Home Office

# Animals (Scientific Procedures) Act 1986

## Non-technical summaries granted during 2013

## Volume 41

## Project Titles and key words

- The modelling and treatment of neurodegeneration in the CNS

  Parkinson's disease, Alzheimer's disease, synuclein, tau, neurodegeneration

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

  Fish, welfare, biomarker, stress, happiness.

- Understanding lung injury, inflammation and fibrosis

  Lung injury, inflammation, fibrosis and TGFbeta

- Improving Outcome in Cutaneous Wounds

  Burns, skin, wound repair, infection

- Breeding and housing of genetically modified and mutant mice

- Novel treatments for dementia therapy

  Dementia, Alzheimer's disease

- Safety & Efficacy in Small Animal Species

  Toxicology, Pharmacology, Safety, Rodent

- A study of myelination

  Myelination, glia cells, axons, time lapse, in vitro

- European eel productivity and escapement in Kent

  European eel PIT tag marshland

- Proteolysis in mouse models of cancer and tissue repair

  Cancer, wound healing, proteases, diet

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The modelling and treatment of neurodegeneration in the CNS</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Parkinson’s disease, Alzheimer’s disease, synuclein, tau, neurodegeneration</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>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>Parkinson’s disease (PD) and Alzheimer disease (AD) are progressive neurodegenerative diseases where the loss of cells results in serious motor and memory impairment. Alpha-synuclein and tau are proteins found in all neuronal cells in the brain; in the neurodegenerative diseases they form sticky clumps (aggregates) inside the neuronal cell where it thought to damage cellular functions leading to the cell death. It is therefore extremely important to study the processes leading to the cellular death after the aggregation of these proteins, as reversing this process will help to cure the disease in humans.<br><br>In this project we will use mice and rats to produce models of AD and PD by generating either genetic model – introducing human alpha-synuclein or tau gene, causing the disease, into genome, by inducing brain cell death after injection of specific toxic substances, or a combination of both. After this we will examine if the cells that are responsible for the memory formation and/or motor control will die after aggregated proteins will form inside, and whether this cell death produces recordable changes in the memory formation and/or motor activity of the animals.<br>Once we are convinced that the changes resemble the once observed in human disease (for example, if we will observe the death of cells in the similar to</td></tr>
</table>

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

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| | human part of brain), we will then look at the ways to restore the damage by re-introducing biologically active molecules or cells that can replace damaged cells of the brain. |
| 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)? | The outcomes of this project will have dual benefit. As an immediate benefit, it will address the mechanisms of disease development similar to one observed in human brain, and will indicate the ways to halt or reverse the process of the cell death. In addition to that, it will offer new understanding of neurobiological processes occurring in the brain after introduction of e.g. proliferating cells. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Mice, 1000 per year<br>Rats, 200 per year |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | In most of the cases the level of severity will not exceed mild, when we will breed transgenic animals and we do not expect significant adverse effects. If animals will develop motor deficit as a consequence of targeted mutation of the gene or neurotoxic insult, it will be considered as moderate level of severity. Other procedures will not exceed moderate level of severity. In the end of the procedures animals will be humanely killed as required. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Unfortunately, there is no valid alternative to reproduce the complexity of the neuronal connections exiting in the brain and cell cultures can only provide a very limited replacement.<br><br>Despite these limitations, we are going to carry on an extensive in vitro program, using immortalized neuronal cell cultures, in order to answer the question of what conditions, substances, chemical, growth factors, etc promote or delay aggregation of proteins we are interested in. This line of work will be used as a basis for choosing the limited list of biologically active compounds to use in animal models, described in this project licence. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Numbers of transgenic animals are minimised by careful monitoring of colony size and breeding, and matching these to the demands of the experiments. We will further decrease the numbers of animals used for tissue acquisition by only buying in animals as required, and we are going to initiate sharing tissues between multiple users and generating a tissue bank from animals. |

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| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Mice and rats will be used for our experiments because they have a nervous system that is sufficiently similar to that of humans that the biology of neurodegeneration, regeneration and plasticity is almost the same. Mice can be genetically manipulated, allowing molecular hypotheses to be tested. In addition, a vast library of live transgenic mice are available for purchase, which will be used in this project for cross-breeding our novel lines.<br>Rats are especially sensitive to the action of several neurotoxins we intend to use in order to model cell death similar to PD.<br>As it is impossible to model the entire complexity of the brain in a cell culture dish, generation of the relevant animal model is vital for the success of this project. Even though it is feasible to model simple question in lower vertebrates (fish) or invertebrates (fruit fly or worms), the rodent brain is functionally and neurochemically similar to humans, therefore it is possible to induce the disease relevant processes and to look at possible treatments in rodent brain.<br>Numbers of animals used in the licence will be minimized by matching numbers to experimental requirements.<br><br>In most of the cases the level of severity will not exceed mild, when we will breed transgenic animals and we do not expect significant adverse effects. If animals will develop motor deficit as a consequence of targeted mutation of the gene or neurotoxic insult, it will be considered as moderate level of severity. Other procedures will not exceed moderate level of severity. In the end of the procedures animals will be humanely killed as required.<br><br>The analgesia and anaesthesia will be used to minimize the suffering of animals when performing invasive and potentially painful procedures.<br><br>We will breed from the mice of a particular phenotype that do not show early clinical progression in order to improve welfare of the animals. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Assessing welfare in fish via application of optimal and sub-optimal holding conditions.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, welfare, biomarker, stress, happiness.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The goal of this research is to discover metabolic indicators of good (and poor) welfare in fish. Currently the holding conditions for experimental fish in research establishments are variable and defined empirically; this research will examine whether (or not) environmental enrichment measurably improves fish welfare.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Our approach has the potential to discover novel biomarkers of fish welfare AND identify methods for their routine measurement by care and health officials within and outside the laboratory establishment (e.g. Home Office Inspectors, Named Animal Care and Welfare Officers, Named Veterinary Surgeons, Auditors).<br>If welfare could be assessed objectively on a routine basis, inappropriate environment and care could be avoided which would:<br>- improve holding conditions for millions of fish held in research aquaria (breeding, stock and experimental fish)<br>- reduce the risks of experimental failure (and therefore the number of fish used for scientific purposes),<br>- reaching wrong conclusions (as meaningful data would be obtained).<br>ALSO, the role of environmental enrichment in fish welfare merits investigation. Evidence of welfare benefits could shape the way fish are maintained in research establishments (as well as in the aquaculture industry which involves far greater numbers).</td></tr>
<tr><td></td><td>Common laboratory fish (i.e. rainbow trout,</td></tr>
</table>

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

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

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Understanding lung injury, inflammation and fibrosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Lung injury, inflammation, fibrosis and TGFbeta</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>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[6]</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>Lung injury and fibrosis are complex processes that occur when wound repair goes wrong. These studies will determine the biological consequences of different types of lung injury that reflect different types of lung disease. The objectives of this project are to understand why lung injury and fibrosis occur and how they may be treated.</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 studies will increase our understanding of how lung injury, inflammation and fibrosis occur. They will increase our knowledge of fundamental wound repair principles in the lung, and will ultimately lead to the development of new therapies so desperately required to treat fibrotic lung diseases that are amongst the most severe disease that people can suffer from.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice and rats will be used and we would estimate that approximately 4000 mice, and 500 rats, will be studied over the course of these experiments.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>All studies require that lung injury is induced which leads to inflammation and lung fibrosis. Previous experience suggests that all animals developing lung injury, inflammation or lung fibrosis, lose weight, get increased breathing rates and some hair standing up on end. However, overall the level of discomfort is moderate and their progress will be carefully monitored to ensure the well being of all animals during the course of these studies. All mice are humanely killed at the end of these studies</td></tr>
</table>

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

| **Application of the 3Rs** | |
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| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Screening or “blue sky” experiments in animals will be replaced by experiments performed in test tubes, or using a new technique we are developing using lung slice experiments. Live animal experiments will only be performed when there is initial evidence that these experiments will lead to meaningful data that may change the way we approach patients who suffer from lung injury and fibrosis. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | To reduce the number of animals used in experiments we use inbred strains of animals to reduce any genetic variability; perform appropriate power calculations to avoid excessive sample sizes; measure endpoints which are reliable and have the lowest variability; use none invasive measures of injury and fibrosis, such as CT and MRI scanning, where possible; and measure as many different endpoints as possible from a single animal. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Mice and rats will be used in these studies as they represent the lowest vertebrate species that develop lung injury, lung inflammation and pulmonary fibrosis in response to various challenges. Rats offer some advantages over mice in terms of size and development of fibrosis in response to certain stimuli. Our laboratory procedures are refined to minimise variability in the measurable outcomes; to find the smallest effective dose of injurious agent or therapy; to minimise the duration of experiment to give meaningful data whilst reducing length of any suffering that might occur. Within the scope of these studies we are refining our studies using new imaging strategies to enable new ways of measuring endpoints of fibrosis that can be repeated on a single animal as well as developing lung slice techniques that will reduce the number of animals required to measure conventional endpoints. |

**Improving Outcome in Cutaneous Wounds**

- Summarise your project (1-2 sentences)

  This project seeks to improve ways of treating wounds created by burns, or other traumatic injury. Burns often heal poorly and get infected which causes disfiguring scarring which we are aiming to reduce.

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

  In the UK alone there are 13,000 hospital admissions every year due to burns, of which 1,000 are due to severe burns. Almost half of these injuries occur in children. It is vital that burn wounds heal rapidly as slow healing leads to scarring and further complications, such as infection can be serious and lead to death. Scarring caused by poor wound healing and infection can also severely affect the psychological well being of the patient and lead to social exclusion and poor quality of life. Scars can also restrict movement due to thickening and tightening of the skin.

  Current research is focussing on developing new ways of regenerating the skin quickly following injury without scarring. Techniques which are being developed to help achieve this include sprayed cells and improved ways of delivering them, new materials to help replace damaged skin and dressings which indicate and treat infections such as MRSA.

- Outline the general project plan.

  This project will focus on three aspects of wound healing and develop new treatments aimed at
  1. Improving healing
  2. Reducing scarring
  3. Treating infection

  After the initial development of treatments, materials and dressing in the laboratory without the use of animals a number of pig experiments will be undertaken to ensure that the treatments are effective and safe. Wounds will be created on the skin while the animal is under anaesthetic. These wounds will then be treated with the new therapy such as cultured / sprayed cells, new materials or new dressings. In some cases the wounds will be infected with a controlled dose of bacteria to mimic a clinical infection. These wounds can then be treated with new dressing material and any reduction in infection measured. Wounds will be measured throughout the experiment to investigate the speed of healing and whether any scarring is occurring. At the end of the experiment and after the animal has been killed, the area of skin which has been treated will be removed and investigated microscopically to see whether the structure of the skin is normal or not.

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

  The creation of small burn wounds (six wounds 4x4cm in size, or twenty four wounds 1cm in diameter) will cause some discomfort and pain to the animal. The level of

discomfort is not expected to be any greater than in a patient undergoing a routine plastic surgery procedure such as removing a skin cancer. When wounds are experimentally infected the wounds may become red and inflamed although the animal will not become ill as a result of the infection. Painkillers will be given to the animal to reduce this as much as possible and their wounds will dressed and treated in very a similar way to those in a patient being treated in a hospital.

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

  Burns are debilitating injuries which often lead to death. Scarring can be unsightly and the thickening and tightening of the skin can cause pain and prevent movement. Infections following burns delay healing and can lead to blood sepsis and death. By developing new treatments to improve healing following burn and skin injuries and reducing the effect of infection we hope to substantially improve the quality of life in patients who have suffered a burn injury.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

  The number of pigs which we plan on using will not exceed 100 in total or approximately 20 a year. Pig skin is very similar to human skin in its thickness and the way that it heals and responds to injury. Research using pigs has lead to advances in the treatment of burn patients and patients with other injuries to their skin. The number of animals will be kept to an absolute minimum by using laboratory techniques to do the majority of the research. Only when it is essential to understand how a whole animal / patient responds to a treatment will animals be used.

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

  The majority of the development of new skin repair treatments will take place in the laboratory and will not use animals. By using human skin discarded with consent following routine hospital procedures we can select only the techniques which have the potential to benefit patients for animal testing. Both human and pig skin cells are used extensively for our research and provide important information which helps ensure that the animal experiments provide information which directly or indirectly improves the treatment of patients. Animals will only be used where wound healing responses and the effects of the circulatory and immune system need to be taken into account.

  The number of animals used is kept to a minimum by ensuring that the number of wounds created and used to test new treatments is the minimum number sufficient to gather relevant data. Where a small wounds are more appropriate less animals will be used with more smaller wounds

  By creating several small wounds per animal a large amount of information can be gathered whilst using the minimum number of animals. As pig skin is very similar to that of humans the data collected on the new treatments under development relates

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| closely to humans. The use of painkillers and protective dressings and jackets reduce as much as possible any pain or discomfort experienced by the animals. |
| • Explain why the protocols and the way they are carried out should involve the least suffering.<br><br>All of the operations, dressing changes and sampling from the wound are performed under general anaesthetic so no discomfort or pain is experienced during the procedure. The administration of painkillers and use of dressings and protective foam jackets minimises as much as possible, any pain arising from the creation of the wounds. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Breeding and housing of genetically modified and mutant mice.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Breeding genetically modified mice</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></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>The purpose of this Project Licence application is to seek authority from the Home Office to maintain lines of genetically altered and mutant mice in order to provide suitable animals, or materials, for research.<br><br>Genetically altered and mutant animals are extremely valuable to scientists. They can provide an insight into fundamental biology and can be used to understand disease mechanisms. The mice bred and housed under this Project Licence will be used for research into addiction, diabetes, atherosclerosis, stem cell research and epilepsy.<br><br>Genetically modified mice bred under this Project Licence will be mated and reared normally. The mice are expected to behave and breed in the same way as normal mice.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>After the mice have been bred they are issued to research scientists either for isolated tissues or transferred onto other Home Office authorised Project Licences. This will allow different researchers and research groups to share resources ultimately leading to a reduction in the numbers of mice bred.</td></tr>
</table>

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

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

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Novel treatments for dementia therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Dementia, Alzheimer’s disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Dementia currently affects around 750,000 people in the UK, and these numbers are likely to increase to 1,000,000 people in 15 years time. Current therapies for dementia are limited in their effects and there is an urgent need to develop new drugs to treat these debilitating disorders.</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 increasing our understanding of some of the mechanisms and receptors involved in neurodegenerative diseases, this may provide targets for intervention in dementia.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats and mice (including genetically modified mice). We expect to use less than 450 per year.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>The animals will undergo procedures that may involve injections and they may experience moderate discomfort as they will experience some symptoms of disease, such as uncoordinated movement, and some of the side effects of the treatment such as excessive movement, weight loss or involuntary movements. Anaesthesia, pain killers and unilateral lesions will be used where appropriate to reduce the pain associated with surgery and the severity of the incapacity. There are also limits to the number and frequency of any injections, blood sampling and behavioural assessment that any one animal can experience. Overall, the severity of this license is expected to</td></tr>
</table>

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

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| | be moderate. At the end of the experiments the animals will be humanely killed and tissues may be investigated biochemically. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Animals need to be used in all these studies because many mechanistic questions require invasive techniques that are not possible or feasible at present in humans. *In vitro* and *in silico* techniques are also not sufficiently advanced (and are not likely to be so for some considerable time) that they can model the integrated actions of the nervous system. Indeed checks carried out during the writing of this application at www.frame.org.uk have revealed no *in vitro* applications able to model dementia. This is largely because our understanding of mechanisms within the nervous system is insufficient to allow effective modelling. Thus, we will undertake some of our work in animals. However, prior to *in vivo* testing compounds will be screened using a range of *in silico* and *in vitro* testing to ensure efficacy. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | For all the experiments proposed we will use a group size which is the smallest compatible with achieving statistically meaningful and robust results using either parametric (Student's t-test, Dunnetts test, ANOVA) or non-parametric (e.g. Mann-Whitney U-test) tests as appropriate. Group sizes will be estimated using data available from existing scientific literature (where available). We will consult statisticians where necessary. However, we have considerable experience in this type of work, and have published extensively in peer-reviewed journals. Thus, we already have a very good working knowledge of the optimal way to design and execute these types of experiment. |
| **3. Refinement**<br>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. | Whilst the animal model that best recapitulates human Parkinson's disease is the MPTP-treated primate, it is unacceptable to use this model at early preclinical stages of therapy. Hence, rodent preclinical models will be used. Mice and rats are often used in studies of this kind and are usually judged the species with the most appropriate neurophysiological sensitivity for providing useful results. The neuroanatomy, neurophysiology and neurogenetics of these rodents are also increasingly well documented. Mice and rats are easily assessed after nervous system injury using behavioural techniques. Our laboratory has extensive experience in the use of these animals in models of nervous system injury. Mice and rats are generally considered the lowest vertebrates suitable for us to perform these studies. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>SAFETY AND EFFICACY IN SMALL ANIMAL SPECIES</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Toxicology, Pharmacology, Safety, Rodent</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>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[12]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project licence authorises the conduct of studies in rodents to evaluate; 1) the safety and efficacy of new pharmaceutical products (or veterinary medicines) prior to administration to humans (or other animals), and; 2) the safety of other substances (industrial chemicals, plant protection products, biocides and food additives) to which humans or animals may be exposed.</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 availability of medicinal products for use by the medical, dental and veterinary professions has the potential to greatly benefit man and animals in terms of new and improved disease treatments. Before medicinal products are administered to patients their safety and efficacy (where possible) must first be evaluated and it is a mandatory legal requirement that toxicity testing is conducted in a rodent species. The studies conducted will help to prevent unsuitable candidates entering development or remove unsafe candidates from development at an early stage, thus saving animals and resources. The studies will identify target organ and system toxicity, and provide biomarkers to allow monitoring and management of human exposure.<br><br>In the case of industrial chemicals, plant protection products, biocides and food additives, achievement of the objectives of the Licence will allow selection of appropriate candidate materials for development, allows an</td></tr>
</table>

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

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| | assessment of safety-in-use for materials, and facilitates hazard classification and marketing authorisation.<br><br>The studies contribute to the establishment of an overall risk/benefit profile of a substance that provides regulatory authorities with the information they need to assess the risks to which humans are exposed when substances are produced, transported or used. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Work will be conducted in rodents, over the 5 year period of this licence estimated numbers are:<br>Rat: 33,000<br>Mice: 26,000<br>Hamster: 8,000<br>GA Mice: 6,500 |
| 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 animals on efficacy or safety studies are expected to show either no effects or mild adverse effects only, such as slight weight loss or changes in appearance or behaviour. A small number of animals in early exploratory toxicity studies or regulated acute toxicity studies where group sizes are small may show more significant adverse effects. This will usually be limited to the highest dose level evaluated and humane endpoints will be adopted or dose levels reduced if animals are deemed likely to exceed the defined severity limits. Longer term studies are expected to have progressively less adverse effects, although there will be an increase in age related conditions. In all cases veterinary advice is available to assess the condition of animals if concern exists.<br><br>With few exceptions animals will be killed at the end of the in-life phase of the study. This is necessary to meet the aims of toxicity studies as post-mortem examination and pathological evaluation of tissues are essential components of the overall safety assessment. The method of killing will always be an approved humane procedure. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Whilst alternatives to *in vivo* animal models are being developed and are used where possible, there are currently no scientific and legally acceptable evaluations of systemic toxicity that will satisfy regulatory requirements other than use of animals. Although molecules for pharmaceutical development are screened by in-vitro techniques, final candidate selection |

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| | based on in-vivo pharmacology and safety data is more stringent and helps to prevent unsuitable compounds going into development and as such reduce animal usage in the long term. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | This project will use the minimum number of animals by following specific published guidelines on study design, statistical analysis and where scientifically acceptable, reduced animal numbers. Studies are designed to provide maximal data and scientific credibility from the minimum number of animals, taking into account that it is essential to use a sufficient number of animals to meet the scientific objectives and avoid the need to repeat the study. Where appropriate, use is made of removing or limiting control groups, combining data from different groups, challenging the need for the use of both sexes. |
| **3. Refinement**<br>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. | Generally the rat is the rodent species of choice in pharmacological and toxicological evaluation. However, mice (or hamsters) may be used where they are considered a more appropriate species, for example, if bioavailability or tolerance is improved. On occasions it may be necessary to use genetically altered animals in order to achieve the objectives of the study. Only animals without an adverse phenotype or one that is mild or moderate will be used.<br><br>Regulatory authorities require characterisation of toxicity at the maximum tolerated dose. Therefore, it is necessary to perform toxicity studies at doses that produce overt toxicity. These studies will involve only a small number of animals and doses will be increased until adverse effects are evident; once signs indicate that a dose is unsuitable for use in a definitive study then action would be taken to alleviate the signs. Usually this would involve termination of the sex/group or may involve reduction of the dose.<br><br>Pivotal studies also require evidence of toxicity to ensure that a sufficiently high dose has been used to allow full evaluation of potential side effects. Typically in these studies, some clinical signs will be seen in the high dose animals. Generally these signs would be expected to be absent or reduced prior to the |

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| | next dose. However, because toxicity can become worse with increased duration of dosing or the exposure of animals to the test substance may increase over time, animals may show significant adverse effects and in this case action will be taken to alleviate the signs such as temporary or permanent withdrawal of the animal from dose, reducing the dose if appropriate or via the application of humane endpoints. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>A study of myelination</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Myelination, glia cells, axons, time lapse, in vitro</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>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[14]</td><td>Yes</td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>A fundamental process in the nervous system is the receiving and transmission of electrical signals around the body. This process is carried out by nerves which are wrapped in an insulating sheath called myelin. Many neurological diseases occur when this myelin sheath is disrupted and demyelination occurs eg. Multiple sclerosis. Myelination is a complex process which involves close interactions between nerve processes (axons) and their support cells known as glial cells. It is the glial cells that produce myelin and wrap axons. However the mechanism by which they wrap axons is not known. The aim of this proposal is to follow myelination using fluorochrome tagged axons or glial cells using novel small animal imaging systems or in petri dishes. If we can understand the process by which myelinating cells ensheath axons then we may be closer in designing strategies to promoting myelination in demyelinating diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The project described in this application will allow us to visualise the process of myelination over time and determine exactly how a glial cell contacts, ensheaths and produce the many layers of myelin necessary for the generation of a myelin sheath that promotes nerve conductance. These studies will allow us to fully understand myelination and identify potential targets and strategies to promote myelination and potential therapeutic interventions in demyelinating diseases.</td></tr>
</table>

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

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| What species and approximate numbers of animals do you expect to use over what period of time? | We will use mice, and over five years no more than1300 |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Most of our use is for production of primary cells from tissue so this has no severity. Wherever possible, and particularly for the transplantation studies, we use neural stem cells, which can be generated in very large numbers from a single animal. This reduces the numbers of neonatal mice that are required as a source of primary cells for each individual transplant. We have developed myelinating cultures from animal embryonic spinal cord which will allows us to examine the effect on growth factors or immune modulators or other myelinating cells to participate in CNS axon myelination prior to translating any findings to the animal. Any that will be used for visualisation of myelination have moderate severity and after imaging will be killed humanely for tissue analysis. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | It is not possible to totally replace animal tissue for use in neuroscience research as the process involved in generating the complex brain and spinal cord cannot be replicated by cell lines. The process of myelination when a nerve gets its insulating sheath necessary for the nerve has never been totally replicated in cell lines. For this reason we still work on cells taken from rodents. Moreover there are a wealth of reagent to study rodent biology and markers that is limited in other animals/systems. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Since we have been able to create complex cell interactions in a Petri dish from rodent cells we are able to so studies in a dish before using an animal and therefore do less testing of ideas and cell types in animal models. This allows a refduction of procedures such that less are carried out under procedure. |
| **3. Refinement**<br>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 animal models of CNS injury as in certain situations it is not possible to carry out complex differentiated functions such as functional nerve repair only in a Petri dish. Transplantation procedures are already established and optimally refined following the guidelines on the NC3RS web site (www.NC3Rs.org.uk). As this is a pilot study information gained will be used to further refine the procedure. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>European eel productivity and escapement in Kent</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>European eel PIT tag marshland</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>1year</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></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>Yes</td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The European eel is critically endangered and research by has discovered a decline in elver (juvenile eel) recruitment by up to 95% since the 1980’s. This low level of recruitment could have a prolonged impact upon yellow (growth-phase) and silver (adult) eel escapement/migration for at least the next 20 years. Very little research has been carried out on silver/yellow eel behaviour in Europe, thus there are large knowledge gaps in the regional and national Eel Management Plans. Identification, protection and suitable-management of favourable yellow/silver eel habitat is essential for the conservation of the species as a whole. This project will monitor yellow/silver eel productivity and escapement in the Kent Marshland, an area identified as a particularly productive habitat for yellow/silver eels. The main objectives for this project are four-fold:<br>• Collection and analysis of the first robust data on yellow/silver eel escapement from marshland in the UK.<br>• Research on the impact/efficiency of outfall pipes for silver eel escapement.<br>• Identification of cost-effective engineering and management options across the marsh area to benefit the eel stock.<br>• First eel stock estimate and growth rate analysis for yellow/silver eels in the Kent marsh area.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>The understanding of habitat preference and behaviour of yellow/silver European eels will be significantly improved by this project and will enable us to determine other priority sites needing</td></tr>
</table>

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

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| animals could benefit from the project)? | protection, to conserve this critically endangered species. On-going monitoring will also allow us to understand growth rates of eels, and determine whether the marshes are significantly more productive, and produce significantly better condition eels than those in the river itself.<br><br>All the data collected will be included in the Thames River Basin District EMP, the UK EMP and EU-wide datasets. We will endeavour to use this to improve management procedures and better the conservation of the European eel. |
| What species and approximate numbers of animals do you expect to use over what period of time? | 100 European eels (*Anguilla anguilla*) will be tagged using passive integrated transponder (PIT) tags. The tagging procedure, from removal from nets to release, should take no longer than 15 minutes, after which previous studies indicate that eels can live unaffected by the present of the tag until they leave freshwater. The eels will then be monitored every month using fyke netting. |
| 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? | Although deemed unlikely, potential adverse effects are:<br><br>1. Adverse response to anaesthesia<br>This is unlikely to occur due to the wide usage of the chosen anaesthetic (MS-222) on fish and the fact that it is frequently used on eels in published research.<br><br>2. Excessive bleeding<br>This is unlikely to occur due to the fact that the surgery is only minimally invasive. The incision that will be required to insert the will be 2-3mm long and in an area chosen to minimise the chance of puncturing any major blood vessels.<br><br>3. Wound infection<br>We do not expect there to be a significant chance of this occurring as each surgery will use a sterile scalpel blade and PIT tag to minimise the chances of infection.<br><br>Once the PIT tagging procedure is finished, the eels will be released back into the area from which they were removed. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | This project is species specific (European eel) and we must study the behaviour of the eels in their natural environment to complete our objectives. No non-animal alternatives can be used. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | 100 eels will be tagged, as per the protocol of Riley *et al. (*2011) study which tagged 101 eels, which is achievable in the time period and will allow robust statistical analysis. |

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| **3. Refinement**<br>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 whole procedure will take place on-site to reduce the amount of time the eels are out of their natural environment. A small number of eels will be processed at any one time, to minimise handling time and to avoid crowding in holding tanks. pH, temperature and oxygen levels of holding/anaesthetic tanks will be monitored continuously, to ensure they do not deviate significantly from ambient. Any eels that we have concerns over will be removed from the experiment to a holding tank to recover immediately. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Proteolysis in mouse models of cancer and tissue repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, wound healing, proteases, diet</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[17]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[18]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall goals of our work are to identify ways to prevent or treat cancer novel, and to improve the healing of skin wounds. Our approaches involve studying the functions of specific genes in cancer development or in wound repair using genetically altered mice, or mice that have been injected with tumour cells, or exposed to skin wounds. We study both cancers and skin wounds because the molecular events taking place are remarkably similar, except that "cancers are wounds that don't heal".<br><br>The focus of our research is a class of molecules called proteases, that act outside the cell and influence the way cells interact with their surroundings. Some of these enzymes seem to aid in the spread of cancer cells through the body, while others hold tumours in check. We want to find out more about how these proteases and related molecules work in the development of cancer. We will study their functions using mice that predictably develop cancers in particular tissues, such as the breast. We can genetically cross these mice with others that are deficient in one of our genes of interest, and in the resulting offspring we can observe the consequences of loss of a particular gene. We will also use human cancer cells to over- or under-produce copies of the molecules encoded by our genes of interest and see how these changes affect the growth of tumours when we inject the cells into appropriate strains of mice</td></tr>
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[17] Delete Yes or No as appropriate.
[18] At least one additional purpose must be selected with this option.

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| | (those that lack an immune system).<br><br>We will then use mice that develop tumours to test the ability of new drugs or other sorts of therapy based on our genes of interest to block the formation and spread of tumours, or to promote the healing of skin wounds. Some of these experiments will involve testing of new classes of agents called nanoparticles that we wish to design to home to tumour cells or be activated when near them.<br><br>In another aspect of our experiments, we will look at the ability of diets that include certain sorts of plants enriched in specific nutrients for their ability to prevent the development of cancer. |
| 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)? | The work will provide essential new information on the functions of genes that are instrumental in both normal tissue repair or the development of cancers. this information could lead to the design of new drugs that target these molecules, or other sorts of drugs that mimic their actions, depending on the effects we find. Our wound healing studies could lead to the development of new medicines to assist in the healing of chronic skin ulcers, which are a major problem for people with diabetes.<br><br>Finally, we are working with plant scientists who have made genetically modified tomatoes. Extracts from these tomatoes block the growth of cancer cells in culture and we now want to see if these tomatoes have cancer preventive properties in mice. If this is successful it will lead to trials of the tomatoes in humans who are at risk of developing cancer. This may have profound impact in several areas, including the public perception of the value of genetically modified foods. |
| What species and approximate numbers of animals do you expect to use over what period of time? | We will use mice, to a maximum of 15,600 animals over the duration of the 5 year 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? | We will use mice that develop tumours, either spontaneously or following injection of cancer cells. Also we will generate skin wounds on some animals (not the mice that develop cancer however). The mice that develop tumours may display a moderate level of severity as a result, which is accompanied by signs such as by weight loss of up to 20% and a reduction eating and water consumption. Mice will be killed to prevent suffering |

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| | beyond a moderate level.<br><br>Mice that receive skin wounds are at a low risk of getting infections in the wounds. This happens in less than 5% of animals and is minimized by ensuring strict sterile surgical technique, including sterilisation of all instruments after each animal to prevent cross contamination, and the use of sterile dressings and adhesive.<br><br>Some mice will be bred to provide animals for experiments. When these animals no longer breed well or develop age-related adverse signs, they will be killed, as will all animals that are used in experiments involving tumour development or skin wounds. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | We always first study the effects of our genes of interest, or new drugs, or the modified tomato plants that we have generated by looking at the effects on cells in culture. Only after we have convinced ourselves that the genes or other materials are having an important action on cell growth or some other aspect such as the way that cancer cells move or the way they make blood vessels grow - only then do we look at their effects in animals. It is essential to study these processes in whole animals because we can then see the interplay between the various types of cells present in a tumour or in normal tissue such as skin. We cannot do these sorts of experiments in humans (ie clinical trials) without first having done the pre-clinical studies in mice to show that the treatments work in the whole organism. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | We use the least number of mice possible in order to achieve statistically significant results. This is helped by good design of experiments with appropriate controls. |
| **3. Refinement**<br>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 types of animals that are genetically identical and develop tumours in very predictable ways, so the numbers of animals needed are kept low, as the variation between animals is as low as possible. The advantage of using tumour-prone mice is that we can look at all of the stages of tumour formation, for the very earliest changes through to growth of small localized lesions, to formation of invasive cancers that spread to other sites. It is not possible to observe all of these events in the petri dish, and human clinical specimens provide only snapshots, usually at the endpoints of disease. Thus the mouse is an excellent choice of species, in which the events of |

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| | cancer development and skin wound healing show good correspondence with those that take place in ourselves.<br><br>The mice are kept in a special facility in cages where they receive clean filtered air and sterile food, and their environments are secure and enriched by the provision of nesting material. We use anaesthetic wherever necessary for the minor treatments that the mice receive.<br><br>We try to use mice such that the tumours they develop can be easily monitored, for instance because they occur in the mammary glands or are implanted under the skin where they can be visible to the scientists. We hope to acquire new imaging equipment that will allow monitoring of tumours in deeper organs in living mice, without needing to kill the animals to observe the tumours or their spread. |