Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 4

Project title and keywords

- **Cardioprotection and regeneration of the injured heart**

  cardiovascular disease, myocardial infarction, epicardium, regeneration

- **HPIVs, virulence and therapy**

  Parpainfluenza, HPIV, HPIVs

- **Development of Radiopharmaceuticals for Molecular Imaging of Cancer**

  Molecular Imaging, Cancer, Early detection, Theranostics, Cancer Therapy

- **Mouse models to study mammary gland morphogenesis**

  mammary gland, GFP, transgene, fibroblasts, epithelium

- **Anticancer effects of cannabinoids and radiation**

  Glioma, cannabinoids, radiation, combination

- **Targeting Wnt signalling in colorectal cancer**

  Wnt signalling, intestinal regeneration, colorectal cancer

- **Hepatocyte Transplantation**

  Liver Disease, Transplantation, Hepatocyte

- **Testis development and role in post-natal health**

  Testis, androgen, brain, development, health

- **Oral vaccine research studies in badgers (Meles meles)**

  Badgers, bait, vaccine, BCG, oral

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Cardioprotection and regeneration of the injured heart</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">cardiovascular disease, myocardial infarction, epicardium, regeneration</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>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></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">As it stands the adult human heart is unable to repair itself following a “heart attack” (myocardial infarction). Heart transplantation remains the only cure, but is compounded by a lack of donor hearts and the requirement for transplant patients to take toxic immune-suppressive drugs to prevent rejection of the donor organ. Hence, there is an urgent need to identify alternate cell-based methods of restoring lost heart muscle and/or blood vessels following a heart attack. The epicardium, an outer layer of cells surrounding the muscle of the heart contributes cardiovascular cells to the forming heart during pregnancy and has recently emerged as a source of resident progenitor cells in the adult mouse heart which can be activated to contribute new muscle and blood vessel cells following a heart attack. This direct contribution is currently inadequate for full repair, but initiated by switching on embryonic epicardial genes; a characteristic event in the adult zebrafish and one-day old mouse pup (neonatal mouse), both of which completely regenerate their hearts following injury. We propose to study adult zebrafish and neonatal mice, in combination, to identify evolutionary-conserved epicardial signals and investigate how epicardial derived cardiovascular cells might be instrumental in driving heart repair. We will screen both models for key molecular changes in the epicardium coincident with ongoing wound healing and tissue restoration and will test the ability of identified</td></tr>
</table>

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

	factors to promote heart muscle and blood vessel growth. We will then apply these “regenerative” epicardial factors (signals) to adult mouse hearts (and ultimately human patient cells) to identify those which might induce therapeutic cardiovascular repair.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A short term benefit will manifest in terms of basic scientific discovery: evidence that the epicardium can be stimulated to either directly contribute new cardiac cells and/or signal to condition repair of the heart will focus the field on an emerging concept of stimulating resident cardiovascular stem/progenitor cells towards repair; as opposed to the alternative of cell transplantation or engraftment which to-date has largely failed in human patient clinical trials. By studying the potential of the epicardium during inherent repair of adult zebrafish and neonatal mouse hearts in parallel we predict the identification of functionally conserved “regenerative signals” which when applied to non-regenerative adult mouse and human model systems may significantly enhance the prospect of identifying drugs to stimulate the epicardium of human patients to invoke cardiovascular repair.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse (*Mus musculus*) and zebrafish (*Danio rerio*). The expected usage of animals is approximately 750 adult mice, 300 neonatal mice and 1360 adult zebrafish per annum (not including breeding and maintenance) over the 5-year lifetime of the project (totals of 3750 adult mice; 1500 neonatal mice and 6800 zebrafish).
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?	We will induce cardiac injury in all three model organisms: adult zebrafish, neonatal and adult mice. This will be done via either excisison removal of a proportion of the heart ventricle muscle, cryo-injury by application of a frozen probe to the muscle or ligation of a coronary artery to block blood flow and induce an infarction (heart attack). These modes of heart injury carry a substantial severity rating and there is a risk of an animal showing signs of premature heart failure after surgery, which will be monitored carefully during the immediate recovery phase and beyond. Animals may also receive substances at the time of operation, and for fixed periods following surgery to assess induction of heart repair; the administration of substances via routes to include directly into the heart muscle carries further risk of bleeding, which will be carefully monitored. After each surgery animals either be humanely culled to collect tissues for assessment or will undergo monitoring of heart function followed by culling according to approved methods.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The heart is a complex organ containing many cell types which interact extensively in a 3D environment to maintain normal heart function. Arguably the most important cells are the myocytes, responsible for the pumping function of the heart and the endothelial and smooth muscle cells, which make up the coronary blood vessels. Many of the experiments we propose will be carried out on isolated pieces of cardiac tissue or cell cultures of cardiomyocytes, vascular or epicardial cells studied in the laboratory. However cells in a test tube or in a tissue culture dish cannot be used to study the complex changes occurring in the heart following injury nor will they enable accurate testing of activated cells or administration of substances which might contribute to wound healing, restore lost tissue and improve overall heart function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All animal studies are informed by prior cell culture assays to test epicardial cell behaviour and the potential of activating factors to induce either pro-reparative signals or the differentiation of epicardial cells into cardiovascular cell types. This ensures predictive insight into responses of the target cell population in the hearts of animal models and, therefore, reduces the need to treat above a minimal number as determined by power calculations. Our exclusive use of highly skilled investigators to carry out the surgical procedures for inducing cardiac injury and treatments ensures minimal variation in the extent of initial injury and the subsequent downstream wound healing response. A low level of baseline variation in injury response means we can minimise each subsequent treatment group against recording a significant effect on cell activation, tissue repair and heart function in vivo.
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 choice of species is based on our proposed study of regenerative animal models, the adult zebrafish and neonatal mouse, which have been extensively characterised in terms of inherent heart repair and subsequent extrapolation to the non-regenerative setting, in this case the adult mouse which fully recapitulates the response to ischaemia and cardiac injury in humans. The genetic tools (transgenic and knockout lines/strain in both fish and rodents) alongside the ease of manipulation of individual cell populations and protein components both in circulation and resident within the heart make these models extremely tractable in establishing a basis for translation to humans. In addition, the imaging techniques used to investigate the human heart (for example ultrasound,

	echocardiography, ECG and Magnetic Resonance Imaging, MRI) can now be applied directly to the adult mouse (and are under development for application to zebrafish and neonatal mice) making findings of direct relevance to that observed in human patients. To minimise harm: i) all surgery will only be carried out by highly trained personnel who have specific experience with the heart injury models proposed; ii) aseptic techniques will be employed throughout to minimise risk of infection; iii) animals will be routinely provided with pain-relief by appropriate analgaesics; iv) animals will be monitored post-operatively for signs of bleeding and distress indicative of heart failure: including reduced activity and breathlessness; v) animals will be humanely killed immediately if there are any adverse signs as indicated under iv).

<table>
<tr><td>Project Title (max. 50 characters)</td><td>HPIVs, virulence and therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Parpainfluenza, HPIV, HPIVs.</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>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>1) To develop methods by which the virulence of newly emerging strains of HPIV could be predicted.
2) To evaluate the efficacy of existing antiviral agents for the prevention and treatment of human paprainfluenza virus infection.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The successful completion of objective 1 should lead to the development of rapid screening techniques that can be used both to improve the clinical treatment of individual patients and to trigger the implementation of barrier nursing techniques to minimise the spread of infection within a hospital setting. This would therefore benefit not only the recovery of the individual patients but also reduce the risk of the spread of HPIV within the hospital setting and thus protect high risk patients from infection.

The successful completion of objective 2) would considerably strengthen the case for clinical trials of drugs which so far have only been shown to be active against HPIV using in-vitro techniques (Alymova et al., 2005): at present there are no antiviral agents approved for clinical treatment of HPIV. The benefit would therefore be an enormous step forward in having drugs to improve the clinical outcome of infection in high risk patients and to minimize the risk of spreading the infection within the hospital setting.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Hamester, estimated number 384</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] 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 severity limit for the procedures conducted under this licence are mild. Overt signs of suffering are not expected as a result of these procedures. At the end of the study the animals will be killed under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The proposed study aims to identify genes imparting virulence to Human parainfluenza viruses (HPIVs) that can be used to develop a bioinformatics program for predicting the potential virulence of newly-emerging HPIVs. The required data includes quantifiable indices of disease severity and viral shedding. It is not possible to obtain this data using *in vitro* systems, however some data on viral replication will be generated using *in vitro* assays although in the absence of animal studies the value of this data would be very limited. The studies will also assess the efficacy of existing antiviral agents for the treatment and/or prevention of HPIV infection: all of the agents to be tested have already been shown to inhibit HPIV replication *in-vitro* and have been shown to be safe in rodents, but it is now essential to test them *in vivo*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot studies will be conducted at the outset of this work and the data generated will be used to determine the most appropriate group size and sampling points for obtaining statistically valid data. The sampling point chosen for the main studies will be at the earliest time following challenge which is consistent with obtaining valid data. A bio-statistician will be consulted from the outset of the study to ensure the validity of the experimental design. The anti-viral agents used in this study are either already licenced for use in humans or are currently undergoing clinical trials, so they are available in a form that has been manufactured to standards required for medicines. The viral agents will be prepared using standards compliant with good laboratory practice.
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	Human parainfluenza viruses (HPIVs) can infect a number of animal species including primates and ferrets, however hamsters are the species with the lowest neurophysiological sensitivity in which these viruses can replicate and induces discernable pathology. The severity of the disease caused by HPIVs infections in hamsters is mild and overt clinical signs are not expected to occur.

(harms) to the animals.	Nevertheless to minimise suffering the earliest time point consistent with obtaining valid data will be used as the end point for the studies. The group size and sampling times for the study will be determined from data generated in pilot studies. The study period for each of the studies will not exceed four weeks and in most case should be considerably shorter. Overt clinical signs of disease are not anticipated based on the information available in the literature however the animals used in the pilot study will be closely monitored to determine whether any clinical signs are apparent that might be useful indicators of disease severity. Any animal that shows signs of suffering, e.g. hunched posture, lack of responsiveness, pilo-erection etc. will be killed using a schedule 1 technique.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Development of Radiopharmaceuticals for Molecular Imaging of Cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Molecular Imaging, Cancer, Early detection, Theranostics, Cancer 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)[5]</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[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>This project aims to find better molecules for the diagnosis, early detection, screening, and therapy of cancer.</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>New radiolabelled proteins may be used for the diagnosis, early detection and even therapy of cancer in patients.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>It is estimated that up to 7,350 mice may be used for this project in 5 years. Mice were selected because human cancers can be grown in athymic mice, and many genetically altered mice exist that mimic human cancer development. Mouse numbers will be minimised based on power calculations on preliminary experiments, and using previous experience in doing similar studies.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>To study radiolabelled proteins that visualise tumour biology, mice bearing tumours are needed. Tumours will be introduced by injection of human cancer cell cultures into the flanks of mice. Genetically altered mice with specific cancer-inducing mutations also develop tumours. This results in moderate levels of harm to the animals, and they will be euthanized before tumour burden becomes a hindrance to their natural behaviour.

The measurements made using small-animal</td></tr>
</table>

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

	scanner, and any surgical therapy are performed under general anaesthesia. The radiolabelled proteins themselves are not toxic. Mice may be treated with chemotherapy or radiotherapy, but at levels which have been proven not to cause any side-effects in mice.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Initial evaluation of the radiolabelled proteins evaluation in this project will be performed using cancer cell cultures. Only if successful in these in vitro experiments, will they be evaluated in mice. The stability and biological behaviour of radiolabelled proteins can only be tested in vivo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be based on power calculations. Where no preliminary data is available for power calculations, pilot studies will be performed to provide data for power calculations. Pilot studies will use animal numbers based on experience with similar radiopharmaceuticals. Management procedures are in place to reduce the number of genetically modified animals bred under this PPL. The number of animals bred will be regularly reviewed through discussion with the Oxford University Biomedical Services and animals will only be bred if an experimental requirement has been established, to avoid excess stock. Furthermore, most of the genetically modified animals bred under this licence (protocol 2) will only experience a mild severity limit, with only a fraction (<10%, those spontaneously developing internal tumours) reaching moderate severity limits.
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 procedures used in this project will be the least invasive and least harmful possible. If less invasive or harmful alternatives exist to the procedures described above, they will be explored. If the scientific outcome to any procedure in not altered by a less harmful procedure, the alternative will be used. An example of this is the use of non-invasive imaging with SPECT or PET as an alternative to dissection. After every procedure, animals will not be returned to their housing until fully recovered from any procedure. Any surgery will include the use of the appropriate level of local analgesia. The impact of all procedure is evaluated by frequent visual assessment of animals, in collaboration with the NACWO. Furthermore, continuous evaluation and revision of the procedures and protocols, in collaboration with the NACWO(s), licence deputies, Biomedical Services, and veterinary services at our institution will continually decrease the impact and severity limits.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mouse models to study mammary gland morphogenesis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>mammary gland, GFP, transgene, fibroblasts, epithelium</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></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>The mammary gland develops mainly after birth. During puberty a rudimentary tissue grows into the surrounding fatty tissue to form a network of milk-transporting ducts. During pregnancy, these ducts grow out to form the milk secreting cells. This growth is regulated by hormones, growth factors, and cell to cell communication. These processes are often deregulated during breast cancer formation and development, and cell to cell communication is crucial for controlling cancer growth and tumour spread. It is therefore vital to learn more about these processes and how they are controlled. The aims of the projects are therefore to i) assess the role of identified potential regulatory genes in mammary gland development through gene deletion or insertion, ii) establish how specific cells contribute to the formation of the glandular tissues by specific labelling and following their cell fate, and iii) study the relationship and communication between the different cell types of the growing and non-growing structures within the developing mammary gland through cell-type specific isolation and characterisation.</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>Breast Cancer remains the most common cancer in the UK, with more than 48,000 people being diagnosed each year. Despite improved treatments 12,000 women still die of breast cancer every year, showing the imminent need for better treatment. In order to find improved treatments for breast cancer patients it is important to learn more about the biology of the normal mammary development and to characterise those cells from which different</td></tr>
</table>

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

	breast cancer are understood to originate. The results of this work will give valuable insights into key developmental processes involved in tissue growth and into the cells of origin of particularly aggressive breast cancer. This understanding could help to identify new breast cancer treatments, and will be of great interest to cancer scientists and developmental biologists alike.
What species and approximate numbers of animals do you expect to use over what period of time?	2500 mice 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?	Some animals will be injected with drug to induce transgene activation (eg tamoxifen). This may slow down pubertal mammary gland development temporarily, but the mammary gland will catch up again so that at adulthood the glands of treated and untreated mice look identical. There are no other expected adverse effects. The animals will be sacrificed by humane killing at the time of analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mammary gland is a highly complex organ with many different cell types, controlled by a numerous networks of signals, which cannot be replicated in a cell culture system. It has also been shown that cell fates, which we aim to study, can vary between those cells that have been isolated and cultured and those that remain in the tissue. Cell cultures therefore cannot fully replicate the situation in the body. Further, it is not possible to study the relationship between different cell types within the mammary gland as cultures cannot recapitulate the full complexity of the tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Since the speed and extent of mammary gland development can vary between mice pilot studies will establish the minimum amount of animals required on the basis of technical and biological variability to obtain meaningful data. The experiments will be terminated as soon as this number of mice has been studied. Embryos/sperm will be frozen from lines not immediately required for scientific studies. The use of fluorescent mice will allow us to clearly define the tissues of interest, thereby reducing the amount of technical variation between replicates, and therefore of the amount of animals required. As we are also seeking to develop a cell culture model to study cell to cell interactions where possible, this project may help in reducing the amount of mice necessary for future studies.
3. Refinement	The mouse mammary gland model is the best

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.	available model for mammary gland development. Our previous results were all obtained from mice and therefore any new results will be directly comparable. This licence further uses genetically altered mice in which only the subsets of cells of interest are targeted, thereby reducing any non-specific side effects often observed when all cells in the body are targeted. None of our protocols exceed a moderate severity level. To minimise any unforeseen suffering all mice will be constantly monitored and humanely sacrificed when exhibiting signs of altered health status or other specified end-point is reached. The animal units involved are proactive in enrichment with tissue, fun tunnels and nesting material. For all of our studies we will ensure best working practice, consult the NC3Rs guidelines and monitor refinement where such practices are published (NC3Rs/LASA websites).

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Anticancer effects of cannabinoids and radiation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Glioma, cannabinoids, radiation, combination</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">Three years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td colspan="3"><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[10]</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 colspan="3">High-grade glioma is one of the most aggressive cancers in adult humans and long term survival rates are very low. Treatment can consist of surgery, radiotherapy and/or chemotherapy, however, due primarily to the intricate localisation of the tumour in the brain and its invasive behaviour, these treatments remain largely unsuccessful. The need for new treatments is thus important.
Cannabinoids have a number or anti-cancer properties, which have previously been shown to reduce glioma growth both in vitro and in vivo. However, their value in this cancer type remains poorly established.
The overall objective of this project is to assess the benefit of cannabinoids in glioma treatments. In addition to an active in vitro research strategy, we have developed an in vivo approach that supports the translation of our research into the clinic. To this end, we have two specific questions, which are to: 1) establish the effect of the cannabinoid agents alone using a mouse affected by glioma; 2). assess whether co-administering these drugs with radiation therapy will improve treatment outcome compared to if the treatments were used separately.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">We believe that these studies will allow us an improved insight into how cannabinoids can be employed in treatment regimens in patients that have cancer. In particular, the way in which these agents can be employed in combination with existing treatments is particularly useful in terms of developing novel therapy approaches.</td></tr>
<tr><td>What species and approximate numbers of</td><td colspan="3">Approximately 400 C57BL/6 mice will be used over a three year period.</td></tr>
</table>

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

animals do you expect to use 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?	Animals being injected with any substance could suffer distress as a result of that injection. For our intracranial injection techniques, careful handling of the animal should be sufficient to prevent distress prior to anesthetisation. Mice will be monitored for outward signs of discomfort and distress after the procedure. To reduce pain and distress caused by the injection itself, the needle used will be the appropriate gauge for the species and route of injection. The volume of liquid being injected is another potential cause of pain or distress. For this reason, the volume of any substance given will be as small as is practicable for the procedure. This volume will not exceed 10µl. Furthermore, our previous experience with a similar orthotopic model suggests minimal effects after the procedure, however, we will monitor carefully for signs of distress and adverse responses to the procedure throughout the course of the study. For example, possible adverse effects can include: paresis or ataxia, seizures, circling, head tilting and other alterations to awareness. If one or more of these are detected, which results in distress in the animal, then the animal will be killed by a schedule 1 method. When a general anaesthetic is administered animals could potentially suffer distress as a result. For example prolonged recovery times could lead to semi-conscious animals lying in their own urine or faeces, or getting bedding in their eyes or nose. Hypothermia could also result from anaesthesia of mice. To prevent this, an appropriate anaesthetic, e.g. an inhalation anaesthetic agent, will be used to reduce recovery times. Furthermore, animals being anaesthetised will be monitored closely and appropriate measures taken to ensure body temperature do not drop (e.g. animals may be placed on a heat mat during the procedure and recovery period). Animals will be monitored regularly during the recovery period to ensure they are not lying in urine or faeces and bedding does not get into eyes or noses. Animals challenged with live tumour cells could potentially suffer adverse effects as a result. The development of solid tumours after injection of live tumour cells could cause distress or pain. To minimise suffering, animals will be monitored daily for signs of distress, and if there is evidence of

	impaired mobility, lameness or pain, then the animal will be killed by a Schedule 1 method. The agents tested may cause adverse effects, and although we expect only minimal side effects, we will follow our local rules to deal with any of these adverse effects. If we see anything more than subtle changes in the clinical condition or the behaviour of the animal then the animal will be killed by a Schedule 1 method. Radiotherapy has previously been applied to murine studies with minimal adverse effects reported. Although exposure to radiotherapy is not expected to last for longer than 1 min, body temperature will be monitored. At the first sign of temperatures exceeding 41.5°C, the source will be removed, and the areas cooled with water. The endpoint of the experiment has been chosen to ensure that the size of the tumour causes no undue distress to the animal. The experiment will be terminated at a maximum of 120 days after administration of tumour cells if no apparent tumour is detected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although cannabinoids have previously been shown to reduce tumour size in certain xenograft models, these agents have not yet been studied in combination with standard anticancer therapies in syngeneic in vivo tumour models. Our in vitro data already shows potential synergistic interactions between these agents and conventional treatments, and for that reason we believe it is now important to study the same combination using in vivo models which will allow us to understand better the therapeutic benefits of these interactions. In addition, this mouse model would be useful for unravelling the toxicity of such drugs to normal tissues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary in vitro and prior in vivo studies will allow us to establish the optimal concentration of cannabinoid agent to be used in our studies. Prior in vivo studies have already identified the optimum amount of cell line to be injected into the animals to maximise the chance that tumours will reach the desired sizes for our studies. These cells are always used freshly resuscitated and are at passage <20. This ensures that they are at ideal growth state, which increases the chances that they will be taken in the animals. The proposed work has been discussed by senior members of the research team, and the numbers of

	experiments required to reach statistical significance has been discussed. Generally, the number of experimental groups has been kept low to ensure that analysis of variance and t-test examinations are achievable with the smallest number of animals. The level of significance will be 5%. Exact numbers of animals for each group may vary; however, we will monitor these numbers on a weekly basis to ensure the lowest number of animals is used to draw adequate conclusions. For each and every experiment, as part of good laboratory practice, we write an experimental protocol that is discussed with members of the research team. This ensures that we work to specific objectives with clearly defined hypotheses. This ensures that only work that is necessary to draw conclusions are performed. The team also meets regularly, and experiments covering such matters as experimental strategy and analysis of results are discussed.
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 experimental approach employed in this study relies on the successful formation of a syngeneic orthotopic tumour, which allows for a more realistic assessment of whether the combination strategy will affect tumours in situ. For this reason, C57BL/6 mice will be used along with the Glioma-261 mouse tumour cell line. The stereotactic method of injection minimises animal suffering as well as employing precise, regimented introduction of the tumour cells to the animal which increases reproducibility and statistical significance. This will allow for minimal numbers of animals to be used. Through regular assessment of the tumour volume by MRI scanning techniques we will also allow be able to monitor tumour development and animal wellbeing and ensure animal suffering is kept to a minimum.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Targeting Wnt signalling in colorectal cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Wnt signalling, intestinal regeneration, colorectal cancer</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)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>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 aim of our overall research programme is to identify druggable molecules in the Wnt signalling pathway, and to demonstrate that blocking these molecules – by genetic manipulation or with chemical drugs – will slow down or prevent the development of intestinal tumours. Our focus is on newly discovered regulators of oncogenic □-catenin, because these have as yet untapped potential in the therapy of colorectal cancer, which is almost inevitably caused by aberrant activation of this Wnt signalling molecule.

Our animal work has three specific objectives: (i) to test Wnt signalling molecules for their oncogenic activity in driving the development of intestinal tumors in a mouse tumour model (called Apc-Min model) that closely approximates the human disease of hereditary colorectal cancer; (ii) to define the functions of these molecules in normal stem cells and tissue regeneration of the mouse intestine; (iii) to use our mouse tumour model for drug trials, to test the therapeutic benefits of novel Wnt signalling inhibitors discovered by us and others.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Our research could open up new avenues for the therapy and prevention of colorectal cancer in humans. In particular, it could pave the way for developing novel rationally-designed anti-cancer drugs, to combat this disease which remains the third most common cause of cancer death in the UK.</td></tr>
</table>

[11] Delete Yes or No as appropriate.
[12] 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?	To achieve our objectives, we require genetically altered mice, maximally 20'000 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of our mice (>80%) are not expected to experience any adverse effects. However, a small fraction of them (<20%) will develop intestinal tumours. They may therefore show moderate signs of this disease, including pale feet, ruffled fur, inactivity and lack of appetite. All mice will be humanely killed at the end of each experiment, or as soon as they present with overt disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexities of the normal and cancerous human intestine cannot be modelled adequately *in vitro* or *ex vivo*. It is therefore essential that we use an animal model that allows us to determine the physiological role of signalling molecules in the most appropriate tissue setting in a living organism, to validate these molecules as therapeutic targets and to test possible inhibitors of these molecules as anti-cancer drugs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Wherever possible, we use models and approaches in the laboratory that include biochemical, biophysical and structural analysis, cell-based assays in human cell lines and functional tests in the fruit fly *Drosophila.* Our experimental design follows well-established protocols, based on minimal groups sizes that allow statistically significant results. We hold regular meetings to optimise our breeding strategies and the management of our mouse colonies. Only where it is appropriate do we use our well-established tumour model, allowing us to rely on extensive previous experience to ensure that we use the minimum number of mice. Pilot experiments with small group sizes and statistical analysis will be used to minimise animal numbers. Cryopreservation of strains will be used to avoid having to keep live stock unnecessarily. We will collect tissue samples from multiple body sites, and provide tissues and mouse strains to other scientists, to maximise the information from a single animal or strain.
3. Refinement Explain the choice of species and why the animal model(s)	Mice have become *the* leading vertebrate model, due to their relative ease of breeding and genetic manipulation, and because they are the lowest

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.	sentient mammal that is closely related to humans. Much is known about mouse development and tissue regeneration, and sophisticated genetic technology is available that allows rigorous and highly refined experimentation. Our model provides a close approximation to the human disease, and we expect the insight we gain from this model to be highly relevant for human therapy. Our experiments require only minimal invasive procedures, mostly intraperitoneal injections, which will be carried out by highly trained staff and will typically cause no more than transient discomfort. All mice will be housed and maintained to the highest international standards for welfare. By choosing a well-established model, we minimise unknown effects on mice and subsequently pain, distress and suffering. The signs of the neoplastic disease in our model are well known, and obvious on inspection. Mice carrying the mutation will be monitored daily for signs of the disease, and humanly killed following disease onset. We will only subject animals to neoplasia studies once we have sufficient evidence from our biochemical work and i*n vitro* cell assays that our genetic manipulations or drug treatments will alleviate their disease.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>HEPATOCYTE TRANSPLANTATION</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Liver Disease, Transplantation, Hepatocyte</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</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[14]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Liver cell transplantation is being developed as an alternative to whole organ transplantation for patients with certain types of liver disease. There are a number of limitations to the technique, which cannot be investigated in humans. A major hurdle is the lack of sufficient donor liver tissue to isolate hepatocytes, the main metabolic cells in the liver, of sufficient quality for transplantation. Studies are needed to improve cell function and to find different sources of cells.

Acute liver failure is a severe clinical condition with a high mortality. This project will investigate encapsulation of hepatocytes in alginate beads, which protects the cells so that they can be administered into the abdomen. This has the advantage that the hepatocytes are not directly in contact with the patient’s immune system, so that the potentially toxic anti-rejection drugs do not have to be given for the duration of the patient’s life.

Anti-rejection drugs are given after liver transplantation, but it may be possible to avoid this by giving back the patient their own hepatocytes isolated from their original liver removed at the transplantation operation. Their own cells will not be rejected by the immune system.

Overall this project will investigate methods to improve the outcome of hepatocyte transplantation.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>This project is expected to help optimise the techniques of hepatocyte transplantation, in particular to improve the outcome of this treatment</td></tr>
</table>

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

advanced or humans or animals could benefit from the project)?	in acute liver failure. It is expected that the advances made will be translated to human application. The results will be disseminated to other groups working in this field of medical research and published in international scientific journals.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice will be used, including mice with genetic alterations affecting the immune system. Up to 150 animals could be used annually in this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There is a small chance of complications occurring during surgery, such as cardiac or respiratory arrest, or haemorrhage. Careful monitoring of the rats will be carried out with at least daily observations. A full program of peri-operative and post-operative analgesia will be employed. Treatment with hepatotoxic agents to induce acute liver failure in rodents will produce the symptoms of this condition. The animals are likely to become agitated followed by coma and may show signs of hypotension, hypothermia and hypoglycaemia. Potentially there is a high mortality from liver failure without cell transplantation. As a result the animals will be closely monitored and if they become too sick, they will be killed humanely to prevent suffering. It is expected that up to one third of the animals would experience the highest level of severity. All animals will be killed once the protocol is completed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to perform these experiments in the cell culture laboratory alone. However, as much preparative work as possible will be done to limit the number of animal experiments required, particularly in optimising the cell culture conditions where different types of cells are cultured together. Cells must be shown to engraft in the liver and their effects and interactions with other cells and organs can only be determined in the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	No more than 3 animals will be used per group initially. For comparisons using larger groups, group size will be estimated by power analysis statistics. The minimum number of animals will be used to obtain statistically valid results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	These models using rats and mice are established by the scientific community for studies on cell transplantation Rats will be used as a source of livers to isolate

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	hepatocytes. The animals will be killed immediately by an approved method or anaesthetised so that the liver cells can be isolated without recovery of consciousness. Liver cells of human or rat origin will be administered to recipient animals under general anaesthesia via a surgical procedure. In a smaller number of animals liver transplantation will be performed. Strict sterile conditions will be used and animals will receive analgesics to reduce pain and antibiotics if required. Animals will receive injections of a hepatotoxic agent to induce acute liver failure. Hepatocytes will be administered in the form of small beads into the abdomen. Restoration of liver function and improvement of outcome will determine the efficacy of hepatocyte transplantation. This is a severe protocol, where there is a high potential mortality rate without treatment as seen in the clinical condition. Criteria have been developed to monitor the animals so that they will be killed if they become very sick. Recovery, if the liver regenerates, is usually rapid. In all protocols, the effects of hepatocyte transplantation will be monitored by taking blood samples for liver function assays. Animals will be humanely killed at different time points and the organs collected for analysis.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Testis development and role in post-natal health</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Testis, androgen, brain, development, health</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td colspan="3"><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[16]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">During development of the male fetus the testes are highly active producing the male hormone androgen. This function of the testes continues after birth and up to adulthood. If there is a problem with androgen production then the baby will be born with genital abnormalities and is likely to be infertile as an adult. In addition, the overall health and wellbeing of this individual is likely to be reduced. Currently our understanding of what controls testis development and production of androgens is limited and we do not know how a reduction in androgen production by the fetus or the newborn baby acts to affect later health in the adult. This project is designed to study the basic mechanisms which control these processes.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Human infertility is an increasing problem in the developed world with the majority of the problems coming from the male. In addition to infertility, however, there is also good evidence that the general reproductive health of men (eg increased testicular cancers, reduced androgen levels) is declining with the cause of this decline likely to be due to problems in testicular development. Finally, reduced androgen levels in adult males have now been shown to be a cause of increased incidence of metabolic syndrome – a condition which is linked to increased levels of cardiovascular disease, diabetes and dementia. There is, therefore, an essential need for basic understanding of the mechanisms regulating testis development, adult testis function and the link between fetal androgen levels and adult health. The studies of which this project will be part are designed to improve our</td></tr>
</table>

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

	understanding of basic testis biology (in some cases in a quite fundamental way) and the role of androgens in male development and post-natal health. These studies will not directly affect human care but will increase our understanding of the underlying biology which will allow appropriate interventions to be developed.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use laboratory rodents (rats and mice). It is expected that 400 mice and 150 rats will be used over the 5 year lifespan of the project
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The most severe procedures that will be carried out will be surgical operations (under anaesthesia) to remove the testes or to move them from their normal scrotal position into the abdomen. Adverse effects from this procedure are those that would be expected from abdominal surgery such as post-operative pain and this will be controlled with analgesics. Other procedures, such as hormone injections, are relatively mild and will lead to only transient discomfort. All animals will be killed by a humane method 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	This project is designed to study how different systems interact with each other. This interaction will be either at the level of the tissue (eg how do the cells of the testis communicate to each other to increase androgen production) or at the level of the whole animal (eg how do androgens from the testis act to alter development of the brain). Considerable progress has been made in the development of techniques which allow culture of testicular cells but it remains very difficult to maintain the normal function of the cells in culture for more than a few days at a time. In addition, it is very difficult to study the normal interactions between cells in culture as the normal architecture of the tissue has been destroyed. For these reasons, study of cell-cell interactions in the testis requires use of experimental animals in which the normal processes can be disrupted *in vivo*. Studies into whether and how androgens affect post-natal development and health generally are currently not feasible using any methods other than experimental animals. The complexity of organ and tissue development and interaction in the whole animal during fetal and post-natal growth cannot yet be

	modelled effectively using computers and tissue or cell culture systems are of limited value. For this reason it is necessary to use live animals to study how testicular function affects overall health.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All studies undertaken as part of this project will be subject to statistical analysis in advance to determine the minimum number of animals necessary to show an effect or an interaction between cells or tissues. Showing an effect depends upon the variation in response between animals and we have good estimates of this variation from previous studies so that we are able to predict with accuracy 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.	All of the studies to be carried out in this project will use rodents. Rats and mice are the most commonly used experimental species and there is a very large amount of background information available on all aspects of their biology. In addition, there are a large number of mouse lines available which have been genetically altered in such a way as to help answer a number of the questions posed by this study. Use of larger animal species would not add substantially to what can be gained from the project, they take longer to mature so that certain developmental studies may not be feasible and they are significantly more costly to maintain. Harm to the animals will be minimised through use of best practice (eg use of analgesics post-operatively) and through monitoring of animal welfare and health.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Testis development and role in post-natal health</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Testis, androgen, brain, development, health</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[17]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[18]</td><td>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>During development of the male fetus the testes are highly active producing the male hormone androgen. This function of the testes continues after birth and up to adulthood. If there is a problem with androgen production then the baby will be born with genital abnormalities and is likely to be infertile as an adult. In addition, the overall health and wellbeing of this individual is likely to be reduced. Currently our understanding of what controls testis development and production of androgens is limited and we do not know how a reduction in androgen production by the fetus or the newborn baby acts to affect later health in the adult. This project is designed to study the basic mechanisms which control these processes.</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>Human infertility is an increasing problem in the developed world with the majority of the problems coming from the male. In addition to infertility, however, there is also good evidence that the general reproductive health of men (eg increased testicular cancers, reduced androgen levels) is declining with the cause of this decline likely to be due to problems in testicular development. Finally, reduced androgen levels in adult males have now been shown to be a cause of increased incidence of metabolic syndrome – a condition which is linked to increased levels of cardiovascular disease, diabetes and dementia. There is, therefore, an essential need for basic understanding of the mechanisms regulating testis development, adult testis function and the link between fetal androgen levels and adult health. The studies of which this project will be part are designed to improve our</td></tr>
</table>

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

	understanding of basic testis biology (in some cases in a quite fundamental way) and the role of androgens in male development and post-natal health. These studies will not directly affect human care but will increase our understanding of the underlying biology which will allow appropriate interventions to be developed.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use laboratory rodents (rats and mice). It is expected that 400 mice and 150 rats will be used over the 5 year lifespan of the project
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The most severe procedures that will be carried out will be surgical operations (under anaesthesia) to remove the testes or to move them from their normal scrotal position into the abdomen. Adverse effects from this procedure are those that would be expected from abdominal surgery such as post-operative pain and this will be controlled with analgesics. Other procedures, such as hormone injections, are relatively mild and will lead to only transient discomfort. All animals will be killed by a humane method 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	This project is designed to study how different systems interact with each other. This interaction will be either at the level of the tissue (eg how do the cells of the testis communicate to each other to increase androgen production) or at the level of the whole animal (eg how do androgens from the testis act to alter development of the brain). Considerable progress has been made in the development of techniques which allow culture of testicular cells but it remains very difficult to maintain the normal function of the cells in culture for more than a few days at a time. In addition, it is very difficult to study the normal interactions between cells in culture as the normal architecture of the tissue has been destroyed. For these reasons, study of cell-cell interactions in the testis requires use of experimental animals in which the normal processes can be disrupted *in vivo*. Studies into whether and how androgens affect post-natal development and health generally are currently not feasible using any methods other than experimental animals. The complexity of organ and tissue development and interaction in the whole animal during fetal and post-natal growth cannot yet be

	modelled effectively using computers and tissue or cell culture systems are of limited value. For this reason it is necessary to use live animals to study how testicular function affects overall health.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All studies undertaken as part of this project will be subject to statistical analysis in advance to determine the minimum number of animals necessary to show an effect or an interaction between cells or tissues. Showing an effect depends upon the variation in response between animals and we have good estimates of this variation from previous studies so that we are able to predict with accuracy 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.	All of the studies to be carried out in this project will use rodents. Rats and mice are the most commonly used experimental species and there is a very large amount of background information available on all aspects of their biology. In addition, there are a large number of mouse lines available which have been genetically altered in such a way as to help answer a number of the questions posed by this study. Use of larger animal species would not add substantially to what can be gained from the project, they take longer to mature so that certain developmental studies may not be feasible and they are significantly more costly to maintain. Harm to the animals will be minimised through use of best practice (eg use of analgesics post-operatively) and through monitoring of animal welfare and health.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Oral vaccine research studies in badgers (Meles meles)</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Badgers, bait, vaccine, BCG, oral</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years.</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[19]</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>Yes</td><td></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[20]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The project is aiming to:
1.) Determine the most suitable bait and oral vaccine formulation for BCG delivery to badgers.
2.) Determine efficacy of oral vaccines in badger in experimental vaccine efficacy studies.

Lead candidate baits will contain live BCG at a protective titre for their entire shelf-life; will be attractive and palatable to badgers, adequately labelled, economical, safe for users and non-target species. In this project, baits will be developed and optimised to achieve these requirements. The protective efficacy of the oral vaccines will be tested in captive badgers against experimental challenge with Mycobacterium bovis.

The overall aim of this project is to continue research and development, on behalf of Defra, on an oral vaccine against tuberculosis in badgers and generate data suitable for submission to the Regulatory Authority in pursuance of a National Marketing Authorisation (MA) for the vaccine.</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 trend of cattle TB incidence in England has been rising for 25 years (DEFRA, 2011). This adversely affects animal health and welfare, and is a cause of considerable economic loss to farmers and Government. Although transmission of M. bovis between cattle is an important factor in the spread of the disease, badgers represent an additional wildlife source of recurrent M. bovis infection to cattle in the UK. Vaccination of badgers against bovine TB is a possible means to</td></tr>
</table>

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

	contribute to the reduction and control bovine TB alongside other control measures. It has long been recognised that the oral delivery of a vaccine in a bait holds the best prospect for vaccinating badgers over a wide geographical area and has proved highly successful for mass vaccination of other wildlife species against rabies. In the short to medium term, the TB vaccine (BCG) represents the best available option for vaccination of badgers. The Veterinary Medicines Directorate, UK (VMD) will be responsible for evaluating the application to obtain a licence for the oral badger TB vaccine. Data required for the licence application are prescribed and include demonstration of the safety of the vaccine to the target species (both captive and wild animals) as well as efficacy (from experimental studies).
What species and approximate numbers of animals do you expect to use over what period of time?	The work is structured so that the minimum number of studies will be performed in order to achieve the objectives. Approximately 80 captive badgers will be used in total, over a period of a minimum of 3 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?	Only qualified, experienced staff will conduct and supervise the proposed experiments. The severity limit for the work is moderate, with the most likely adverse effects associated with non-recovery from general anaesthesia, which is necessary for collecting samples (blood from superficial vessels, mucus from tracheal, urine and rectum) during the course of the study. The number of anaesthetic procedures will be minimised in this project and anaesthesia only used where data cannot be obtained by other means (e.g. using remote video surveillance). Badgers involved in studies aiming to develop and optimise baits will not be submitted to protocols more severe than general anaesthesia and manual delivery of bait components, and will therefore be reused. Badgers will not be released at the end of the vaccine efficacy studies, but will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The main requirement is to generate data for consideration by the VMD for the granting of a licence for an oral badger vaccine for badgers. Non-animal gut models are used when possible to study to which extent the bait constituents may

	contribute to BCG survival after consumption. However, the protective immune responses generated in badgers after vaccination are only partially understood and cannot be modelled artificially; therefore studies to measure the protective efficacy of BCG in badgers require the use of live animals. As the principle aim of this programme of work is to generate data for consideration by the VMD for the granting of a licence for an oral badger vaccine, there is no alternative than to perform efficacy studies in the target species. It is not possible to optimise bait or vaccine formulation in a surrogate species, as bait preference is peculiar to the species under evaluation, as is the response to vaccination.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have sought statistical advice to ensure that the minimum number of badgers is used to generate sound and valid data. The protocol for each study is scrutinised in the context of the whole R&D programme in order to focus on the most relevant questions to answer.
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.	As the principle aim of this programme of work is to generate data for consideration by the VMD for the granting of a licence for an oral badger vaccine, there is no alternative than to perform efficacy studies in the target species. The majority of data for bait development will be collected without direct intervention (by CCTV footage under infrared illumination). When testing for the presence of biomarker in blood is required the number of anaesthetic procedures will be minimised. For vaccine efficacy studies, it is intended to allow conscious badgers to consume the bait. If gavage is required, up to 200µl vaccine (liquid presentation) and/or up to 1ml for paste-like consistency presentation (minced bait or concentrated BCG) will be placed directly into the mouth. Infection with *M. bovis* challenge strain is not expected to induce clinical signs of disease during the 12 week interval between challenge and necropsy. The infection model was developed prior to 2005 and has successfully been used for all the Vaccine Efficacy Studies conducted at AHVLA. One aim of the virulent challenge is the development of visible lesions as consistent as possible within the non-vaccinated control group, without generating clinical signs of disease. Since badgers have to be anaesthetised for

	handling, care will be taken to group procedures to minimise the number of anaesthetic events.