Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 17

Project Titles and key words

- Mechanisms of ageing

  Ageing, lifespan, health span, metabolism, diet

- Understanding liver injury and disease

  Liver, fibrosis, myofibroblast

- Making and freezing of genetically altered animals

  Transgenic cryopreservation rat mouse

- Understanding microglia-tumour interactions

  brain tumour, immune cells, zebrafish

- The biological significance of DNA methylation

  Rett Syndrome, epigenetics, mouse models

- Cardiac Development and Regeneration in Zebrafish

- Breeding and Maintenance of Genetically Altered Mice

  Mice, Breeding ,Vaccine, Research

- Mechanisms of immunity to Leishmania infection

  Immunology, Leishmania, Microscopy

- The ecology and biology of UK seals
  marine mammals, diving, foraging, behaviour, population dynamics

- Stem Cells in Organ Repair and Scarring

  Liver, cancer, regeneration, repair

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<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms of ageing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ageing, lifespan, healthspan, metabolism, 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)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td>~~No~~</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall objective of this project is to identify the mechanism/s that cause ageing in mammals.</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>How ageing affects animals appears very similar across different species from microscopic worms to humans. Consequently, if we can understand the ageing process in mice then this information should firstly help us understand more about the ageing process in humans. Secondly, it should help identify realistic interventions to help extend the period of life that humans can enjoy free from age-related disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice
5400</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 environmental and genetic manipulations we will use will primarily extend lifespan and improve health in mice, and so our animals will not be subjected to unnecessary suffering or illness. Consequently the level of severity will be mild. Some animals may be exposed to a diet rich in fat, with the major side-effect being that these animals will become obese. Animals for ageing studies will be maintained until they die naturally or will be culled if they show any evidence of debilitating disease or pathology. All other animals will be terminated at the end of each experiment in order to provide biological material for further studies.</td></tr>
</table>

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

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Ageing acts on the whole animals but affects different cells, tissues and organs in different ways. Therefore, to understand ageing you need to study multiple systems and study how these interact with one another over time. As a result the fundamental biological processes that occur during ageing are best studied in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental protocols within this project are based upon well-established methods refined to optimize experimental design and minimize animal suffering. We will use power analysis throughout this project to make sure we use the minimal number of animals required to produce meaningful data. Pilot studies will be run in such a way that the data they produce will be added to any main study wherever possible, in order to further reduce the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have been shown to be highly effective model organisms to study the basic mechanisms of ageing and have generated seminal findings on the physiological and genetic factors governing longevity. In addition, the majority of ageing changes seen in mice are also seen in humans. A set of highly refined procedures and extensive training of staff will be used for all studies to ensure consistency in the data output.

Understanding liver injury and disease
Liver, fibrosis, myofibroblast.

- Summarise your project (1-2 sentences)

This project seeks to identify ways in which to treat chronic liver disease such as fibrosis, for which there are currently no clinical treatments available. The project also seeks to understand how the liver can become more susceptible to damage, and how we may be able to use stem cells to treat patients with liver failure.

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

We are doing this project because liver disease is the fifth most common cause of premature death in the western world and the only one in the top five predicted to increase over the next few decades. A common consequence of liver injury of any type is fibrosis, for which there is currently no treatment. This project seeks, in part. to find treatments for fibrosis. It also seeks to establish whether a reduction in fibrosis can reduce cancer in the liver, and enhance liver cell function.

An additional component of the project will examine the potential for dietary constituents such as alcohol to alter the absorption of chemicals like food additives and colourings, from the gut. This could result in liver damage and/or increased exposure for people and is something that is not taken into consideration by regulatory authorities when they evaluate safe levels of exposure of dietary additives. We therefore need to establish whether this may be the case and could be the explanation for liver diseases such as primary biliary cirrhosis, for which the cause is not known.

- Outline the general project plan.

The general project plan will be to use established liver injury approaches to generate liver fibrosis in mice or rats. This may be induced by treating the animals with a selected number of toxins at a dose that specifically damages the liver without causing more than transient moderate adverse effects. Alternatively, the liver may be exposed to a transient loss of blood flow to part of the liver to model the effects that liver tissue undergoes prior to transplantation (as this can result in liver fibrosis in the recipient patient's new liver).

These models will be used to test potential drugs for their ability to reduce fibrosis.

In addition, the laboratory has developed an antibody-based strategy to target therapeutics to the cells that cause liver fibrosis. This strategy will be used to examine the role of myofibroblasts (and fibrosis) in a range of diseases such as cancer or stem cell engraftment in the liver and may demonstrate that an anti-fibrotic treatment may also help to reduce cancer growth in the liver or increase cell engraftment.

The project will also examine the potential for alcohol to modulate the uptake of chemicals in the diet and to increase the risk tf the chemical causing liver injury and fibrosis.

- 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.

Procedures include causing liver damage using chemicals or stopping the blood flow through a part of the liver for a period of time to initiate and sustain a fibrosis in the liver tissue. In some experiments, animals will be injected with tumour cells or stems cells to see if fibrosis affects them. No procedure exceeds moderate severity. In the absence of changes in body condition scoring and general clinical appearance, body weight losses of up to 25% of controls may occur.

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

The benefits of this project include a greater understanding of the function of the cells which cause fibrosis; the identification of drugs with the potential to treat fibrosis in man; the potential for improved stem cell therapies and the identification of a mechanism by which the population may be exposed to harmful chemicals present in our diet.

- 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.

Rats, mice and transgenic mice only will be used as these are established and effective models for human liver disease research. Approx 260 rats will be used. Approx 1080 mice will be used, 580 in experiments with approx 500 used for breeding transgenic mice, of which a proportion will be re-used in other experiments. In some cases, transgenic animals may be used if they enable a mechanism of action for a drug to be identified or confirmed. Transgenic mice may also be used if they enable live imaging experiments to be performed that relate to the degree of liver injury. This is because they provide more information about the injury and mean that we can use fewer animals. We have also replaced a severe model of liver injury(bile duct ligation) with a moderate replacement which markedly reduces mortality and provides a more controllable refined injury. These developments ensure that we will use the minimum number of animals necessary.

- 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.

We use a panel of in vitro assays to identify potential anti-fibrogenics or potential liver toxins. In this way, we reduce our reliance on animal models as much as possible. However, liver injury and liver fibrosis is a multi-cellular and multi-tissue process which cannot be completely replicated in vitro. Accordingly, key observations such as the identification of an efficacious anti-fibrogenic, need to be tested in animal models before they could approach Pharma development and clinical trials.

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

It is clear from animal studies and in man that significant liver injury can occur for many years without any symptoms because the liver regenerates (with the development of fibrosis). The liver injury protocols are designed to damage the liver sufficiently to cause fibrosis without causing more than transient moderate stress. So we can be reasonably confident that the animals used in these study protocols suffer the minimal degree of suffering required to undertake these studies.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Making and freezing of genetically altered animals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Transgenic cryopreservation rat mouse</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five 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></td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
</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 aims to produce mice and rats with altered genes to enable the study of biology and disease processes. These studies will increase our understanding of human diseases because of the similarities in the genes of mice, rats and humans. These species are already established as model systems for the study of some aspects of human biology.

The study of patients with naturally occurring gene mutations has helped us to understand the physiology involved, and the nature of the defect causing the disease. We do not have natural mutations in all human genes. Our ability to manufacture specific genetic alterations in the genetic material of mice and rats allows us to study a new disease model without having to screen thousands of animals looking for a naturally occurring mutation. The knowledge gained from creating genetic alterations has already led to tremendous advances in medical understanding and treatment. Now that the DNA sequence from humans, mice and rats is known, it is possible to design very powerful experiments to study disease genes.</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>Not all laboratories have the facilities to produce the genetically altered models of disease. This project is for us to produce new strains of organism for other people to study who are experts in their own fields. This is the most efficient way to create disease models, by minimising the amount of training that each group needs to do. It is also important to archive</td></tr>
</table>

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

	these types of research models. Archiving makes resources available to a wider group of researchers across the globe, and minimises the numbers of animals used just for breeding. Sometimes studies on humans with disease are not possible, because the tissues needed are not accessible, or there are insufficient patients willing to take part. The close similarities between the genetics and biology of mice and men mean that information from animals can guide the research on people so that the best treatments are found more quickly. Our models will help a variety of projects aimed at understanding basic processes, and will also help hospital-based researchers directly. Each of the experiments performed is reviewed by an independent panel and the work must have a good probability of answering the questions posed. Most of the genetic alterations that will be made under this licence will not result in abnormality that affects the animal. All our animals are monitored very carefully so that ill health and suffering is minimised. When physiology is affected to mimic human diseases there are trained people, such as vets, whose job it is to minimise suffering. These stages of the work will be done under other licences linked to this one, with experts in each field responsible for the animal care. The mouse is the model organism that we know most about in genetic and biochemical terms, after humans. Nowadays, the genetics of the rat are also well developed and we already know a lot about its physiology and biochemistry. It is this genetic ability that makes these organisms the best ones for research.
What species and approximate numbers of animals do you expect to use over what period of time?	During the five year duration of this licence up to 6,000 mice and 6,000 rats may be born, and up to 15,500 other mice and 8,000 rats used in processes regulated by this document. These animals will on the whole be distributed to other projects for further study, and will have very little analysis performed under this licence. Mostly, these animals will have non-invasive studies performed, such as direct visualisation. The tissues isolated from these animals after death will be most useful. It is hoped that medical knowledge will advance because of these new resources, and lead to deeper understanding of complex diseases and to the invention of new

	drugs and treatments. This type of work is possible now that the human genome (and those of the model organisms used here) have been determined. The important issues now are how all the many genes and environmental factors interact to regulate normal growth and life, and how these networks go wrong in disease.
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 adverse effects that may be seen are limited to surgical discomfort (managed by anaesthesia and analgesia), which is classified as mild or moderate severity. The effects of genetic alteration are not known before the start, and so each new strain of animal is carefully monitored by highly trained people (such as vets). Any animal that experiences an unexpected adverse effect will be humanely killed immediately so that suffering cannot occur. All animals used in these procedures will be humanely killed using methods that do not involve any animal suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project is to provide a core service of highly trained and efficient people to make genetically altered mice and rats. This cannot be done without using animals. The research done using these animals undergoes separate ethical review.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We know what is required to generate new strains by comparison with all other transgenic groups in the world. This has been done, and standards are now set within which we operate. In our own work, we constantly review the way we work and find ways to use fewer animals. New technologies are introduced as soon as they are available, many of which are designed to reduce further the number of animals we need.
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 kinds of research projects this work supports are those which look at the physiology of animals in health and disease, using mice and rats as models. Alzheimer's disease, cardiovascular disease, studies of learning and memory, for example, cannot be completed on cells in culture or by computer modelling. When more refined models exist, the scientists we work with are encouraged to use them for preliminary work at least, which leads to better, and more informative animal experiments. Animal welfare costs are minimised in our work by careful attention to aseptic techniques during surgery, and close supervision and monitoring of all animals used.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Understanding microglia-tumour interactions</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>brain tumour, immune cells, zebrafish</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></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 aims to study the role of a special type of immune cells (microglia) during tumour growth in the brain. The microglia normally serve as guardians of the brain and respond immediately to infections and injuries for example. This reaction is essential to remove any abnormalities and to allow brain recovery afterwards. However during tumour growth in the brain these defensive cells change their behaviour and begin to support the tumour causing it to grow and to spread. The reasons for this are not understood. Furthermore high levels of microglia within Glioblastomas (the most common malignant brain tumours) are very often correlated with a poor prognosis for the patient. Thus understanding the reasons of this pro-tumoural behaviour is the essential first step to develop therapeutic strategies to interfere with these cells to finally inhibit tumour growth.
We will study the interactions of microglia and tumour cells using the zebrafish larvae as a model. Due to the optic transparency of the zebrafish larvae we will be able to observe these interactions in the living animal as they occur in real-time. Using this model we aim to understand the signals that attract microglia to the tumour and influence them within the tumour. Furthermore due to the good accessibility of the zebrafish larvae for chemicals we can test which drugs influence the interactions of microglia and tumour cells.</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>Our studies will provide a comprehensive understanding of microglia functions, a cell type of high scientific and medical interest due to its tremendous repertoire of functions. To understand</td></tr>
</table>

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

animals could benefit from the project)?	the regulation of these cells within a tumour provides the basis for the development of future therapeutic interference. Here the obvious aim is to find treatments that interfere with microglia functions specifically within the tumour environment, instead of applying suppressive treatments for the entire immune systems. Such treatments could be an alternative treatment for glioblastomas, the most common malignant brain tumours, since these tumours still resist standard therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use the zebrafish (Danio rerio) for our studies. Adult fish will only be kept for breeding whilst experiments will be performed only in larval fish between day 3 and day 10 post fertilization. For the duration of the project (5 years) we estimate to keep approximately 6000 adult fish and to use approximately 3000 larvae for experiments.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most adult fish kept for breeding will be transgenic. Generally, the introduction of a transgene has no deleterious effect on fish health and well-being. Thus keeping genetically modified zebrafish is considered to be a mild procedure. No line will be kept in which insertional effects occur, which are deleterious to fish health. Tumour growth will be induced in larval zebrafish. This is a moderate procedure and larvae will bi killed by the end of the experiments, latest by day 10 post fertilization. Depending on the method used to induce tumour growth in larval fish additional injuries or infections might occur. Fish will be closely monitored and if abnormalities are observed fish will be killed immediately.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We aim to study the interactions of cells of the immune system (microglia) and brain tumours. Since microglia are, as parts of the immune system, very sensitive to any alteration in their environment these studies can not be done in cell culture. The zebrafish larva offers the unique opportunity to observe interactions of the immune system and tumours in real time as they occur in the living animal. This is essential to understand these processes and to develop future therapies. However, the majority of our experiments will be performed in larval zebrafish below the age of independent feeding and replaces the use of adults. Furthermore before doing experiments in larval zebrafish we will test the suitability in cell culture. Only treatments that show clear results in cell culture will be tested in the zebrafish larva.
2. Reduction Explain how you will assure	To strongly reduce the number of animals we will follow statistical guidelines for experimental design,

the use of minimum numbers of animals	which ensures that the minimal number of animals is being used to reach significant results. In case of necessary drug treatments dose finding studies will be performed *in vitro* beforehand to further reduce the number of animals. .
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 optically transparent zebrafish larva is an excellent model for studying the interactions of microglia and brain tumours in the living animal. In particular the opportunity to image cellular interactions as they occur in real time in the living animal, combined with the possibility of intervening genetically and pharmacologically, make the zebrafish the model of choice for our studies. This is not possible in any mammalian model. Furthermore the zebrafish has been established as a productive and informative cancer model. Multiple studies in recent years have led to zebrafish models for different types of human cancer, ranging from B-Cell/T-Cell leukaemia and melanoma to glioma. We will minimise animal suffering by performing the majority of experiments in larval zebrafish between day 3 and day 5-post fertilization and only occasionally larvae up to day 10 will be used. To minimise animal suffering larvae will be anaesthetised during experimental procedures and will be allowed to recover afterwards. Furthermore, upon injections we will treat the fish water with a suitable anti-fungal/anti-bacterial agent to prevent infections. Under such conditions, infections are almost never observed. Should infection occur despite all preventive measures, the fish will be killed by a schedule 1 method. All larvae will be closely monitored during the course of experiments and killed immediately if any adverse effects are observed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The biological significance of DNA methylation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Rett Syndrome, epigenetics, mouse models</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>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[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>Without changing the basic sequence of DNA it can still be chemically altered in ways which effect whether or not a particular gene is expressed. We would like a greater understanding of the function of one such chemical modification of DNA; methylation. Our research will focus on how DNA acquires this signal and in turn how cells then read and act upon this signal.</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>Defects in genes involved in the interpretation of methylation are associated with human diseases such as cancer and Rett Syndrome. Similar work in our lab has already established that Rett Syndrome is potentially curable. A better understanding of the underlying biology of such diseases will improve future therapeutic approaches.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice
4750 per annum</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>Rett syndrome is a disabling neurological disorder which primarily affects girls. It is known that mice with genetically altered copies of the Mecp2 gene develop clinical signs similar to Rett syndrome, after several weeks. The resultant severity in mice is moderate. The majority of the animals generated will be on breeding protocols, to maintain the line, with nothing happening to them. Animals will not be allowed to develop clinical signs unless this state is specifically required for an experiment. Some animals will develop signs such as impaired mobility. A minority of animals will be administered</td></tr>
</table>

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

	prospective therapeutic substances via injections into the brain. This is done under anaesthesia and using adequate analgesia to limit post-operative pain as advised by a veterinary surgeon. All animals will be euthanized using authorised procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We wish to study the consequences of the proposed genetic alterations in organs such as the brain. The complex biology of such multi-cellular organs cannot be modelled *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful consideration will be given to all breeding strategies to ensure we can produce sufficient animals with the appropriate genetic status in the most efficient way, thereby reducing the numbers of animals produced overall. When novel approaches are being tested, we will conduct pilot studies with smaller groups of animals prior to further experiments. Careful experimental design and statistical analyses will enable us to determine the smallest number of animals required to give us meaningful 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 objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse offers the best system in which to study the consequences of changes to genes such as Mecp2 as it provides us with the best characterised model of the human disorder, Rett Syndrome. Mice will not be allowed to develop clinical signs unless specifically required for an experiment and in all experiments use of early humane end-points is implemented using clearly defined scoring systems to limit suffering.

Cardiac Development and Regeneration in Zebrafish

- Summarise your project (1-2 sentences)
  The project looks at how malformations of the heart might be caused by genetic and environmental effects. It also investigates how genetic diseases of the heart muscle cells (cardiomyopathy) affect the heart and how aging, exercise and injury might improve or worsen the disease process.

- 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.
  It is largely unknown how heart malformations arise and how genetic abnormalities of the heart muscle cause their effects. Importantly it is not known whether the effects of age exercise and injury –such as heart attacks – can affect these processes. By using zebrafish we can analyse the basic processes involved in these conditions in order to eventually devise new treatments.

- Outline the general project plan. In order to understand heart malformations we will analyse the function of zebrafish genes either known to be malfunctioning in patients or within genetic pathways that are known to form the heart. In a similar way we will look at diseases of muscle cells (cardiomyopathies) by investigating the function of zebrafish genes either known to cause cardiomyopathy or new genes within relevant genetic pathways. We will ask what happens to these hearts with aging, exercise or with the equivalent to a heart attack and try and identify if and how they regenerate.

- 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.
  Most of the experiments can be carried out in very early embryos as the heart is forming but before the fish are capable of feeding. In general, disease causing genes are expressed or normal genes supressed to see what effects occur on the heart. In older fish the additional effect of exercise will be assessed by comparing the structure and function of the heart in fish kept in moving water as opposed to still waters – both natural habitats for zebrafish. The effect of injury on the heart can be assessed in a controlled way by making a small cut in the skin between the gills, to expose the heart. After damaging the tip of the heart the skin heals quickly and the fish are able to swim and feed normally.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.
  By understanding which genes cause heart disease we can understand the pathways involved and devise treatments or diagnostic tests.

- 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.
  Zebrafish are used as they have a simple, yet very similar heart to people. Most experiments can be done on embryos. Some experiments need to be done on older fish. In all cases we determine how many animals will be needed to answer scientific questions. By carefully planning the experiments we can use the minimum number of fish, but get a clear answer, reducing numbers of animals used, financial costs and researchers time.

- 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.
  Where possible we use cells grown in dishes but where questions relate to the working of heart we have to use animals. Many of our experiments are done in early embryos which are not animals protected under ASPA. We reduce our use by carefully designing experiments and also use the most modern microscope technologies.

- Explain why the protocols and the way they are carried out should involve the least suffering.
  Most experiments are designed to keep the fish healthy – for example swimming in flowing or still water. We use a fish anaesthetic that is put into the water when we are examining fish; on moving the fish to fresh water they wake up and swim again. At all stages we carefully watch them and if there is any evidence of them looking unwell we provide euthanasia by an overdose of anaesthetic.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Breeding and Maintenance of Genetically Altered Mice</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Mice Breeding Vaccine Research</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 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">Infectious diseases are still a major cause of death and illness in both high and low income countries and further development of new treatments and vaccines are desperately needed. We are a specialist centre for studying infectious diseases of global importance to public health including malaria and tuberculosis. The mice bred under this licence will allow us to determine the role of specific genes and host defences in resistance to these organisms and aid in the development and evaluation of new drugs and vaccines.

A single Project Licence for this purpose allows effective colony management as personnel with experience in breeding and husbandry of Genetically altered (GA) mice are in full control of the breeding. This has the potential effect of reducing the number of animals produced under one licence rather than producing the same line of animals under several different projects.</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">GA mice allow specific manipulation of a gene or gene elements and the subsequent examination of gene activity in a complex physiological environment, and provide a valuable method of understanding the function of particular genes in the development of disease. In this establishment the main aims are to study the biology of infectious disease including immunology, pathogenesis, chemotherapy and vaccination. For example, our previous work has helped to screen live vaccines for safety in recipients that have defects in their immune system. This allowed us to progress some</td></tr>
</table>

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

	candidates to clinical trials in humans but to hold back others which had an unacceptable level of residual virulence.
What species and approximate numbers of animals do you expect to use over what period of time?	For each strain of mouse, the experimental design will be determined by the expected breeding performance. This will be determined on a case-by-case basis from knowledge of the strain, previous experience and the required breeding strategy for the particular genetic alteration. Using this analogy, it will determine the numbers of breeding pairs that need to be set up to produce the required number of offspring. As this licence covers such a wide variety of strains of GA mice, we estimate on past breeding colony records, that a maximum of 5000 mice a year could be produced.
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?	Due to the nature of the genetic alteration, there are no adverse effects expected. The overall level of severity for this licence is mild. However should any mouse show any unexpected behaviour or signs of ill health the Named Veterinary Surgeon will be consulted. The fate of these mice will be used for research under other approved Project Licences.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	During the development of new vaccines and treatments of infectious diseases, the study of the effects of genetic alteration often needs to be addressed as the 'whole' animal. Wherever possible, other laboratory based techniques will be used, including cell lines but in many cases they must be obtained by the use of tissues from the living GA mouse.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A centralised facility allows for efficient use of the GA mice produced by controlling the breeding of the mice to match the scientific demand and to allow for the sharing of tissues which will stop the chance of duplication.
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.	GA mice allow for specific manipulation of a gene or gene elements and provide a valuable method of understanding the function of particular genes in the development of disease. This is considerably more efficient and reproducible than previous techniques (such as antibody mediated cell depletion) and allows us to more closely model the pathology of human infection with these pathogens in mice. All mice will be housed in individually ventilated cages (to ensure the exposure to environmental pathogens is minimised), in appropriate sized groups in solid floored cages and supplied with adequate bedding and nesting material and environmental enrichment.

	All new strains of GA mice that are added to this project are quarantined and health checked before being introduced to the main colony, to check that the high health standards are not compromised. Health screens are carried out every 6 months to ensure that the mice are in optimal health so that breeding success is ensured and without any adverse effects due to any incurrent disease or infection. This high health standard allows the results of the scientific studies to be reportable and correct, reducing the number of times experiments need to be repeated.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms of immunity to Leishmania infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunology
Leishmania
Microscopy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five 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>Leishmania is responsible for a major health burden, with up to 20 million people infected globally. The Leishmania parasite has co-evolved with its host over millennia, with a myriad of mechanisms to manipulate the host immune system. However, the pathways by which Leishmania circumvents host immunity to establish chronic infections are not clear. Studying this could allow the development therapeutics designed to prevent Leishmania. Additionally, identifying the mechanisms by which the parasite is able to switch off the immune system could provide unique ways to treat conditions such as arthritis and asthma in which inflammation is triggered inappropriately. Therefore, our primary objective is to identify how Leishmania parasites interact with the host immune system.
It is currently not clear what constitutes a protective immune response against Leishmania, but recruitment and migration of white blood cells to the site of infection plays a key role in determining the outcome of infection. Thus, our second objective is to understand the cell motility during Leishmania infection, applying advanced microscope and imaging methods, allowing informed design of vaccines and drugs.
In parallel with these objectives, we plan to develop novel methods to understand the fundamental aspects of the host-parasite interaction. There is an increasing need for new and improved methods of detecting, targeting and manipulating biological</td></tr>
</table>

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

	processes *in vitro* and *in vivo*, especially in experiments using *in vivo* imaging. Thus, the final component of our project will be to develop physical and chemical approaches for use in addressing biological problems, to validate them using relevant *in vitro* and *in vivo* models and to use them to help in understanding the immune response associated with *Leishmania* infection.
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 primary goal of our work is to develop new knowledge of the fundamental immunology and cell migration and motility in an important infectious disease. Our findings may therefore provide the opportunity to alter the recruitment or function of specific cells with currently-available drugs or to develop new interventions aimed at preventing the immune-evasion by *Leishmania*. Alternatively, manipulating and targeting specific cells could provide new approaches to vaccination to protect against *Leishmania* infection. Understanding the mechanisms by which the parasite evades the immune system may also provide new ways to treat autoimmune diseases. Finally, we will develop new technologies and methodologies for enhanced *in vivo* imaging, creating less invasive approaches for understanding many aspects of biology.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using approximately 7500 mice over the duration of the project. The majority of these will be from breeding genetically-altered and wild-type animals. We plan to only use mice (rather than other rodents) as the *Leishmania* mouse model is well accepted and the availability of genetically-altered strains will help answer important questions. We will minimise the use of animals by seeking *in vitro* alternatives wherever possible and through careful experiment design.
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?	Cutaneous lesions develop at the site of *Leishmania* infection and may ulcerate in a proportion of animals. Animals are monitored regularly and the experiment terminated if lesions develop beyond a certain limit or if mice show lameness or signs of distress. Certain genetic alterations to components of the immune system render animals more susceptible to infection. Where necessary, immune-deficient animals are maintained in filter cages to prevent infection, and any affected animal will be removed from the experiment or receive veterinary attention. Some procedures (especially imaging) require the animals to remain still and are therefore performed under anaesthetic. The level of anaesthesia will be maintained at sufficient depth for the animal to feel no pain, minimizing adverse effects by ensuring accurate dosing.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The interaction between the plethora of immune cells (and their many products) with an invading pathogen represents a complex interplay, with many factors as-yet unknown. Currently, non-animal alternatives cannot adequately replicate this complexity. However, we are developing a number of *in vitro* and *ex vivo* models to visualise the complexity of the immune response to pathogens. Informed by *in vivo* experimentation and imaging, we will continue to improve these *in vitro* models wherever possible, eg. using microfluidic devices to understand cell migration. Using data derived from *in vivo* imaging experiments, we are developing collaborations with experts in mathematical modelling to perform simple *in silico* models and experiments. Providing the capacity to better inform our 'wet-lab' and *in vivo* experiments, these approaches will allow us to both refine and reduce animal usage. These *in vitro* and *in silico* models can only be optimised through input of high-quality data. Given the complexity of the infection site and the number of unknown parameters, it is only through *in vivo* experimentations that we can adequately answer the fundamental research questions to inform the development of such models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Use of *in vitro* and *ex vivo* methods will be used wherever possible to minimise the use of animals. Combining multiple advanced imaging approaches will yield more (and better quality) data per mouse, using fewer animals thanks to the capacity of such systems to make repeated measurements from the same animal throughout the duration of infection. In all experiments, group sizes will reflect the minimum number of animals required to perform proper statistical analysis.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse model of *Leishmania* is well-documented and accepted by international peer-review. It represents the most refined approach as the range of genetically-altered animals greatly improves functional analysis, allowing precise questions to be addressed *in vivo* and reducing the number of animals used overall to obtain an objective. *Leishmania* infection causes relatively little suffering for the mice. We will minimise this by examining the earliest stages of infection wherever possible, often before lesions develop or become ulcerated. Additionally, we will seek to establish infections in sites causing minimal discomfort to animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The ecology and biology of UK seals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>marine mammals, diving, foraging, behaviour, population dynamics</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></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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of the programme is to investigate how environmental change impacts on the ecology, physiology and population dynamics of UK seals. Information on seal abundance, distribution, behaviour and health will be used to provide advice to government departments and conservation agencies on the management of UK seals.

There is a fundamental need to know how marine top predators such as seals are likely to respond to rapid ocean changes and the sort of ecosystem shifts that are now being observed. The dynamics and abundance of the two UK seal species have changed dramatically in the last 10 years. Grey seals have increased or begun to stabilise whereas common (harbour) seals have experienced dramatic declines of up to 90% in some regions. The reasons for these changes remain unknown.

The work will thus focus on determining the mechanisms and processes that lead to the observed dynamics of the seal populations around the UK. It is based on having a fundamental understanding of the biology and ecology of UK seals and requires basic scientific underpinning studies which can provide data on how seals respond to change.

The work will comprise studies at different scales, of individual animals comprising colonies and populations. By combining information collected from different approaches at the individual level,</td></tr>
</table>

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

	population models will allow us to predict responses to environmental change and increased human activity in the oceans (for example, investigating the effect wind and wave power installations, increases in ocean noise, changes in contaminant and toxin inputs, changes in weather and climate patterns and interactions with fisheries). One hypothesis for the decline in UK harbour seals is an increase in competition for limited resources with grey seals. Knowledge of how these two species use the ocean and land is crucial to addressing this question. Sophisticated tracking devices will allow us to investigate for the first time fine scale use of the marine ecosystem by both species. Detailed studies of how the two species use their breeding and haulout sites, the factors affecting their choice of breeding colony and individual reproductive success will continue to be of critical importance to predict the impact of future environmental change. Defining seal habitat requirements is a key component necessary for the conservation and management of the species. Both UK species are listed under the EU Habitats Directive where 'favourable conservation status' should be ensured by member states. Defining the requirements that allow animals to successfully feed, breed and rest will be used by policy makers to fulfil this conservation objective. We also require detailed knowledge of the foraging behaviour of the seals including measurements of the effects of dive duration, activity levels, season and development on energy requirements and foraging capabilities, and on how diet can vary. The work focuses on the two species of seal that are found in UK waters (grey and harbour seals). These are both listed in Annex II of the Habitats Directive. In addition they are covered by the Conservation of Seals Act (1970) and the Marine Scotland (2010) Act. The results from these studies will be used to inform policy makers about the management and conservation of these seal species.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	This project is a response to the needs of government and conservation agencies to maintain a high level of expert knowledge about seals around the coasts of the UK. This need is driven by three main processes: (i) seals, like other marine

project)?	mammals, are now being seen as increasingly important indicators/symbols of the state of our seas. They are at the top of marine food chains and therefore experience outcomes and pollutant flows of many anthropogenic effects; (ii) seals are viewed by some important pressure groups as a pest species within the UK but, on a larger European scale, they are seen as rare species that require protection that will conserve their critical habitat. This leads to conflicting interests in the debate about how best to manage seal populations and this debate needs to be informed by high quality information about the impact of seals upon the environment and the vulnerability of seals to changes in that environment within the UK; (iii) seals are potentially important parts of the structure of the marine ecosystems surrounding the UK. They are clearly vulnerable to natural environmental and prey fluctuations and one species is declining rapidly in some parts of its range for as yet unknown reasons. The work in this study will allow us to assess the effects of human disturbance on seals and help to inform the development of management decisions aimed at improving animal welfare and conservation.
What species and approximate numbers of animals do you expect to use over what period of time?	Typically a few hundred harbour and grey seals may be involved in this research each 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?	Animals may be temporarily restrained in nets, anaesthetised if required then weighed and measured. Blood and tissue samples may be collected and tracking devices may be glued to their fur (these last only until the next moult when the devices fall off). Some animals will be exposed to noise or other stimuli to assess their effect on seals. All animals are released to the wild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to study the behaviour and physiology of the animals directly because there are no alternative approaches that will allow us to answer questions about human impacts on seal populations around our coasts and how they are likely to be affected by increasing pressure on the marine ecosystem. These studies will be carried out in conjunction with sophisticated statistical and mathematical modelling of seal populations, dynamics and movements so we can predict how their distribution and abundance may change in future. We will also continue to develop appropriate

	in vitro approaches so that, for example, the effects of particular contaminants and toxins on seal cells may be assessed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of robust statistical calculations for each stage of the project will ensure the minimum number of animals is used whilst ensuring the scientific questions can be answered with enough power to detect any change.
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.	Grey and harbour seals are chosen due to UK and European conservation legislative drivers. All procedures carried out constitute current best practice, improved with veterinary advice. This process continues by review and refinement among licence holders, ensuring standards continue to improve. Animals may be anaesthetised to reduce stress and handling times kept to a minimum.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Stem Cells in Organ Repair and Scarring</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Liver, cancer, regeneration, repair</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)[15]</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[16]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Liver disease in the UK is increasing in prevalence and as a result the number of transplantable livers is much lower than required. The aim of this work is to find out 1. How liver disease occurs, 2. How the liver repairs itself following injury and 3. What happens when regeneration goes wrong: does it become cancer? By understanding these processes we can design drugs to specifically target these processes with the ultimate goal of preventing disease and enhancing repair in the liver; thus eliminating the requirement for transplantation</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 benefits of this work primarily focus on liver disease and the treatment of such. However many of the processes which occur during liver disease also occur during disease in other tissues so our work in the liver will contribute to disease progression in a range of organs.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will use both rats and mouse and over the five years we will use a maximum of 25000 animals.</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>As we are modelling disease our animals they will progressively demonstrate symptoms of the disease. However we closely manage these symptoms to ensure that the animals do not undergo any undue suffering.</td></tr>
</table>

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

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Regeneration in the liver is a complex, multi-staged process in which there are any different cell types interacting with one and other. It is impossible to model such complexity without using animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is possible to calculate the numbers of animals required for experimentation based on data from previous data. In all cases we ensure that we have calculated the minimum number of animals required for the experiment to give us useful data. This approach reduces the animal numbers required, and also reduces the likelihood that the animal experiment would have to be repeated.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We regularly refine the disease models we use to reduce animal symptoms and to improve the effectiveness of our models. Because of this we can ultimately use fewer animals per procedure to and still generate meaningful and clinically relevant data. As part of this e regularly monitor the body weight, body condition, food and fluid intake of animals as a measure of disease; we set strict limits to ensure that there is limited harm to the animals used.