

The methods outlined in the protocols below have been carefully chosen to fit these objectives while producing the minimal possible animal suffering. Of course, as detailed in the protocols, good surgical technique, animal husbandry and veterinary advice will ensure that all procedures cause the minimal possible suffering.

We do not propose any protocols with substantial severity.

Project Title (max. 50 characters)	Molecular imaging assessment of therapeutic strategies in animal models of disease
Key Words (max. 5 words)	Molecular Imaging, Cancer, Therapy
Expected duration of the project (yrs)	5
Purpose of the project (as in Article 5)[10]	Basic research ~~Yes~~ **No** Translational and applied research **Yes** ~~No~~ Regulatory use and routine production ~~Yes~~ **No** Protection of the natural environment in the interests of the health or welfare of humans or animals ~~Yes~~ **No** Preservation of species ~~Yes~~ **No** Higher education or training ~~Yes~~ **No** Forensic enquiries ~~Yes~~ **No** Maintenance of colonies of genetically altered animals[11] ~~Yes~~ **No**
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to use modern scanning techniques to study the efficacy or mechanism of new treatments for cancer in small animal types of human disease to predict their likely clinical performance in patients or to provide additional useful information on the performance of a drug candidate in a living animal..
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)?	Most well-established anti-cancer therapies are relatively toxic and, unfortunately, have limited efficacy in many sorts of this disease. There is therefore a need for more effective and less toxic cancer treatments. Many new such treatments are currently in development or are expected in future years. However, assessing the utility of these new drugs is complicated, slow and expensive resulting in long delays before patients can claim the clinical benefits. Whole-body scanning is a way of accelerating this process since it enables therapeutic effects to be assessed more rapidly than conventional studies which rely on monitoring the life-span of animals..
What species and approximate numbers of animals do you expect to use over what period of time?	Mice or rats. We envisage using a maximum of about 500 mice and 20 rats 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	To reproduce the human disease in the animal we need to inject potentially harmful agents such as tumour cells or infectious organisms. This may result in the same consequences as those seen in patients with these diseases such as localised or

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

happen to the animals at the end?	more general pain or, as the disease progresses, interference with normal body functions such as excretion or moving around. Animals need to undergo scanning procedures and are anaesthetised to make sure they stay still which may result in some discomfort such as dry mouth and windpipe. We also need to inject drugs to treat the disease which may themselves have side effects. Animals are killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Live animals need to be used for this type of research since we need is to determine the effects of the drugs in a living creature. However a broad range of non-animal studies will be undertaken prior to the procedure to ensure that the study is appropriate and that all the data that can be obtained without the use of experimental animals is available. These include analytical studies to assess the performance of the tracers used for imaging and studies to assess the action of the experimental treatments on cell lines. Only those drugs which perform most successfully in these in-vitro studies will proceed to in-vivo evaluation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical tests will be performed before starting a study to predict the numbers of animals required to produce a certain expected result. One of the advantages of whole-body scanning is that it allows us to perform successive studies on the same animals without any invasive procedures. This significantly reduces the overall numbers of animals required for such 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.	The animal model we use for the imaging procedures must be relevant to human biology. We have therefore chosen the lowest level of mammals available for these studies i.e. mice and rats. We limit the distress caused by inducing human diseases in animals by using pain-killers or other drugs or by killing the animal before the disease progresses too far. We limit the number of anaesthetic procedures that the animals undergo. Wherever possible we use drug treatments that are not expected to be toxic since these are preferred for eventual use in patients however sometimes they have unexpected side effects and animals will be killed if they are judged to be suffering as a result of these

Project Title (max. 50 characters)	**Sleep Behaviour of Zebrafish**
Key Words (max. 5 words)	Sleep, zebrafish, behaviour
Expected duration of the project (yrs)	5 years
Purpose of the project (as in Article 5)[12]	Basic research: Yes / No Translational and applied research: Yes / No Regulatory use and routine production: Yes / No Protection of the natural environment in the interests of the health or welfare of humans or animals: Yes / No Preservation of species: Yes / No Higher education or training: Yes / No Forensic enquiries: Yes / No Maintenance of colonies of genetically altered animals[13]: Yes / No
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Although many sleep-altering drugs are available in the clinic, how these work to alter brain function is unknown. In addition, there is a clinical need for better sleep-regulating drugs, as many of those currently available have unwanted side effects.
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)?	If we understood how the currently available sleep drugs worked in the brain, we may be able to develop more selective drugs with fewer side effects. In addition, there are many forms of insomnia that remain untreated by current medications. Using the zebrafish to search for novel drugs with sleep-altering activity is the first step to identify new potential sleep drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	We are using zebrafish larvae, because they are useful for screening thousands of compounds. We will screen drug effects in thousands of animals over 5 years (~10,000 animals/year). Such a screen would be undesirable in a rodent model because of the cost and number of animals needed.
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?	Because we carefully prescreen for toxic effects, and continuously monitor animals during the experiment, the alterations to the larval behaviour are expected to be mild. The fish will sleep more or less but should otherwise experience few adverse effects. All animals exposed to drugs are sacrificed upon toxic effects, or at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	We are studying the complex interactions that take place in the brain. This is impossible to replicate in a dish of cells. Eventually, we will have collected

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

use non-animal alternatives	enough behavioural data that some aspects of the behaviour could be modelled by computers.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use statistics to ensure the minimum number of animals is used in our experiments while still providing useful data. We design our experiments based on large databases of larval drug responses, which further minimises the number of animals needed.
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.	Zebrafish are the least complex vertebrate model. Because their brain is similar to mammals, they represent the simplest animal model for studying sleep that will be directly relevant to human health. We also perform the experiments at very young larval stages, when their capacity for suffering is minimised. To mitigate any harm to the animals, we constantly monitor our experiments with video and test beforehand on small numbers for toxic drug effects.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Epithelial transport in health and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Diabetes, Chronic Renal Disease, Homeostasis, Epithelial Transport, Gut-Renal Interactions</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)[14]</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[15]</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>It is recognised that the Western diet, particularly the consumption of processed ‘junk’ food increases the risk of developing obesity and type II diabetes, and that this condition increases the individual’s chance of developing chronic kidney disease. Recent findings suggest that imbalance in levels of glucose and phosphate in the body caused by these conditions can have detrimental outcomes on cardiovascular health. It is accepted that the intestine and kidney are the main organs involved in maintaining glucose and phosphate homeostasis but how these organs interact and whether this process is detrimentally altered during disease has not been established.</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 provide new insights into the mechanisms of glucose and phosphate homeostasis and how this process is altered by diet and disease. The aim is to identify novel targets for controlling hyperglycaemia and hyperphosphatemia in diabetes and chronic renal failure. Overall these results are expected to benefit the development of new strategies for preventing the detrimental effects of glucose and phosphate on vascular calcification and cardiovascular health.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately 1500 rats and 370 mice will be used over the 5 year period.</td></tr>
</table>

[14] Delete Yes or No as appropriate.
[15] 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 genetically and surgically altered models that will be used in this proposal have all been characterised in detail by a number of different research groups. In addition, with the exception of the Roux-en-y gastric bypass (RYGB) procedure, the applicant has experience of all the surgical techniques and animal models described in this proposal. An ongoing collaboration with the individual who originally described the RYGB technique is in place which will enable the applicant to learn this technique. Based on our previous studies animals undergoing 5/6 nephrectomy surgery, induction of diabetes using streptozotocin, or receiving dietary manipulation display no adverse effect at the end points proposed in this study. The possibility of post-surgical infection is minimised by using aseptic conditions during surgery and animals will receive subcutaneous analgesic as required to reduce post-operative pain. The techniques described on this proposal are of mild to moderate severity. All animals undergoing a procedure will be weighed at regular intervals and any showing loss of weight greater than 20%, associated with other clinical signs of distress, will be killed by a Schedule I method. In the event that any animal shows signs beyond those described in this licence, the NVS will be contacted for advice and treatment. If such treatment fails to alleviate the symptoms, the animal will be killed by a Schedule 1 method (cervical dislocation or anaesthetic overdose).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	An important aspect of the proposed work is to establish how the intestine and kidney interact to regulate levels of glucose and phosphate in the body. This is likely to involve the intestine releasing factors into the blood which then circulate around the body and act on the kidney to adjust transport. Diabetes and chronic renal failure are likely to affect these interactions. These complex interactions cannot be reproduced under cell culture conditions making this method inappropriate for use in this project. In addition, cell lines available often have different transport and structural properties than native tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preparation of membrane vesicles from rat intestine and kidney is well-established resulting in higher yields than mouse tissue for subsequent in vitro studies. In most instances rats will be used for

	these studies to ensure animal usage is kept to a minimum. Wherever possible changes in renal and intestinal transport will be established in one animal in order to reduce animal numbers. In all instances blood, small intestine and kidney will be harvested from each animal at the end of any procedure in order to correlate changes in transport rates with changes in transporter expression. This approach not only reduces animal usage but also allows more detailed and precise interpretation of data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In most instances the rat is the animal of choice for these studies since there is extensive information available on renal and intestinal glucose and phosphate transport. In addition, surgical or chemical induction of chronic renal failure or diabetes is generally easier and more reproducible in rats. However, genetically modified mice, *such as those lacking the gene for specific transporter proteins* will also be used under this licence. The protocols used in this proposal have been designed to be the most refined possible, using the minimum number of animals, to provide statistically satisfactory results. These protocols have been planned to cause the least pain, suffering or distress whilst adequately addressing the scientific question they have been designed to answer.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Tissue-engineered allografts for congenital heart defects</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem Cell; Congenital Heart Disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>2</td></tr>
<tr><td>Purpose of the project (as in Article 5)[16]</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[17]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This study aims to determine whether the viability of arterial grafts can be improved by coating them with stem cells taken from the recipient prior to implantation.</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>• Tissue-engineered valves, conduits and vascular patches made of living tissue could function like a native structure, with the potential to grow, repair and remodel
• Longer lasting therapeutic effect than the acellular grafts currently used
• Fewer re-operations, better survival and quality of life
• Reduced cost to the NHS</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Landrace piglets
Around 30 piglets in 2 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>We will use the same anesthetic and surgical techniques we use for human congenital heart surgery. Complications might include bleeding, pneumothorax, respiratory distress. All these complications and their treatment have been considered in the PIL application.
The animals will have to be sacrificed after 6-9 months period for the biological assessment of the</td></tr>
</table>

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

	implanted conduits.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have examined the seeded conduits extensively using *in vitro* techniques, but the only way to complete our studies prior to clinical studies is to use animal models. The *in vitro* studies provide proof of concept, but in order to ascertain the safety and efficacy of the proposed interventions they need to be tested in a relevant live animal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We intend to use a minimum number of animals (we will make sure only one animal at times will be done and fully recovered before embarking in subsequent operation), and thus have selected prosthetic valve performance (velocity of blood through the graft) as the primary outcome and marker of success (Leon *et al.,* Eur Heart J 2011; 32:205-17), as it is in clinical practice. Normal peak velocity in clinically successful grafts is ~2.5 m/s: we anticipate that flow in the control grafts at 6 months will be >3.0 m/s, with a standard deviation of 0.2 m/s (approximated from our own clinical data). Thus to ensure a power of 80% (significance level of 0.05, correlation of 0.8), we will need to use at least 6 pigs per group. Assuming a failure rate of approximately 20% we will need 8-10 pigs in each group to adequately.
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 piglet has been chosen for this work because there are no realistic alternatives. Piglets are the most refined model for the purpose of our study because their size and anatomy means that the surgery closely replicates that performed in children. Establishment of CPB and intra-operative manipulations, with consequent trauma due to surgery, will be mimicked very closely. This will enable a study that closely reflects the clinical setting, with the inherent mechanical and physiological insult from use of the CBP apparatus.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Closed-loop BMIs for upper-limb function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Motor disorders, Neural prosthetics, Spinal Cord</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[18]</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[19]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project will develop devices called closed-loop Brain Machine Interfaces (BMIs) which could replace or rehabilitate hand function following paralysis. BMIs use tiny electrodes in the brain to record electrical activity and decode motor intentions. These can be relayed to assistive devices like computers and wheelchairs, or relayed to the spinal cord to create a bridge across damaged regions.</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>These experiments will provide valuable scientific information about how the brain controls natural movements and learns from experience. In addition we hope this project may lead to clinical devices that can be used with a large number of patients to help restore the ability to move following paralysis.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The project will use 12 monkeys over 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The likelihood of adverse effects from implants (e.g. infection) are minimised by implantation in sterile conditions under general anaesthesia as is routinely used for human surgery, with post-operative analgesics. If any infection does not respond to antibiotics, the implant will be removed or reimplanted. Possible adverse effects from brain and spinal cord implants include weakness or sensory loss in the limbs. In the event of severe, persistent paralysis or other signs of major</td></tr>
</table>

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

	compromise to welfare the animals will be killed. The animals will be restrained by a loose neck collar in a chair for experiments, which they are trained to accept using positive reinforcement techniques. The food intake will be controlled so that food reward can be used to train animals to control BMIs. Weight, behaviour and health are assessed regularly to ensure food control is not impacting on the animals' wellbeing. At the end of the experiment the animals are killed painlessly so we can acquire anatomical data to corroborate our findings.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While a clinical device for humans remains the long-term goal of this research, surgically implanting experimental technologies without first demonstrating their effectiveness would be unethical. Electronics can be tested using simulated signals, and we run an active programme of human experiments that use non-invasive techniques, for example recording electromyogram signals from muscles and non-invasive magnetic stimulation of the brain. However, the closed-loop interaction between implanted devices and the brain can only be evaluated using a real system of comparable complexity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use chronic implants and reversible pharmacological inactivation to allow multiple recording sessions to be performed in the same animals. This allows us to maximise the data we collect from each animal and also increases the statistical power of our studies (e.g. allowing within subject statistical comparisons to be made).
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.	Since the goal is to restore hand function to humans, animals with similar upper-limb use must be used. The motor system of the macaque monkey has close homologues to that of man, unlike lower species which use their fore-limbs predominantly for walking. This project utilises a number of technological innovations to reduce animal suffering, including chronic multi-electrode arrays (so awake head fixation is not required). Use of wearable electronics allows some data to be collected during completely unrestrained behaviour in the home cage. Food control is also minimised by attempting to find the least restrictive regime that is consistent with the required number of trials per session. We have developed an automated feeding system that also allows early stages of positive reinforcement training to be conducted in the home cage with no food control. The aim of this project is to test

	devices to restore function with relevance to conditions including spinal cord injury and stroke. We choose to use pharmacological inactivation of a localised area of motor cortex to induce localised motor deficits, because the effect is completely reversible after several hours. This allows us to collect data with relevance to human neurological injury without inducing lasting deficits by lesion or other permanent injury to the animals.